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Ozone impairs the response of isoprene emission to foliar nitrogen and phosphorus in poplar☆

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ABSTRACT

Tropospheric ozone (O_3) impairs physiological processes of plants while nitrogen (N) deposition may cause imbalances in soil N and other nutrients such as phosphorus (P) suggesting an increase of P demand for plants. However, the combined effect of O_3, soil N and P on isoprene emission from leaves has never been tested. We therefore examined isoprene emission in leaves of Oxford poplar clone exposed to O_3 (ambient, AA [35.0 nmol mol^{-1} as daily mean]; 1.5 × AA; 2.0 × AA), soil N (0 and 80 kg N ha^{-1}) and soil P (0, 40 and 80 kg P ha^{-1}) in July and September in a Free-Air Controlled Exposure (FACE) facility. We also investigated the response of isoprene emission to foliar N, P and abscisic acid (ABA) contents in September because the 2-C-methylerythritol-5-phosphate (MEP) pathway of isoprenoid biosynthesis produces ABA. We found that O_3 increased isoprene emission in July, which was associated to increased dark respiration, suggesting an activation of metabolism against O_3 stress as an initial response. However, O_3 decreased isoprene emission in September which was associated to reduced net photosynthesis. In September, isoprene emission was positively correlated with leaf N content and negatively correlated with leaf P content in AA. However, no response of isoprene emission to foliar N and P was found in elevated O_3, suggesting that the isoprene responses to foliar N and P depended on the O_3 exposure levels. Isoprene emission rate in 1.5 × AA and 2.0 × AA increased with increasing leaf ABA content, indicating accelerated senescence of injured leaves to favor new leaf growth when high O_3 and nutritional availability in the soil were combined. Even though foliar N and P usually act as a proxy for isoprene emission rate, the impact of recent abiotic factors such as O_3 should be always considered for modeling isoprene emission under climate change.

1. Introduction

Tropospheric ozone (O_3) is an important contributor to greenhouse radiative forcing (IPCC, 2013; Sitch et al., 2007) and a secondary gas pollutant formed by photochemical reactions with nitrogen oxides (NO_X) and volatile organic compounds (VOCs) (Mills et al., 2018). Ozone damages plant physiological processes causing a decline of growth and productivity (Grulke and Heath, 2020; Hoshika et al., 2020b; Paoletti, 2007). Plants are known to emit VOCs such as terpenes (isoprene and monoterpenes; Sharkey and Monson, 2014), which can further react in the troposphere leading to O_3 formation (Baraldi et al., 2019; Calfapietra et al., 2013; Peñuelas and Staudt, 2010).

Previous studies demonstrated that O_3 may affect VOC emission from leaves, especially that of isoprene (Fares et al., 2010; Feng et al., 2019b). Chronic O_3 exposure, at concentrations lower than 150 nmol mol^{-1} for more than 10 days, decreased isoprene emission rate by 21% (Feng et al., 2019b), although shorter-term acute O_3 exposure may stimulate isoprenoid emissions (Feng et al., 2019b; Velikova et al., 2005). Emission of isoprene may play a role in the

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protection of membranes against the oxidative stress induced by O_3, by scavenging reactive oxygen species (ROS) in the leaf intercellular spaces (Vickers et al., 2009), changing photosynthetic membrane properties therefore affecting photochemistry (Pollastri et al., 2014, 2019) and acting as a signal molecule for defensive metabolism (Harvey and Sharkey, 2016; Zuo et al., 2019).

Anthropogenic nitrogen (N) deposition is a major factor of modification of the plant nutritional balance while other macronutrients such as phosphorus (P) show a less significant atmospheric deposition (Peñuelas et al., 2012). Soil nutrients such as N and P may affect the production of secondary metabolites in leaves (Carriero et al., 2016; Koricheva, 2002; Lin et al., 2010; Peñuelas et al., 2011). Indeed, isoprene emission is affected by soil nutritional conditions (Fernández-Martínez et al., 2018). Previous studies showed that N fertilization increased isoprene emission rates (Fernández-Martínez et al., 2018; Harley et al., 1994; Litvak et al., 1996; Possell et al., 2004). This is because high foliar N increases photosynthesis, which positively correlates with isoprene emission (e.g. Litvak et al., 1996). In contrast, P addition has a negative effect on isoprene emission (Fares et al., 2008; Fernández-Martínez et al., 2018), indicating a decoupling of isoprene emission from photosynthesis under P-rich conditions in the soil (Fares et al., 2008).

Poplars are recognized as a model plant in plant physiology and are used for timber production worldwide, especially in the Northern Hemisphere (Jansson et al., 2010). Poplars are known to be strong emitters of isoprene, and generally sensitive to O_3 (Loreto and Velikova, 2001; Yuan et al., 2020). In addition, poplar plantations are established under various soil N and P conditions (Arevalo et al., 2011; Hoosbeek et al., 2004). Therefore, the combined effects of O_3 and soil nutrients on isoprene emission warrant consideration. In fact, it has been reported that O_3 may change plant responses to soil N and P (Braun et al., 2017; Feng et al., 2019a). However, to the best of our knowledge, there are only a few studies where the combined effects of O_3 and N on isoprene emission from poplar leaves were examined (Yuan et al., 2017, 2020). They reported that O_3 exposure decreased isoprene emission just under limited soil N availability (Yuan et al., 2020). Overall, the interaction between O_3, N and P on isoprene emission from leaves has never been tested.

Ozone may accelerate leaf senescence (Paoletti, 2007). The premature leaf senescence after O_3 exposure may be promoted by nutrient-rich conditions in the soil (Hoshika et al., 2019; Pell et al., 1995). Abscisic acid (ABA) is a major phytohormone relating to leaf aging process (Jibran et al., 2013). Isoprene biosynthesis is closely related to the chloroplastic 2-C-methylerythritol-5-phosphate (MEP) pathway, in which ABA is biosynthesized through carotenoids. In fact, previous studies reported that isoprene emission was correlated with leaf ABA content in water-stressed conditions (Barta and Loreto, 2006; Marino et al., 2017). In addition, isoprene may be associated with the expression of ABA-related genes under stress (Zuo et al., 2019). We therefore hypothesized that O_3 may modify the response of isoprene emission from leaves to soil N and P availability, which is potentially linked with ABA during the process of premature senescence of leaves following O_3 exposure.

Our aim was to investigate isoprene emission rates in an O_3-sensitive poplar clone (Oxford: *Populus maximowiczii* Henry × *P. berolinensis* Dippel) exposed for one growing season to the combinations of O_3, N and P treatments in an O_3 Free Air Controlled Exposure (FACE) experiment. Measurements were conducted during two periods, i.e., in July and September, thus taking into account the impact of different O_3 dose and leaf age on isoprene emission rate (Fares et al., 2010; Hoshika et al., 2019). In fact, previous studies reported that leaf physiological responses to O_3 were different between early summer (July) and late summer (September) in deciduous trees (Hoshika et al., 2019, 2020b). The questions addressed here are: (i) does O_3 affect the response of isoprene emission to foliar N or P? (ii) does ABA correlate with the response of isoprene emission to the combination of $O_3 \times N \times P$?

