Biogeosciences
Discussions

Combined effects of ozone and drought stress on the emission of biogenic volatile organic compounds from *Quercus robur* L.

Arianna Peron[1], Lisa Kaser[1], Anne Charlott Fitzky[2], Martin Graus[1], Heidi Halbwirth[3], Jürgen Greiner[3], Georg Wohlfahrt[4], Boris Rewald[2], Hans Sandén[2], Thomas Karl[1]

[1]Institute of Atmospheric and Cryospheric Sciences, University of Innsbruck, Innsbruck, 6020, Austria
[2]Forest Ecology, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences Vienna (BOKU),Vienna, 1190, Austria
[3]Technische Universität Wien, Institut für Verfahrenstechnik, Umwelttechnik und Technische Biowissenschaften, A-1060, Vienna, Austria
[4]Department of Ecology, University of Innsbruck, Innsbruck, 6020, Austria

Correspondence to: Thomas Karl (Thomas.Karl@uibk.ac.at)

Abstract. Drought events are expected to become more frequent with climate change. To predict the effect of plant emissions on air-quality and potential feedback effects on climate, the study of biogenic volatile organic compound emissions under stress is of great importance. Trees can often be subject to a combination of abiotic stresses, for example due to drought or ozone. Even though there is a large body of knowledge on individual stress factors, the effects of combined stressors are not much explored. This study aimed to investigate changes of biogenic volatile organic compound emissions and physiological parameters in *Quercus robur L.* during moderate to severe drought in combination with ozone stress. Results show that isoprene emissions decreased while monoterpene and sesquiterpene emissions increased during the progression of drought. Exposing plants additionally to ozone, resulted in faster stomatal closure partially mitigating drought stress effects. Evidence of this was found in enhanced green leaf volatiles in trees without ozone fumigation indicating cellular damage. In addition we observed an enhancement in Methyl Salicylate emissions in trees with ozone treatment. Individual plant stress factors are not necessarily additive and atmospheric models should implement stress feedback loops to study regional scale effects.

1 Introduction

Plants in, both natural and managed ecosystems, release biogenic volatile organic compounds (BVOCs), covering over 30,000 known compounds (Peñuelas and Llusiá, 2004). These molecules have different physical and chemical characteristics and they differ in their metabolic origins in plants (Peñuelas and Llusiá, 2001; Laothawornkitkul et al., 2009; Maffei, 2010). An important subset of BVOCs are isoprenoids, such as isoprene (IS), emitted by plants and synthesized by the enzyme isoprene synthase (Silver and Fall, 1991) and via the 2-methylery-thritol 4-phosphate (MEP) pathway (Lichtenhaler et al., 1997; Schwender et al., 1997; Lichtenthaler, 1999) in chloroplasts (Wildermuth and Fall, 1996; 1998). Monoterpenes (MT) and sesquiterpenes (SQT), other classes of isoprenoids, are synthesized through the condensation of isoprene units (allylic isomer dimethylallyl diphosphate, DMAPP and isoprenyl diphosphate, IPP) (Ruzicka, 1953; Cheng et al. 2007). Geranyl diphosphate (GDP) is the precursor of all MT isomers. GDP is formed from IPP and DMAPP driven enzymatically by GDP synthase (Mahmoud and Croteau, 2002). Farnesyl diphosphate (FDP) synthases adds two molecules of IPP to DMAPP for the formation of the SQT precursors, C_{15} diphosphate (Cheng et al., 2007). The estimated global annual flux of IS ranges from 440 to 600 Tg C per year (Guenther et al., 2012). These values correspond to 2 % of the photosynthetically fixed carbon (Lal, 1999) and comprise a significant part of the total annual emission of BVOCs on a global scale of 1150 Tg C (Guenther et al., 1995).

40 The emission of BVOCs is strongly influenced by external factors (Peñuelas and Llusiá 2003; Niinemets et al., 2004; Fitzky et al., 2019). BVOCs are thought to play a role in protecting vegetation from abiotic (Peñuelas and Munné-Bosch, 2005; Velikova et al., 2005) and biotic stress (Berg et al., 2013; Amin et al., 2012; Amin et al., 2013), and to act as a system for plant–plant and plant–animal communication (Baldwin et al., 2006; Filella et al., 2013).

Interactions between abiotic factors due to climate change in plants are of increasing importance and timeliness. The past
45 and projected future increase in average global temperature (IPCC, 2007) is expected to lead to a change in BVOC emissions, subsequently influencing atmospheric chemistry and climate (Tingey et al., 1991; Guenther et al., 1995; Filella et al., 2007; Kiendler-Scharr et al., 2009).

