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Ozone risk assessment is affected by nutrient availability: Evidence from a simulation experiment under free air controlled exposure (FACE)☆

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ABSTRACT

Assessing ozone (O_3) risk to vegetation is crucial for informing policy making. Soil nitrogen (N) and phosphorus (P) availability could change stomatal conductance which is the main driver of O_3 uptake into a leaf. In addition, the availability of N and P could influence photosynthesis and growth. We thus postulated that the sensitivity of plants to O_3 may be changed by the levels of N and P in the soil. In this study, a sensitive poplar clone (Oxford) was subject to two N levels (N0, 0 kg N ha^{-1}; N80, 80 kg N ha^{-1}), three P levels (P0, 0 kg P ha^{-1}; P40, 40 kg P ha^{-1}; P80, 80 kg P ha^{-1}) and three levels of O_3 exposure (ambient concentration, AA; 1.5 × AA; 2.0 × AA) for a whole growing season in an O_3 free air controlled exposure (FACE) facility. Flux-based ($POD_{0\ to\ 6}$) and exposure-based (W126 and AOT40) dose-response relationships were fitted and critical levels (CLs) were estimated for a 5% decrease of total annual biomass. It was found that N and P availability modified the dose-response relationships of biomass responses to O_3. Overall, the N supply decreased the O_3 CLs i.e. increased the sensitivity of poplar to O_3. Phosphorus alleviated the O_3-caused biomass loss and increased the CL. However, such mitigation effects of P were found only in low N and not in high N conditions. In each nutritional treatment, similar performance was found between flux-based and exposure-based indices. However, the flux-based approach was superior, as compared to exposure indices, to explain the biomass reduction when all nutritional treatments were pooled together. The best O_3 metric for risk assessments was POD_4, with 4.6 mmol m^{-2} POD_4 as a suitable CL for Oxford poplars grown under various soil N and P conditions.

1. Introduction

M24 is the mean value of hourly O_3 concentrations during the daily 24 h and its value was about 10 ppb at the pre-industrial era (Voltz and Kley, 1988; Cooper et al., 2014). W126 (the sum of weighted hourly O_3 concentrations, cumulated over the 12-h daylight period from 8:00 a.m. to 8:00 p.m.) is an index designed to reflect the cumulative exposures during three consecutive months in the growing season and is under consideration as secondary standard in the USA (Lefohn et al., 1988; EPA, 2008; EPA, 2015). AOT40 (the accumulated exposure over a hourly threshold of 40 nmol mol^{-1} during the growing season (Fuhrer et al., 1997) was the European legislative standard for plant protection (Directive, 2008/50/EC), and AOT40-based CLs have been proposed for trees (Karlsson et al., 2003) and semi-natural vegetation (Mills et al., 2011). However, such exposure-based metrics lack biological meaning because they disregard the different species- or cultivar-specific sensitivities of plants to O_3 and the effects of environmental and biological variables on O_3 uptake into a leaf (Paoletti and Manning, 2007).

Ground level ozone (O_3) is one of the major air pollutants in many developed or developing countries, and causes serious threat to forest and natural ecosystems (Paoletti, 2007; Ainsworth et al., 2012; Matyssek et al., 2013). A meta-analytic review estimated that the O_3-caused loss in the biomass of trees ranged from 7 to 17% under the current O_3 levels relative to projected levels by 2100 at middle latitude in the northern hemisphere (Wittig et al., 2009; Li et al., 2017). The assessment of O_3 risk to plants including forest

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vegetation is topical in research and policy making nowadays (Mills et al., 2011; Watanabe et al., 2012; De Marco et al., 2015; Sicard et al., 2016b). Several O_3 metrics have been suggested for risk assessments (Paoletti and Manning, 2007). Critical levels (CLs), above which O_3-caused loss would occur, have been developed for identifying areas of CL exceedance (LRTAP Convention, 2010; Mills et al., 2011; Büker et al., 2015).

Ozone enters the leaf through stomata, which are the main regulating apparatus of gas exchange and play key roles in determining the potential O_3 damage (Matyssek et al., 2013). There are many reports that O_3-caused negative effects - such as foliar visible injury, inhibition of photosynthesis and decrease of biomass - are more closely related to the stomatal O_3 flux than to O_3 exposure (Karlsson et al., 2007; Bagard et al., 2015; Sicard et al., 2016b). The accumulated stomatal O_3 flux is called phytotoxic ozone dose (POD) and has a potential to be the best metric for setting future O_3 CLs for forest protection against O_3 pollution (Karlsson et al., 2003; Mills et al., 2011; Sicard et al., 2016a). New CLs are thus suggested as flux although AOT40 still has some fields of application (CLRTAP, 2017).