2. Materials and methods

2.1. Experimental site and plant materials

The study was conducted in an O_3 FACE system located in Sesto Fiorentino, Italy (43°48′49″ N, 11°12′01″ E, 55 m a.s.l.). In December 2015, rooted cuttings of Oxford poplar clone (15.4 cm as an averaged initial height) were planted in 10 L plastic pots (one cutting per pot) filled with a mixture of sand: peat: soil = 1:1:1 (v:v:v) (Zhang et al., 2018a, 2018b). Plants were exposed to three levels of O_3 concentrations: ambient air (AA), 1.5 times ambient O_3 concentration (1.5 × AA), and 2.0 times ambient O_3 concentration (2.0 × AA) from 1st May to 1st October, 2016. Daily mean O_3 concentrations during the experiment were 35.0 nmol mol^{-1} in AA, 51.6 nmol mol^{-1} in 1.5 × AA and 66.7 nmol mol^{-1} in 2.0 × AA (Zhang et al., 2018a). Ozone was generated from pure-oxygen (Mod. TOGC13X, Triogen Ltd., Scotland) and mixed with ambient air. The O_3 mixed air was delivered to plants by 25 Teflon tubes hanging down from a fixed grid above the plants in each plot. The air flow was controlled by a proportional integral derivative (PID) system. The air pressure was tested and controlled to be constant along the Teflon tubes, to allow a similar flow from each microhole for the fumigation of O_3 (0.3 L min^{-1}). The details of the FACE system are described in Paoletti et al. (2017). We used two N treatments (0 and 80 kg N ha^{-1} referred to as N0 and N80), and three P treatments (0, 40 and 80 kg P ha^{-1} referred to as P0, P40 and P80) (Table 1). NH_4NO_3 solutions (0 and 5 mM) and KH_2PO_4 solutions (0, 0.5 and 1 mM) were applied in order to reach the target concentrations at the end of the experiment (NH_4NO_3: every 10 days for a total of 14 applications; KH_2PO_4: every 5 days for a total of 28 applications). To keep the same level of K among all treatments, KCl was also supplied to the soil that did not receive KH_2PO_4. From the point of view of experimental planning, one of the most important strategies is to carry out experiments at the extremes (maximum and minimum settings) of the range of the variables (Fogler, 2016). This is why we tested the effects of minimum/maximum levels of N deposition, which may happen in a real world (0 and 80 kg N ha^{-1}, Fenn et al., 2003; Peng et al., 2017). Although the atmospheric deposition of P is usually less significant, poplar plantation can be established in various soil types such as low-fertilized (e.g., volcanic ash soils, Hoosbeek et al., 2004) and high-fertilized soils (e.g., agricultural field soils, Arevalo et al., 2011). Therefore, the levels of P application were selected to simulate a realistic soil available P. In fact, Zhang et al. (2018a) confirmed that soil P concentrations in our experiment (soil P: 0.5 g kg^{-1} in P0, 0.8 g kg^{-1} in P40, 1.0 g kg^{-1} in P80) were within the range of those observed in various soil types in the world (0.5–1.3 g kg^{-1}, Stevenson and Cole, 1999). There were three replicated blocks (L × W × H: 5 m × 5 m × 2 m) in each O_3 treatment, and each block contained all six N and P combinations (three plants per each combination), for a total of 162 plants. Soil water content in the pots was kept to the field capacity (0.295 m^3 m^{-3}, Paoletti et al., 2017).

2.2. Measurements of gas exchange and isoprene emission

Leaf gas exchange measurements were conducted between 9 and 12 a.m. solar time in two periods, i.e. at the end of July and September 2016, for fully developed healthy leaves (one leaf per

Table 1
Summary of the experimental treatments in this study. Poplars were exposed to three levels of O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrients in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)].

Ozone treatments	Nitrogen treatments	Phosphorus treatments
Ambient (AA), 1.5 times ambient ozone concentration (1.5 × AA), 2.0 times ambient O3 concentration (2.0 × AA).	0 kg N ha^{-1} (N0), 80 kg N ha^{-1} (N80).	0 kg P ha^{-1} (P0), 40 kg P ha^{-1} (P40), 80 kg P ha^{-1} (P80).

one to three plants per each replicated block per each combination of treatments, n = 3 blocks, 5–10th leaf from the tip of a shoot). Leaves flushed at mid-June were used for the measurement in July (5-week old leaves). Leaves flushed at the 1st week of July were used for the measurement in September (10-week old leaves). Net photosynthetic rate (P_N) and stomatal conductance (g_s) were recorded by using a leaf cuvette (6 cm^2) of a portable infra-red gas analyzer (LI6400, Li-Cor Inc. Lincoln, NE, USA). Leaves were kept at a constant conditions of photosynthetic photon flux density (PPFD, 1000 μmol m^{-2} s^{-1}), CO_2 concentration (400 μmol mol^{-1}), leaf temperature (30 °C) and relative humidity (50–60%). Dark respiration (R_{n30}) was assessed by measuring the CO_2 efflux from the leaf after switching off the light source for 30 min under the same conditions of temperature, CO_2 concentration and relative humidity.

Isoprene emissions were measured in real-time, simultaneously to the gas exchange on the same leaves and plants, by connecting the inlet of a Proton Transfer Reaction–Mass Spectrometer (PTR-MS, Ionicon Analytik GmbH, Innsbruck, Austria) directly to the LI6400 cuvette with a Teflon tube. During measurements, the PTR-MS was set to operate in Selected Ion Monitoring (SIM) mode acquiring the protonated ion 69+ (Lindinger et al., 1998). Isoprene concentration was quantified through a gas standard (Apel Riemer, Broomfield, CO, USA) after subtracting the background signal recorded from the empty cuvette before starting the measurements. To avoid any contamination with isoprene coming from ambient air, VOC-free air was provided to the LI6400 system by a custom made catalytic converter (Brilli et al., 2011).

2.3. Abscisic acid, nitrogen and phosphorus contents of leaves

In September, after the measurements of gas exchange and isoprene emission, all leaves used in leaf-level measurements were collected. Leaves were immediately frozen in liquid N and stored at −80 °C until analysis. The concentration of free forms of ABA was determined on freeze-dried leaves according to the protocol reported by Brilli et al. (2019). The identification and quantification of ABA was performed by using a GC-MS system (7890A-5975C, Agilent Technologies, US). The levels of endogenous hormones were estimated from the corresponding peak as determined by the principles of isotope dilution (Cohen et al., 1986). ABA concentrations were calculated on a dry weight basis (nmol gdw^{-1}).

Half of the leaf samples were dried in an oven at 70 °C to reach constant weight. Leaf N concentration was then determined on dried, grounded leaves using the modified Kjeldahl method (Cools and De Vos, 2010). Leaf P concentration was determined with an inductively coupled plasma–optical emission spectroscopy (ICP–OES, iCAP7000, Thermo Fisher Scientific, Waltham MA, USA). In addition, photosynthetic nitrogen use efficiency (PNUE) and phosphorus use efficiency (PPUE) were calculated as P_N divided by the area-based N and P content (N_{area} and P_{area}), respectively.

2.4. Statistical analysis

The experiment had a replicated split-plot design with a full factorial combination of treatment levels. The whole-plot treatment comprised three levels of O_3 with three block replications for a total of nine blocks. The sub-plot treatment consisted of six combinations of the nutritional treatments (two levels of N and three levels of P), which were randomly assigned to 18 pots (three pots per nutritional combination, distributed among the three blocks of each O_3 level, in total, 162 pots distributed among nine blocks). Data were tested for normal distribution (Kolmogorov-Smirnov D-test) and homogeneity of variance (Levene's test). Since all data were normally distributed, they were analyzed with a full-factorial split-plot three-way analysis of variance (ANOVA) with O_3, N and P as fixed factors, and the block as random factor nested in O_3. To assess relationships between isoprene emission and photosynthetic parameters or leaf traits P_N or R_{n30} in July and September, we conducted simple linear regression analyses. In addition, simple linear regressions were also applied to the relationships between isoprene emission rate and leaf nutrients (N or P) or ABA in September. When significant lines were obtained, the effect of O_3 on the relationships between isoprene emission rate and leaf nutrients or hormones was tested by analysis of covariance (ANCOVA). Quadratic functions were applied to the relationships of isoprene emissions with leaf N:P ratios in September. Results were considered significant at $p < 0.05$. All statistical analyses were performed in R 3.5.1 (R Core Team, 2018).