Based on the algorithm by Guenther et al. (1995) it has be shown that BVOC emissions linked to temperature have increased by 10% over the last 30 years, (Peñuelas and Staudt, 2010). Future climate scenarios with expected temperature increases
50 between 1.8 and 4°C (IPCC, 2007) suggest an additional enhancement of global BVOC emissions between 30 to 45 % (Peñuelas and Lluisiá, 2003). This increase is explained by direct temperature effects and longer growing longer seasons (Peñuelas and Filella, 2001), and indirect effect by the response to (enhanced) abiotic stresses, such as tropospheric ozone and drought (EEA, 2017), (Müller et al., 2008; Loreto and Schnitzler, 2010; Dai, 2013; Unger et al., 2013; Sindelarova et al., 2014). Drought stress can also alter the composition of BVOCs emitted by plants, depending on the nature of stress
55 (Niinements, 2010). Pegoraro et al. (2004) and Beckett et al. (2012) have shown that the gradual suppression of physiological processes of plants in response to drought stress initially leads to an increase in isoprene emissions, followed by a tapering off of isoprene emissions. In the initial phase of stress, the plant responds via a reduction of stomatal conductance leading to reduced transpiration rate; this results to an increase in temperature at the leaf level and a decrease of carbon assimilation (Siddique et al., 2000). Although emissions tend to increase initially due to reserves of reduced carbon
60 present in the plant, isoprene emissions decrease under severe drought stress (Tingey, 1981; Pegoraro et al., 2004).

Besides increasing temperature and more severe droughts, future climate scenarios predict increasing ozone concentrations (Bowen, 1926; Kangasjärvi et al., 1994; Hollaway et al., 2012). Long-term elevated tropospheric ozone concentration affect BVOC emissions (Peñuelas et al., 1999), and induce alterations in photosynthetic performance increasing the production of
65 reactive oxygen species (ROS) (Cotrozzi et al., 2017; Jolivet et al., 2016).

Ground-level O_3 concentrations in the pre-industrial period were around 10 ppb in Europe (Volz and Kley, 1988; Royal Society, 2008). For the period 2000–2014, the average ozone concentrations during the growing season (April to September) in European forests were 36.2 ppb, ranging from 14.5 to 70.1 ppb (Schaub et al., 2018). Instance of severe ozone pollution were recorded during the heatwave of summer 2003 in Europe, with peaks > 100 ppbv in UK (Lee et al., 2006).

70

Among plants, trees are the dominant source of BVOC emissions (Guenther et al., 1995), and they are not often subject to only one stress but to a combination of stresses (Fitzky et al., 2019). For example, drought and ozone stresses can often occur in parallel. The combinations of these stress factors are difficult to understand because ozone and drought stress individually lead to similar symptoms, such as cell dehydration, early senescence and cell necrosis (Chaves et al., 2003).
75 Emissions of green leaf volatiles (GLVs) are indicators for damage of cellular membranes (Hatanaka, 1993; Croft et al., 1993). GLVs are released once the membrane is injured independently of the stress that caused the damage (Heiden et al., 2003). The release of GLVs is related to the degree of damage, and high emissions are linked to high membrane degradation (Fall et al., 1999; Beauchamp et al., 2005; Behnke et al., 2009). Other BVOCs are the product of metabolic processes in plants such as transcription and enzymatic activity which are induced by various stimuli, for example ozone (Betz et al.,
80 2009). An example of such a BVOC is Methyl Salicylate (MeSa), produced by the shikimate pathway (Kessler and Balwin, 2001), which fixes 20 % of the carbon obtained from photosynthesis (Bentley, 1990; Herrmann and Weaver, 1999).

Few studies have analyzed the effects of plant emissions from a combination of drought and ozone stress (Vitale et al., 2008; Yuan et al., 2016). Studying *Quercus ilex*, Vitale et al. (2008) reported that drought stress leads to stomatal closure therefore reducing stress by ozone as it is restricted to enter the leaf. Yuan et al. (2016) found that drought increased isoprene

85 emissions in a hybrid poplar deltoid species, but that isoprene emissions decreased under moderate drought stress combined with long-term ozone fumigation.

In this work our hypothesis was that different abiotic stresses in plants are not necessarily additive and that the plant's response to drought and ozone exposure can result in an alteration of characteristic BVOC emission strengths. As a model plant we chose *Quercus robur* L., a widely distributed isoprene emitting oak species in Europe (Barstow and Khela, 2017).

90 **2 Materials and Methods**

2.1 Plant species and stress treatments

Q. robur is a broad-leaf tree species widely distributed in Europe growing in mixed and deciduous forest ranging from sea level up to 1200 m ASL (Ülker et al., 2018). According to Ellenberg (1988), *Q. robur* has a high tolerance to drought due to its fast-regulated transpiration rates and stomatal conductance, and low susceptibility of water embolism in the xylem (Van

95 Hees, 1997).

Fourteen 2-year-old *Q. robur* seedlings were planted in 7 L pots in March 2019. The substrate consisted to one-thirds of soil used by the city gardeners for city trees in Vienna and two-thirds of quartz sand to improve drainage. The plants were fertilized once after planting (universal fertilizer NovaTec, Compo, Münster, Germany) and from thereon kept well-watered in a greenhouse at near ambient light and slightly increased temperature conditions (Tulln, Austria). At the beginning of

100 July, two weeks before the measurement campaign, the trees were moved to Vienna into another close-by greenhouse. Dust was removed from the leaves by showering the trees before starting the drought stress. For the biochemical assays eight trees were used as reference, four well-watered plants (C) and four well-watered plants receiving one time 100 ppb ozone for one hour (OS) inside the enclosures.