Nitrogen (N) deposition is increasing mainly due to elevated anthropogenic N emissions such as nitrogen oxides (NOx) emission from combustion of fossil fuels (Galloway et al., 2004). The background N deposition in some areas, such as Sichuan basin in China and California central valley in USA, was even higher than 80 kg N ha^{-1} yr^{-1} (Fenn et al., 2003; Peng et al., 2017). It has been indicated that nutrient availability dominates carbon retention in forests (UNECE, 2011; De Vries, 2014). However, N deposition of more than 26 N ha^{-1} $year^{-1}$ was negatively related with basal area increment in forests of Switzerland (Braun et al., 2017). Since nitrogen oxides (NOx) are the main O_3 precursor, O_3 exposure and N deposition are often interrelated (Ollinger et al., 2002). Nitrogen is considered as an important modifier of plant responses to O_3 (Schulze et al., 1994; Utriainen and Holopainen, 2001a). There are some reports that additional N supply may increase the tree sensitivity to O_3 (e.g. Watanabe et al., 2012). On the other hand, some opposite responses were also found such as in hybrid aspen (Häikiö et al., 2007). In contrast to N, trees are facing phosphorus (P) deficiency by depletion, soil barriers, low-P parent materials, and sink-driven and anthropogenic limitations (Vitousek et al., 2010; Peñuelas et al., 2012). Phosphorus is an essential macronutrient for physiological functions, including photosynthesis (Larcher, 2003). Phosphorus availability in the soil is an important regulator of the aboveground net primary productivity of forests (Domingues et al., 2010). Soil acidification induced by excess N deposition may cause P limitation for tree growth (Huang et al., 2016; Braun et al., 2017). Phosphorus availability may affect stomatal conductance (Fitter, 1988; Tissue and Lewis, 2010; Cernusak et al., 2011; Zimmerli et al., 2012), which modifies stomatal O_3 flux. Therefore, risk assessment of O_3 pollution must not overlook interactions with N and P availability (Matyssek et al., 2013). However, to our knowledge, there are only few reports about the impacts of N and P availability individually or in combination, on the O_3 risk assessment of forest species (Utriainen and Holopainen, 2001b; Wallin et al., 2002). Most of these studies were exposure-based assessment rather than flux-based one. The influences of N or P availability on risk assessment based on stomatal O_3 flux are urgently needed to be investigated.

Among tree species, poplar has been paid particular attention because it is widely used for wood production and as a model system in plant biology (e.g., Christersson, 2010). However, the knowledge of O_3 dose-response relationships for poplar is still limited (Marzuoli et al., 2009; Hu et al., 2015; Hoshika et al., 2018a).

The objectives of the present study were: (1) to derive exposure based and flux based dose-response relationships for poplar biomass responses to O_3 and nutritional availability; and (2) to define O_3 CLs for protecting poplar from O_3 stress under different nutritional conditions.

2. Materials and methods

2.1. Plant material

Rooted cuttings of Oxford poplar clone (*Populus maximoviczii* Henry × *Populus berolinensis* Dippel) were propagated in December 2015, and then transplanted in 10 l plastic pots filled with a mixture of sand:peat:soil = 1:1:1 (v:v:v) in April 2016. Plants were irrigated sufficiently to field capacity every 2–3 days to avoid water stress.

2.2. Ozone and nutrient treatments

Ozone treatments were applied at three levels: ambient air concentration (AA), 1.5 × AA and 2.0 × AA, from 1st May to 1st October, 2016, 24 h per day, in a free air controlled exposure (FACE) system located in Sesto Fiorentino, Florence, Italy (43° 48′ 59″ N, 11° 12′ 01″ E, 55 m a.s.l.). Details on this FACE facility can be found in Paoletti et al. (2017). Three replicated 25 m^2 blocks were assigned to each O_3 concentration, with 18 plants in each block. Three of the plants in each block were randomly assigned to each of six nutritional treatments as explained below. The pot position was changed every two weeks within each block to eliminate possible positional effects due to irrigation or light. Ozone was generated by a generator (TGOC13X, Triogen ltd., Glasgow, UK). A mixture of O_3 and ambient air was distributed into the O_3 FACE by a system of 25 teflon tubes in each block hanging down from a fixed grid above the seedlings (Paoletti et al., 2017). Ozone concentration at plant height was continuously monitored by O_3 analyzers (Model 202, 2B Technologies Inc., Boulder, Colorado, USA).

Two levels of N concentration (N0: 0 kg N ha^{-1}, i.e. 0 mg N $seedling^{-1}$; N80: 80 kg N ha^{-1}, i.e. 392.5 mg N $seedling^{-1}$) were supplied as NH_4NO_3 (0 and 5 mM solution) according to Thomas et al. (1994). Three levels of P concentration (P0: 0 kg P ha^{-1}, i.e. 0 mg P $seedling^{-1}$; P40, 40 kg P ha^{-1}, i.e. 196.3 mg P $seedling^{-1}$; P80: 80 kg P ha^{-1}, i.e. 392.5 mg P $seedling^{-1}$) were added using KH_2PO_4 (0, 0.5 and 1.0 mM solution) according to Lewis and Strain (1996). Based on P affinity constant and adsorption maxima, these levels of P were selected to simulate a realistic range in soil available P (Yu et al., 2017). Therefore, there were 6 combinations of nutrient treatment, i.e. N0P0, N0P40, N0P80, N80P0, N80P40, N80P80. In detail, 200 ml solution of NH_4NO_3 or KH_2PO_4 with different concentrations as described above were added into the soil twice a week during the whole treatment period. At the same time, KCl was supplied into the soil that did not receive KH_2PO_4 to keep an equal amount of K among all treatments (Tissue and Lewis, 2010; Mao et al., 2014). In total, 162 plants (3 levels of O_3 × 2 levels of N × 3 levels of P × 3 replicated blocks × 3 plants in each block) were used. At the end of the experimental period, soil was sampled for measuring the pH of the soil aqueous solution using a pH meter (Model MP220, Mettler Toledo, Switzerland). In addition, contents of N and P in the soil were measured. Total N content was determined using modified Kjeldahl method. In this method, titanium dioxide was used as catalyst instead of selenium (classic Kjeldahl method), distillation apparatus being Gerthardt (Cools and De Vos, 2010; ISO 11261). Content of total P was determined by inductively coupled plasma–optical emission spectroscopy (ICP–OES, iCAP7000, Thermo Fisher Scientific, Waltham MA, USA). During the whole treatment period, the hourly mean, maximum, and minimum temperature were 22.9, 37.4, and 9.3 °C, respectively, and the precipitation was 226.6 mm. The volumetric soil water content (SWC) was measured in the root layer (5 cm depth in pots) by EC-5