3. Results

3.1. Abscisic acid, nitrogen and phosphorus contents of leaves

Nitrogen addition increased leaf ABA content especially in P0 plants (Fig. S1: +241% in AA, +204% in 1.5 × AA, +95% in 2.0 × AA, as N80P0 compared to N0P0). However, this positive effect of N addition was not clearly observed under P80 treatments. The interaction of O_3 × N × P was significant for foliar ABA content so that 2.0 × AA O_3 exposure significantly increased it when supplying N and P together (N80P80).

Nitrogen treatments enhanced leaf N content and P treatments increased leaf P content (Fig. S2). However, this increase of foliar P by P treatment was limited at the highest level of O_3 treatment (2.0 × AA), as confirmed by a significant interaction between O_3 and P. Leaf N:P ratios were in a range of 6.9–21.6. The N:P ratio was significantly increased by N treatments but decreased by P treatments.

3.2. Effects of ozone, nitrogen and phosphorus on leaf gas exchange

Net photosynthetic rate (P_N) values were significantly affected by O_3, N and month (Fig. 1; Table 2). P_N decreased with advancing season due to leaf senescence. Ozone decreased P_N not in July but in September, as confirmed by the statistical significance of O_3 × Month. The effect of N on P_N varied with O_3 treatments and measuring month. In July, Tukey's test indicated that N increased P_N only at 2.0 × AA. On the other hand, in September, N increased P_N at AA and 1.5 × AA while such positive effect was not found at 2.0 × AA. In September, O_3 induced a significant reduction of

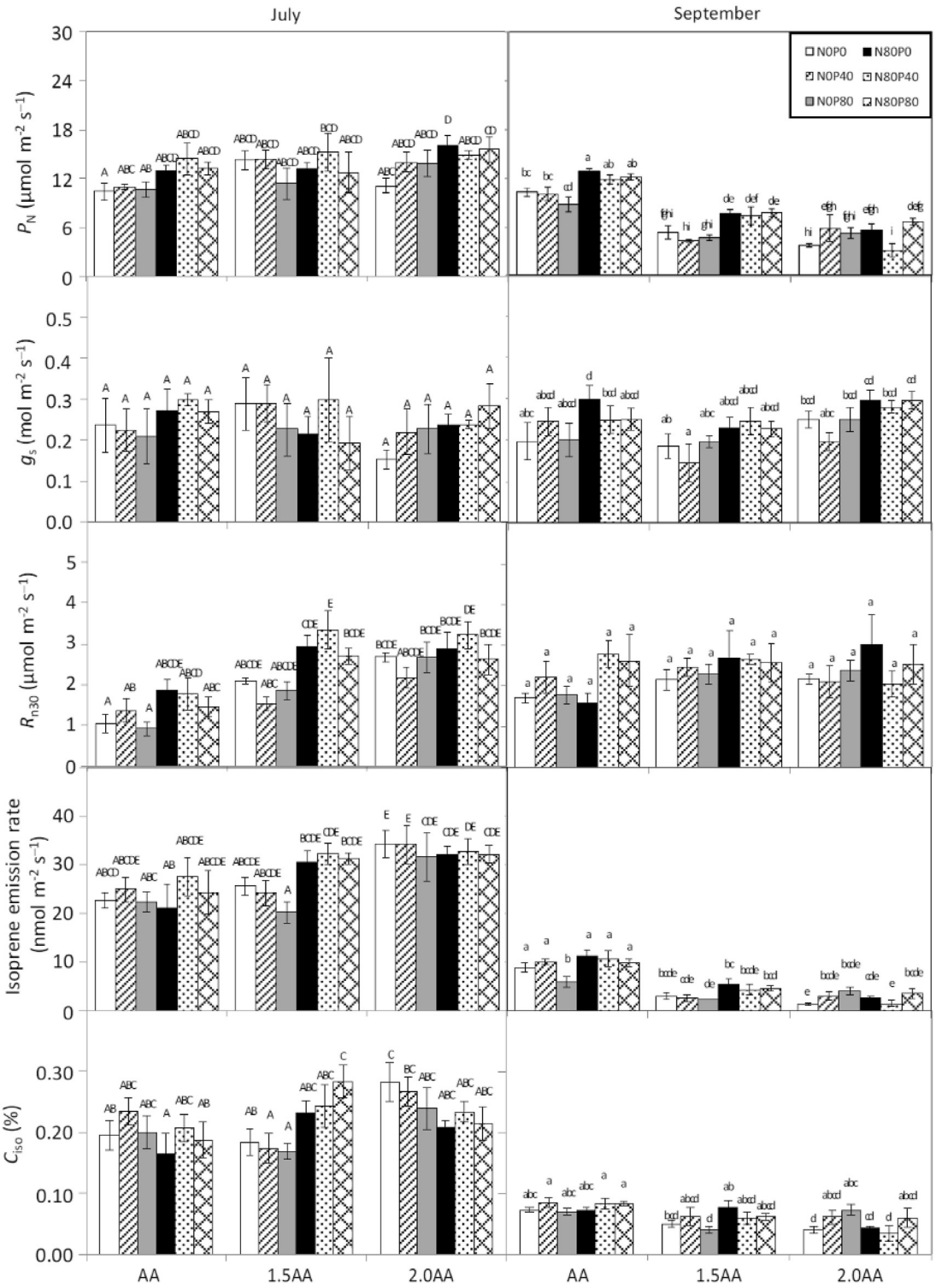

Fig. 1. Net photosynthetic rate (P_N), stomatal conductance (g_s), dark respiration rate at 30 °C (R_{n30}), isoprene emission rate and the percentage of photosynthetic carbon lost as isoprene (C_{iso}) of poplar Oxford clone in July and September 2016 under three O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrients in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)]. The bars represent mean ± S.E. (n = 3 blocks). ANOVA results are shown in Table 1. Different letters show significant differences among treatments in each month ($p < 0.05$, Tukey test).

photosynthetic nutrient use efficiency (PNUE and PPUE) (Table 3). A significant interaction of the three factors was found for PNUE, suggesting that there was a remarkable reduction in PNUE due to 2.0 × AA O_3 exposure in N80P40 plants (−79%). Nitrogen addition increased PPUE whereas P addition decreased it. The reduction of PPUE due to P addition was not found in 2.0 × AA as indicated by the significant interaction of O_3 × P. Stomatal conductance (g_s) was stimulated by N treatments. Dark respiration rate at 30 °C (R_{n30})

Table 2
Statistical significance of O_3, N, P and measured month and their interactions on net photosynthetic rate (P_N), stomatal conductance (g_s), dark respiration rate at 30 °C (R_{n30}), isoprene emission rate and the percentage of photosynthetic carbon lost as isoprene (C_{iso}) of poplar Oxford clone grown with three O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrients in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)]. Asterisks show the significance of ANOVA tests: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: not significant.

Treatments	P_N	g_s	R_{n30}	Isoprene emission rate	C_{iso}
O_3	***	ns	***	*	ns
N	***	**	***	**	ns
P	ns	ns	ns	ns	ns
Month	***	ns	ns	***	***
O_3 × N	ns	ns	ns	**	***
O_3 × P	ns	ns	ns	ns	ns
O_3 × Month	***	ns	**	***	***
N × P	ns	ns	ns	ns	ns
N × Month	ns	ns	ns	ns	ns
P × Month	ns	ns	ns	ns	ns
O_3 × N × P	ns	ns	ns	ns	ns
O_3 × N × Month	*	ns	ns	ns	**
O_3 × P × Month	ns	ns	ns	ns	ns
N × P × Month	ns	ns	ns	ns	ns
O_3 × N × P × Month	ns	ns	ns	ns	ns

was increased by O_3 in July. However, no significant effect of the combination of O_3, N and P treatments on R_{n30} was found in September.

3.3. Effects of ozone, nitrogen and phosphorus on isoprene emission rate

Ozone effects on isoprene emission were different between the months. In July, a significant increase of isoprene emission due to O_3 exposure was found (Fig. 1, for N0P0, +13% in 1.5 × AA and +51% in 2.0 × AA compared to AA) in association with an increasing percentage of photosynthetic carbon lost as isoprene (C_{iso}). In September, however, O_3 significantly decreased isoprene emission (for N0P0, −66% in 1.5 × AA and −84% in 2.0 × AA compared to AA). Isoprene emission rate in July was positively correlated with R_{n30} while it was not significantly dependent on P_N (Fig. 2A). In contrast, in September there was a significant positive relationship between isoprene emission and P_N, while R_{n30} was not correlated with the isoprene emission rate in this month (Fig. 2B).

We found a significant N effect on isoprene emission rate (Fig. 1, Table 2). This was confirmed by the positive correlation between isoprene emission and leaf N content in September (Fig. 3). However, such a positive relationship was not observed at the highest O_3 level (2.0 × AA). In September, isoprene emission decreased with increasing P content in AA, while there were no significant relationships between isoprene emission and leaf P in poplars treated with the higher O_3 levels (1.5 × AA and 2.0 × AA) (Fig. 3). Still in September, the response of isoprene emission to leaf N:P ratio in AA and 1.5 × AA showed a upward convex curve with a vertex as maximum isoprene emission rate where the N:P ratio was approximately 15 in AA and 18 in 1.5 × AA. Such a response curve was not obtained in 2.0 × AA.

Isoprene emission rate and C_{iso} in 1.5 × AA and 2.0 × AA were significantly correlated with leaf ABA content (Fig. 4). However, no significant relationship was found between isoprene emission and foliar ABA content at the low O_3 level (AA).

4. Discussion

4.1. Effects of ozone on isoprene emission and leaf gas exchange

Twice ambient O_3 exposure enhanced the isoprene emission rate in July (Fig. 1). This increase of isoprene emission did not correlate with net photosynthesis, but rather with an increase of

Table 3
Photosynthetic nitrogen use efficiency (PNUE) and phosphorus use efficiency (PPUE) of poplar Oxford clone in September 2016 under three O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrient treatment in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)]. The values represent mean ± S.E. (n = 3 blocks). Asterisks show the significance of ANOVA tests: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: not significant. Different letters show significant differences among treatments ($p < 0.05$, Tukey test).

Treatment		PNUE ($\mu mol\ g^{-1}\ s^{-1}$)		PPUE ($\mu mol\ g^{-1}\ s^{-1}$)	
AA	N0P0	12.1 (±0.7)	ab	129.4 (±7.3)	bcde
	N0P40	11.2 (±0.3)	ab	134.2 (±14.7)	bcd
	N0P80	9.9 (±0.5)	b	70.2 (±17.2)	fg
	N80P0	13.2 (±0.6)	a	213.0 (±35.7)	a
	N80P40	12.6 (±1.3)	a	175.9 (±37.1)	ab
	N80P80	12.9 (±0.6)	a	103.7 (±7.7)	cdef
1.5 × AA	N0P0	6.5 (±0.6)	c	82.9 (±20.4)	defg
	N0P40	5.0 (±0.4)	cde	40.7 (±5.8)	g
	N0P80	5.4 (±0.1)	cd	40.0 (±5.1)	g
	N80P0	7.2 (±0.5)	c	154.4 (±18.5)	bc
	N80P40	6.9 (±0.9)	c	96.7 (±17.7)	defg
	N80P80	5.6 (±0.4)	cd	60.4 (±3.8)	fg
2.0 × AA	N0P0	3.8 (±0.5)	de	46.8 (±3.7)	fg
	N0P40	6.5 (±2.0)	c	73.6 (±24.6)	efg
	N0P80	4.8 (±0.6)	cde	57.3 (±2.2)	fg
	N80P0	4.9 (±0.7)	cde	87.8 (±8.2)	defg
	N80P40	2.7 (±0.9)	e	39.1 (±16.2)	g
	N80P80	5.4 (±0.4)	cd	82.3 (±12.1)	defg
ANOVA results					
	O_3	***		***	
	N	ns		***	
	P	ns		***	
	O_3 × N	*		ns	
	O_3 × P	ns		**	
	N × P	ns		ns	
	O_3 × N × P	*		ns	

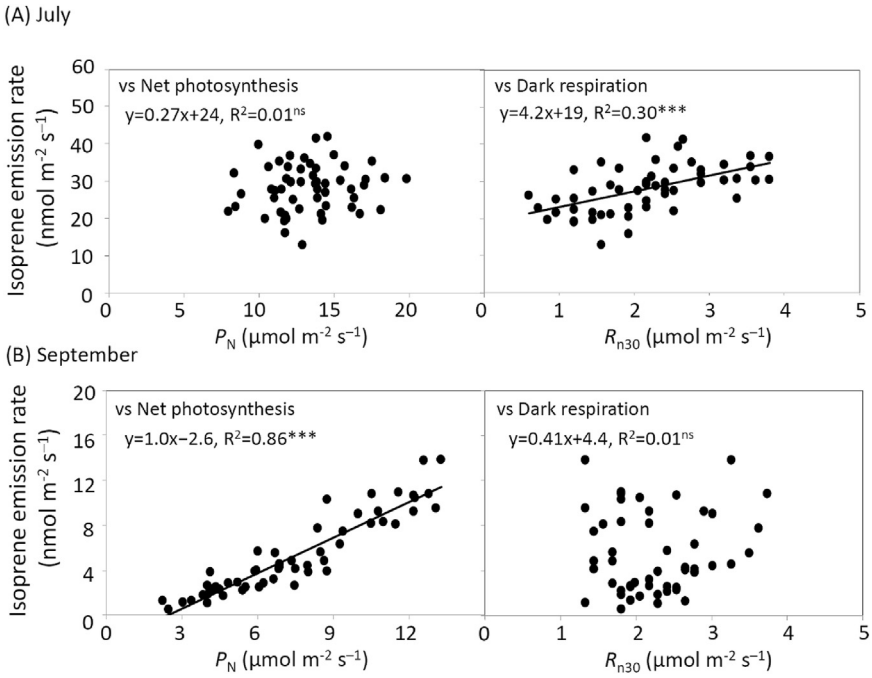

Fig. 2. Relationships between net photosynthetic rate (P_N) or dark respiration rate at 30 °C (R_{n30}) and isoprene emission rate of poplar Oxford clone in (A) July and (B) September 2016 under three O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrients in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)]. Simple linear regressions were applied. *** $p < 0.001$, ns: not significant.

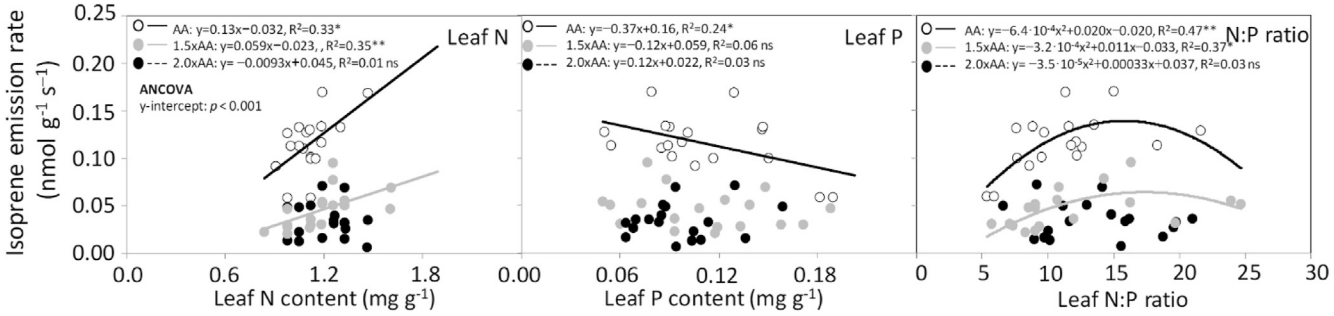

Fig. 3. Relationships between isoprene emission rate and leaf N, P and N:P contents of poplar Oxford clone in September 2016 under three O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrients in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)]. Simple linear regressions were applied in the relationships with leaf N or P contents. Quadratic functions were applied in the relationships with leaf N:P ratios. ** $p < 0.01$, * $p < 0.05$, ns: not significant.

dark respiration rate (Fig. 2A). In fact, O_3 enhanced the rate of dark respiration although it did not affect net photosynthesis in July. Elevated dark respiration after O_3 exposure was similarly reported in Japanese Siebold's beech (*Fagus crenata*; Hoshika et al., 2013), European beech (*Fagus sylvatica*; Kitao et al., 2009) and Scots pine (*Pinus sylvestris*; Skärby et al., 1987). The positive relationship between dark respiration and isoprene emission is in agreement with the fact that respiration and the MEP pathway for isoprene biosynthesis share a common substrate as previously discussed by Loreto et al. (2007). The increase of respiration may indicate a raised metabolic activity of detoxification processes to cope with the oxidative stress induced by O_3 (Matyssek and Sandermann, 2003). This implies that leaves may be able to detoxify and repair O_3 damage thanks to catabolic respiratory processes while emitting isoprene (Fares et al., 2006; Loreto and Velikova, 2001).

Ozone may promote a decrease of physiological parameters as an accelerated leaf senescence (Paoletti, 2007). In September, as leaves aged, isoprene emission rate was decreased due to the associated decrease in P_N and C_{iso} (Fig. 1; Table 2). In addition, after prolonged exposure, O_3 induced decreases of these parameters in this month. Ozone-induced injury may occur when O_3 dose exceeds antioxidant defense capacity (Paoletti, 2007). It has also been reported that the defense capacity against oxidative stress declined in older leaves (Fares et al., 2010; Peltonen et al., 2005). As a result, O_3 increased isoprene emission in July but decreased it in September, indicating that the isoprene response to O_3 was hormetic, which is generally caused by the possible biological acclimation to stressors (Agathokleous et al., 2019). In September, the decrease of isoprene emission rate due to O_3 exposure correlated with a reduction of net photosynthesis, while dark respiration was no more significantly correlated with isoprene emission (Fig. 2B). A decrease in isoprene emission has been associated to a damage to photosynthesis by abiotic stress such as drought in manipulative experiments (e.g., Brilli et al., 2007), and confirmed by a recent meta-analysis (Feng et al., 2019b). In particular, Calfapietra et al. (2007) reported that the inhibition of isoprene emission after O_3

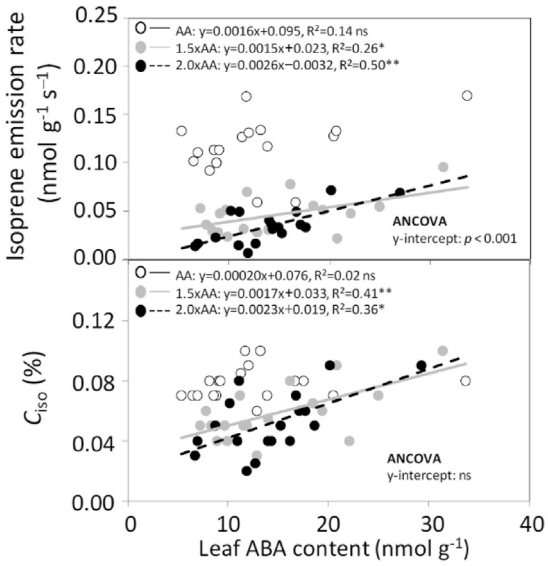

Fig. 4. Relationships between isoprene emission rate or the percentage of photosynthetic carbon lost as isoprene (C_{iso}) and leaf ABA contents of poplar Oxford clone in September 2016 under three O_3 concentrations (AA, ambient O_3 concentration; 1.5 × AA; 2.0 × AA) and six combinations of nutrients in the soil [two levels of N (0 and 80 kg N ha^{-1}; N0 and N80) and three levels of P (0, 40 and 80 kg P ha^{-1}; P0, P40 and P80)]. Simple linear regressions were applied. ** $p < 0.01$, * $p < 0.05$, ns: not significant.

exposure may be due to a reduction of isoprene synthase protein levels.

4.2. Effects of nitrogen and phosphorus on isoprene emission and leaf gas exchange

High N availability in the soil may stimulate photosynthesis, which, in turn, results in higher rates of isoprene emission (Litvak et al., 1996; Monson et al., 1994). In fact, isoprene emission rate was positively correlated with leaf N content in control plants (AA) in September (Fig. 3). This is consistent with previous findings for other species (*Mucuna* sp.: Harley et al., 1994; *P. tremuloides* and *Quercus rubra*: Litvak et al., 1996; *P. cathayana*: Yuan et al., 2017). Such positive effect of N on isoprene emission was not clear at AA in July. Monson et al. (1994) suggested that the N effect on isoprene emission may vary with leaf developmental stage.

Another important macronutrient influencing the change of isoprene emission was phosphorus (P). Tukey's test revealed that P80 treatment decreased isoprene emission rate at AA in September (Fig. 1) although the main effect of P was not statistically significant (Table 2). In fact, isoprene emission was negatively correlated with leaf P content in September (Fig. 3). This was supported by the finding in *Phragmites australis* (Fares et al., 2008) and by a meta-analysis (Fernández-Martínez et al., 2018). This negative relationship between isoprene emission and leaf P content was not explained by a photosynthetic limitation, because we did not find a negative relationship between P_N and foliar P (data not shown). Fernández-Martínez et al. (2018) mentioned the possibility that high pyruvate content in P-treated plants may efficiently control mitochondrial respiration competing with the MEP pathway for isoprene synthesis. However, mechanisms to explain the decoupling of isoprene emission from photosynthesis under P-rich conditions remain unclear (Fares et al., 2008).

Isoprene emissions showed quadratic responses to leaf N:P ratio in control plants (AA) (Fig. 3), with an optimal N:P ratio (approximately 15) where isoprene is emitted at the highest rate. Fernández-Martínez et al. (2018) found that the optimal N:P ratio for isoprene emission ranged from 10 to 40, depending on plant species. The N:P ratio may not be always suitable for plants to discuss the nutrient limitation because we sometimes do not find any significant relationships between the N:P ratio and physiological parameters. However, we found the optimal N:P ratio where isoprene was emitted at the highest rate as reported by Fernández-Martínez et al. (2018). According to the general theory by Koerselman and Meuleman (1996), it was suggested that N:P ratios <14 indicate limitation by N and those >16 indicate limitation by P for plant productivity while 14< N:P ratios <16 imply co-limitation. The observed optimal N:P ratio therefore indicated that isoprene emission from poplar leaves was co-limited by N and P under ambient O_3 (AA).

4.3. Interactive effect of ozone, nitrogen and phosphorus on isoprene emission and leaf gas exchange

An interaction of O_3 and N on isoprene emission occurred in the later growing season (Figs. 1 and 3). In September, in fact, no significant relationships between isoprene emission and leaf N content was found at the highest O_3 level (2.0 × AA). Ozone did not significantly affect leaf N content (Fig. S2), but decreased PNUE (Table 3). We therefore postulate that the decrease of isoprene emission at 2.0 × AA O_3 exposure was not mainly due to the decrease in leaf N content, but due to the decrease of PNUE. The decrease of PNUE might be due to a reduced allocation of N to the photosynthetic machinery following O_3 exposure (Shang et al., 2019; Watanabe et al., 2013).

Ozone also impaired the isoprene emission response to foliar P at high O_3 concentrations (1.5 × AA, 2.0 × AA) in September. Ozone suppressed the positive effects of soil P treatments on leaf P content (Fig. S2). No response of isoprene emission to P may be indirectly caused by impairment of root functioning for P acquisition under elevated O_3. Indeed, O_3 may affect P uptake through changes of ectomycorrhizal activities in roots, which helps host plants to absorb essential nutrients, such as P (Grebenc and Kraigher, 2007). Our previous investigations confirmed that the colonization rate of ectomycorrhizal fungi in our poplar roots was higher in P0 than in P80 for the Oxford poplar clone (Mrak et al., 2020).

It has been reported that the optimal N:P ratio for plant growth and physiology may depend on geography, temperature, and other climatic factors (Reich and Oleksyn, 2004). Fernández-Martínez et al. (2018) postulated that evolution of isoprenoids may be linked to nutrient availability. However, they also mentioned that isoprene emission is highly variable, suggesting an influence of specific factors such as recent abiotic stressors (i.e. air pollution). In fact, there was no dependency of isoprene emission on leaf N:P ratio in 2.0 × AA. Although foliar N and P can be used as a proxy for the isoprene emission (Fernández-Martínez et al., 2018), the results indicate that O_3 impaired the isoprene emission response to foliar N and P in Oxford poplar clone.

4.4. Is isoprene emission rate under high ozone, nitrogen and phosphorus related to leaf abscisic acid content?

The variation of isoprene emission rate after O_3 exposure in September was correlated with leaf ABA content (1.5AA and 2.0AA) (Fig. 4). It is known that isoprene metabolism is linked with ABA biosynthesis as indicated by previous studies (e.g., Barta and Loreto, 2006). Ozone increased leaf ABA content in plants grown with high nutritional availability (Fig. S1), which relates to a premature leaf senescence due to O_3 exposure (Cotrozzi et al., 2017; Podda et al., 2019). Plants with high soil nutrition often favor the replacement of damaged leaves by new leaf growth to alleviate the productive

decline induced by O_3 (Pell et al., 1995). This is confirmed by the fact that O_3 increased leaf shedding and new leaf growth in Oxford clone when grown under high nutritional conditions in the soil (Hoshika et al., 2019). As a result, O_3 may modify the biosynthesis of ABA eventually in association with isoprene metabolism through the activity of MEP pathway. No significant difference in C_{iso} was found among O_3 treatments in plants with high nutritional availability (e.g. N80P80) (Fig. 1). This indicates that those poplar plants maintained the allocation of photosynthates to isoprene emission even in O_3 damaged leaves, which may help to control the amount of ROS during a cellular degradation process such as a premature senescence after O_3 exposure (Sun et al., 2012). This hypothesis will be validated in further studies.

5. Conclusions

Ozone increased isoprene emission of poplar leaves in July but decreased it by the end of the growing season (i.e., September). In July, the increase of isoprene emission was not related to an enhanced photosynthetic rate, but to an increase of dark respiration rate, suggesting an activation of metabolism against O_3 stress. In contrast, in September, the decrease of isoprene emission rate due to O_3 was related to an impaired photosynthesis, while dark respiration did not significantly correlate with isoprene emission. The seasonal response of isoprene may be related to the different doses of O_3 and leaf ages (Fares et al., 2010; Feng et al., 2019b).

Ozone modified the response of isoprene emission to different nutritional availabilities due to an impairment of efficient nutrient use or nutrient uptake. Nitrogen increased the rate of isoprene emission, while P decreased it. However, those responses to N or P were not observed at the highest level of O_3. Isoprene emission rate under elevated O_3 increased with increasing leaf ABA content, suggesting an acceleration of leaf senescence in the combination of O_3 and high nutritional availability in the soil (Podda et al., 2019). The replacement of damaged leaves by new leaf growth may be a response to alleviate the productive decline induced by O_3 (Pell et al., 1995; Hoshika et al., 2020a). The variation of isoprene emission under high O_3, N and P may affect such an ecophysiological response of coping with O_3 stress through driving the turnover of the leaves.

The results suggest that soil N and P availability can be used as a factor to influence the changes in isoprene emission rates, and that interaction of O_3 and nutritional conditions should be considered for estimating future isoprene emission rate and atmospheric chemical processes.

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Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115679.

References

Agathokleous, E., Belz, R.G., Calatayud, V., De Marco, A., Hoshika, Y., Kitao, M., Saitanis, C.J., Sicard, P., Paoletti, E., Calabrese, E.J., 2019. Predicting the effect of ozone on vegetation via linear non-threshold (LNT), threshold and hormetic dose-response models. Sci. Total Environ. 649, 61–74.

Arevalo, C.B.M., Bhatti, J.S., Chang, S.X., Sidders, D., 2011. Land use change effects on ecosystem carbon balance: from agricultural to hybrid poplar plantation. Agric. Ecosyst. Environ. 141, 342–349.

Baraldi, R., Neri, L., Costa, F., Facini, O., Rapparini, F., Carriero, G., 2019. Ecophysiological and micromorphological characterization of green roof vegetation for urban mitigation. Urban For. Urban Gree. 37, 24–32.

Barta, C., Loreto, F., 2006. The relationship between the methyl-erythritol phosphate pathway leading to emission of volatile isoprenoids and abscisic acid content in Leaves. Plant Physiol. 141, 1676–1683.

Braun, S., Schindler, C., Rihm, B., 2017. Growth trends of beech and Norway spruce in Switzerland: the role of nitrogen deposition, ozone, mineral nutrition and climate. Sci. Total Environ. 599–600, 637–646.

Brilli, F., Barta, C., Fortunati, A., Lerdau, M., Loreto, F., Centritto, M., 2007. Response of isoprene emission and carbon metabolism to drought in white poplar (*Populus alba*) saplings. New Phytol. 175, 244–254.

Brilli, F., Pollastri, S., Raio, A., Baraldi, R., Neri, L., Bartolini, P., Podda, A., Loreto, F., Maserti, B.E., Balestrini, R., 2019. Root colonization by *Pseudomonas chlororaphis* modulates differently primary and secondary metabolism of *Lycopersicum esculentum* plants and enhances tolerance to water stress. J. Plant Physiol. 232, 82–93.

Brilli, F., Ruuskanen, T.M., Schnitzhofer, R., Müller, M., Breitenlechner, M., Bittner, V., Wohlfahrt, G., Loreto, F., Hansel, A., 2011. Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction "time-of-flight" mass spectrometry (PTR-TOF). PloS One 6, e20419.

Calfapietra, C., Fares, S., Manes, F., Morani, A., Sgrigna, G., Loreto, F., 2013. Role of Biogenic Volatile Organic Compounds (BVOC) emitted by urban trees on ozone concentration in cities: a review. Environ. Pollut. 183, 71–80.

Calfapietra, C., Wiberley, A.E., Falbel, T.G., Linskey, A.R., Mugnozza, G.S., Karnosky, D.F., Loreto, F., Sharkey, T.D., 2007. Isoprene synthase expression and protein levels are reduced under elevated O_3 but not under elevated CO_2 (FACE) in field-grown aspen trees. Plant Cell Environ. 30, 654–661.

Carriero, G., Brunetti, C., Fares, S., Hayes, F., Hoshika, Y., Mills, G., Tattini, M., Paoletti, E., 2016. BVOC responses to realistic nitrogen fertilization and ozone exposure in silver birch. Environ. Pollut. 213, 988–995.

Cohen, J.D., Baldi, B.G., Slovin, J.P., 1986. 13C6-[benzene ring]-indole-3-acetic acid. Plant Physiol. 80, 14–19.

Cools, N., De Vos, B., 2010. Sampling and analysis of soil. Manual Part X. In: Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests. UNECE, ICP Forests, Hamburg, ISBN 978-3-926301-03-1, p. 208. http://www.icp-forests.org/Manual.htm.

Cotrozzi, L., Remorini, D., Pellegrini, E., Guidi, L., Lorenzini, G., Massai, R., Nali, C., Landi, M., 2017. Cross-talk between physiological and metabolic adjustments adopted by *Quercus cerris* to mitigate the effects of severe drought and realistic future ozone concentrations. Forests 8, 148.

Fares, S., Barta, C., Brilli, F., Centritto, M., Ederli, L., Ferranti, F., Pasqualini, S., Reale, L., Tricoli, D., Loreto, F., 2006. Impact of high ozone on isoprene emission, photosynthesis and histology of developing *Populus alba* leaves directly or indirectly exposed to the pollutant. Physiol. Plantarum 128, 456–465.

Fares, S., Brilli, F., Nogués, I., Velikova, V., Tsonev, T., Dagli, D., Loreto, F., 2008. Isoprene emission and primary metabolism in *Phragmites australis* grown under different phosphorus levels. Plant Biol. 10, 38–43.
Fares, S., Oksanen, E., Lännenpää, M., Julkunen-Tiitto, R., Loreto, F., 2010. Volatile emissions and phenolic compound concentrations along a vertical profile of *Populus nigra* leaves exposed to realistic ozone concentrations. Photosynth. Res. 104, 61–74.
Feng, Z., Shang, B., Li, Z., Calatayud, V., Agathokleous, E., 2019a. Ozone will remain a threat for plants independently of nitrogen load. Funct. Plant Ecol. 33, 1854–1870.
Feng, Z., Yuan, X., Fares, S., Loreto, F., Li, P., Hoshika, Y., Paoletti, E., 2019b. Isoprene is more affected by climate drivers than monoterpenes: a meta-analytic review on plant isoprenoid emissions. Plant Cell Environ. 42, 1939–1949.
Fenn, M.E., Baron, J.S., Allen, E.B., Rueth, H.M., Nydick, K.R., Geiser, L., Bowman, W.D., Sickman, J.O., Meixner, T., Johnson, D.W., Neitlich, P., 2003. Ecological effects of nitrogen deposition in the western United States. Bioscience 53, 391–403.
Fernández-Martínez, M., Llusiá, J., Filella, I., Niinemets, Ü., Arneth, A., Wright, I.J., Loreto, F., Peñuelas, J., 2018. Nutrient-rich plants emit a less intense blend of volatile isoprenoids. New Phytol. 220, 773–784.
Fogler, H.S., 2016. Elements of Chemical Reaction Engineering, fifth ed. Pearson Education Inc., New Jersey.
Grebenc, T., Kraigher, H., 2007. Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentration. Plant Biol. 9, 279–287.
Grulke, N., Heath, R.L., 2020. Ozone effects on plants in natural ecosystems. Plant Biol. 22, 12–37.
Harley, P.C., Litvak, M.E., Sharkey, T.D., Monson, R.K., 1994. Isoprene emission from velvet bean leaves. Interactions among nitrogen availability, growth photon flux density, and leaf development. Plant Physiol. 105, 279–285.
Harvey, C.M., Sharkey, T.D., 2016. Exogenous isoprene modulates gene expression in unstressed *Arabidopsis thaliana* plants. Plant Cell Environ. 39, 1251–1263.
Hoosbeek, M.R., Lukac, M., van Dam, D., Godbold, D.L., Velthorst, E.J., Biondi, F.A., Peressotti, A., Cortufo, M.F., de Angelis, P., Scarascia-Mugnozza, G., 2004. More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect? Global Biogeochem. Cycles 18, GB1040. https://doi.org/10.1029/2003GB002127.
Hoshika, Y., Carrari, E., Mariotti, B., Martini, S., De Marco, A., Sicard, P., Paoletti, E., 2020a. Flux-based ozone risk assessment for a Plant Injury Index (PII) in three European cool-temperate deciduous tree species. Forests 11, 82.
Hoshika, Y., De Carlo, A., Baraldi, R., Neri, L., Carrari, E., Agathokleous, E., Zhang, L., Fares, S., Paoletti, E., 2019. Ozone-induced impairment of night-time stomatal closure in O_3-sensitive poplar clone is affected by nitrogen but not by phosphorus enrichment. Sci. Total Environ. 692, 713–722.
Hoshika, Y., Fares, S., Pellegrini, E., Conte, A., Paoletti, E., 2020b. Water use strategy affects avoidance of ozone stress by stomatal closure in Mediterranean trees—a modelling analysis. Plant Cell Environ. 43, 611–623.
Hoshika, Y., Watanabe, M., Inada, M., Koike, T., 2013. Model-based analysis of avoidance of ozone stress by stomatal closure in Siebold's beech (*Fagus crenata*). Ann. Bot. 112, 1149–1158.
IPCC, 2013. Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Jansson, S., Bhalerao, R., Groover, A.T., 2010. Genetics and Genomics of *Populus*. Springer, New York, NY, USA.
Jibran, R., Hunter, A.D., Dijkwel, P.P., 2013. Hormonal regulation of leaf senescence through integration of developmental and stress signals. Plant Mol. Biol. 82, 547–561.
Kitao, M., Löw, M., Heerdt, C., Grams, T.E.E., Häberle, K.-H., Matyssek, R., 2009. Effects of chronic elevated ozone exposure on gas exchange responses of adult beech trees (*Fagus sylvatica*) as related to the within-canopy light gradient. Environ. Pollut. 157, 537–544.
Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33, 1441–1450.
Koricheva, J., 2002. The Carbon-Nutrient Balance Hypothesis is dead; long live the carbon-nutrient balance hypothesis? Oikos 98, 537–539.
Lin, Y., Liu, X., Zhang, H., Fan, H., Lin, G., 2010. Nutrient conservation strategies of a mangrove species *Rhizophora stylosa* under nutrient limitation. Plant Soil 326, 469–479.
Lindinger, W., Hansel, A., Jordan, A., 1998. On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) medical applications, food control and environmental research. Int. J. Mass Spectrom. Ion Process. 173, 191–241.
Litvak, M.E., Loreto, F., Harley, P.C., Sharkey, T.D., Monson, R.K., 1996. The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. Plant Cell Environ. 19, 549–559.
Loreto, F., Centritto, M., Barta, C., Calfapietra, C., Fares, S., Monson, R.K., 2007. The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. Plant Cell Environ. 30, 662–669.
Loreto, F., Velikova, V., 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 127, 1781–1787.
Marino, G., Brunetti, C., Tattini, M., Romano, A., Biasioli, F., Tognetti, R., Loreto, F., Ferrini, F., Centritto, M., 2017. Dissecting the role of isoprene and stress-related hormones (ABA and ethylene) in *Populus nigra* exposed to unequal root zone water stress. Tree Physiol. 37, 1637–1647.
Matyssek, R., Sandermann, H., 2003. Impact of ozone on trees: an ecophysiological perspective. In: Esser, K., Lüttge, U., Beyschlag, W., Hellwig, F. (Eds.), An Ecophysiological Perspective. Progress in Botany, vol. 64. Springer-Verlag, Berlin Heidelberg, pp. 349–404.
Mills, G., Pleijel, H., Malley, C.S., Sinha, B., Cooper, O., Schultz, M., Neufeld, H.S., Simpson, D., Sharps, K., Feng, Z., Gerosa, G., Harmens, H., Kobayashi, K., Saxena, P., Paoletti, E., Sinha, V., Xu, X., 2018. Tropospheric Ozone Assessment Report: present day tropospheric ozone distribution and trends relevant to vegetation. Elementa: Sci. Anthropocene 6 (1), 47. https://doi.org/10.1525/elementa.302.
Monson, R.K., Harley, P.C., Litvak, M.E., Wildermuth, M., Guenther, A.B., Zimmerman, P.R., Fall, R., 1994. Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. Oecologia 99, 260–270.
Mrak, T., Eler, K., Badea, O., Hoshika, Y., Carrari, E., Paoletti, E., Kraigher, H., 2020. Elevated ozone prevents acquisition of available N due to smaller root surface area in poplar. Plant Soil 450, 585–599. https://doi.org/10.1007/s11104-020-04510-7.
Paoletti, E., 2007. Ozone impacts on forests. CAB Rev.: Perspect. Agric. Veterinary Sci. Nutrition Nat. Resour. 2, 68.
Paoletti, E., Materassi, A., Fasano, G., Hoshika, Y., Carriero, G., Silaghi, D., Badea, O., 2017. A new-generation 3D ozone FACE (free air controlled exposure). Sci. Total Environ. 575, 1407–1414.
Pell, E.J., Sinn, J.P., Johansen, C.J., 1995. Nitrogen supply as a limiting factor determining the sensitivity of *Populus tremuloides* Michx. to ozone stress. New Phytol. 130, 437–446.
Peltonen, P.A., Vapaavuori, E., Julkunen-tiitto, R., 2005. Accumulation of phenolic compounds in birch leaves is changed by elevated carbon dioxide and ozone. Global Change Biol. 11, 1305–1324.
Peng, Y., Chen, G., Chen, G., Li, S., Peng, T., Qiu, X., Luo, J., Yang, S., Hu, T., Hu, H., Xu, Z., Liu, L., Tang, Y., Tu, L., 2017. Soil biochemical responses to nitrogen addition in a secondary evergreen broad-leaved forest ecosystem. Sci. Rep. 7, 2783. https://doi.org/10.1038/s41598-017-03044-w.
Peñuelas, J., Sardans, J., Llusia, J., Owen, S.M., Niinemets, Ü., 2011. Lower P contents and more widespread terpene presence in old Bornean than in young Hawaiian tropical plant species guilds. Ecosphere 2, 45.
Peñuelas, J., Sardans, J., Rivas-Ubach, A., Janssens, I.A., 2012. The human-induced imbalance between C, N and P in Earth's life system. Global Change Biol. 18, 3–6.
Peñuelas, J., Staudt, M., 2010. BVOCs and global change. Trends Plant Sci. 15, 133–144.
Podda, A., Pisuttu, C., Hoshika, Y., Pellegrini, E., Carrari, E., Lorenzini, G., Nali, C., Cotrozzi, L., Zhang, L., Baraldi, R., Neri, L., Paoletti, E., 2019. Can nutrient fertilization mitigate the effects of ozone exposure on an ozone-sensitive poplar clone? Sci. Total Environ. 657, 340–350.
Pollastri, S., Jorba, I., Hawkins, T.J., Llusiá, J., Michelozzi, M., Navajas, D., Peñuelas, J., Hussey, P.J., Knight, M.R., Loreto, F., 2019. Leaves of isoprene-emitting tobacco plants maintain PSII stability at high temperatures. New Phytol. 223, 1307–1318.
Pollastri, S., Tsonev, T., Loreto, F., 2014. Isoprene improves photochemical efficiency and enhances heat dissipation in plants at physiological temperatures. J. Exp. Bot. 65, 1565–1570.
Possell, M., Heath, J., Hewitt, C.N., Ayres, E., Kersteins, G., 2004. Interactive effects of elevated CO_2 and soil fertility on isoprene emissions from *Quercus robur*. Global Change Biol. 10, 1835–1843.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from. https://www.R-project.org/.
Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proc. Nat. Acad. Sci. USA 101, 11001–11006.
Shang, B., Xu, Y., Dai, L., Yuan, X., Feng, Z., 2019. Elevated ozone reduced leaf nitrogen allocation to photosynthesis in poplar. Sci. Total Environ. 657, 169–178.
Sharkey, T., Monson, R.K., 2014. The future of isoprene emission from leaves, canopies and landscapes. Plant Cell Environ. 37, 1727–1740.
Sitch, S., Cox, P.M., Collins, W.J., Huntingford, C., 2007. Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448, 791–794.
Skärby, L., Troeng, E., Boström, C.-A., 1987. Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration in Scots pine. For. Sci. 33, 801–808.
Stevenson, F.J., Cole, M.A., 1999. Cycles of Soils: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients, second ed. John Wiley and Sons Publishers, Hoboken.
Sun, Z., Copolovici, L., Niinemets, Ü., 2012. Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? J. Plant Res. 125, 263–274.
Velikova, V., Pinelli, P., Pasquali, S., Reale, L., Ferranti, F., Loreto, F., 2005. Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. New Phytol. 166, 419–426.

Vickers, C.E., Possell, M., Velkova, V.B., Laothawornkitkul, J., Ryan, A., Mullineaux, P.M., Hewitt, C.N., 2009. Isoprene synthesis protects transgenic tobacco plants from oxidative stress. Plant Cell Environ. 32, 520–531.
Watanabe, M., Hoshika, Y., Inada, N., Wang, X., Mao, Q., Koike, T., 2013. Photosynthetic traits of Siebold's beech and oak saplings grown under free air ozone exposure. Environ. Pollut. 174, 50–56.
Yuan, X., Li, S., Feng, Z., Xu, Y., Shang, B., Fares, S., Paoletti, E., 2020. Response of isoprene emission from poplar saplings to ozone pollution and nitrogen deposition depends on leaf position along the vertical canopy profile. Environ. Pollut. 265A, 114909.
Yuan, X., Shang, B., Xu, Y., Xin, Y., Tian, Y., Feng, Z., Paoletti, E., 2017. No significant interactions between nitrogen stimulation and ozone inhibition of isoprene emission in Cathay poplar. Sci. Total Environ. 601–602, 222–229.
Zhang, L., Hoshika, Y., Carrari, E., Badea, O., Paoletti, E., 2018a. Ozone risk assessment is affected by nutrient availability: evidence from a simulation experiment under free air controlled exposure (FACE). Environ. Pollut. 238, 812–822.
Zhang, L., Hoshika, Y., Carrari, E., Cotrozzi, L., Pellegrini, E., Paoletti, E., 2018b. Effects of nitrogen and phosphorus imbalance on photosynthetic traits of poplar Oxford clone under ozone pollution. J. Plant Res. 131, 915–924.
Zuo, Z., Weraduwage, S.M., Lantz, A.T., Sanchez, L.M., Weise, S.E., Wang, J., Childs, K.L., Sharkey, T.D., 2019. Isoprene acts as a signaling molecule in gene networks important for stress responses and plant growth. Plant Physiol. 180, 124–152.