For six plants the drought stress was initiated 10 days before the VOC measurements started and was maintained by keeping

105 the soil water content at 4-5 vol.% using a soil moisture probe (Fieldscout TDR100, 20 cm probe depth, Spectrum Technologies, UK), whereas 100 % field capacity was 13.4 vol.%. Approximately 24 hours before the air gas exchange and VOC measurements, the plants were moved from the greenhouse to an indoor climate chamber (Fitotron Weiss Gallenkamp, UK) which was kept at 25°C, ~60 % rH and 300 µmol m^{-2} s^{-1} PAR at mid canopy height, to adapt to constant air temperature.

110 To study the effect of ozone exposure of trees during increasing drought, the six trees above mentioned, were separated into two groups, three trees were drought stressed and fumigated with 100 ppb O$_3$ (DS×OS) inside the enclosure for one hour each day after the measurement of BVOCs. The other three trees were drought stressed but not fumigated with ozone (DS). To continuously increase the drought stress, the plants were not watered and the humidity in the climate chamber was decreased to 40 % after the first day of measurements and temperature was increased to 30°C. At the end of the experiment

115 leaves were harvested for leaf area and enzyme analysis. Values of the enzymatic activity of C and OS were compared to DS and DS ×OS to investigate the effect of ozone fumigation.

2.2 Measurement of leaf gas exchange and BVOC fluxes

Throughout the increasing drought stress, tree leaf gas exchange and BVOC emissions were measured for two sets, DS and

120 DS×OS, over a seven-day period, one in the morning and one in the afternoon alternating daily. The plants were taken out of the climate chamber and kept inside custom-made plant enclosures (Appendix A Figure A1; TC-400, Vienna Scientific

Instruments GmbH, Alland, Austria) for 2-3 hours each day in order to measure their gas exchange along with key physiological parameters (soil moisture and stem water potential). The plant enclosures covered most of the plant material excluding a few leaves (about 7 on each tree) to allow determination of stem water potential (SWP). Each day, one leaf was

125   wrapped in aluminum foil and placed in a plastic bag for equilibrating to SWP (Williams and Araujo, 2002). After darkening for 30 minutes the leaf was cut off and SWP was measured by using a Scholander pressure bomb (Soil moisture Equipment Corp., Goleta, CA, USA).

The four custom-made plant enclosures (12 liters) were lined with PTFE and sealed on top with 55×60 cm PET-bags. The plant enclosures were continuously flushed with 10 l min^{-1} of ambient outside air that was previously passed through a cold

130   trap to remove water and an activated carbon filter (360 m³ h^{-1}, PrimaKlima Trading, Radnice, CZ) to remove VOCs and O_3. This resulted in 32 % rH air and ~370 ppm CO_2 entering the enclosures (experimental conditions in Appendix A, Table A2). The flow rate of 10 l min^{-1} assured that no condensation of water occurred in the tubing and enclosures, as well as resulted in a slight overpressure preventing the entry of room air into the enclosures. Three of the enclosures were used to measure the air gas exchange of the plants and the fourth enclosure was kept empty as a reference to allow continuous monitoring of the

135   air entering the enclosures. Trees inside the enclosure were LED-irradiated with 1450 μmol m^{-2} s^{-1} at the mid-point of the enclosure (Eckel Electronics, Trofaiach, Austria). Leaf temperature was monitored in each enclosure by placing a calibrated (±0.1°C) thermocouple (type k, PTFE IEC wire; Labfacility Ltd, Bognor Regis, West Sussex, UK) on the abaxial side of a mature mid-canopy leaf.

An automated valve system allowed the consecutive analysis of air exiting each enclosure for 5 minutes each, leading to a 20

140   minutes cycle through the four enclosures. Before inserting the three trees into the enclosures, background measurements of the empty enclosures were carried out. After inserting each plant into one enclosure, the plant was allowed to acclimatize for approximately two hours and the following 40–60 minutes of data was analyzed to determine plant CO_2 assimilation, transpiration and BVOC emissions rates. After the measurements, the trees of DS×OS were fumigated for one hour with 100 ppb of ozone each day.

145   CO_2 and H_2O mixing ratios in the air leaving the enclosures were measured using a CIRAS-3 SC PP System (Amesbury, MA, USA). Ozone measurements before and after the enclosures were conducted continuously in all enclosures with an ozone monitor (six channel ozone monitor BMT 932, BMT Messtechnik, Berlin, Germany). BVOC measurements were made using a proton transfer reaction time of flight mass spectrometer (PTR-Tof-MS, PTR-TOF6000X2, IONICON Analytik GmbH, Innsbruck, Austria; Graus et al., 2010) operated at 350 V drift voltage, ion funnel settings of 1 MHz and

150   35V amplitude as well as 35 VDC, and 2.5 mbar drift pressure. These settings are comparable to an E/N of 100 Td in a PTR-TOF8000 with no ion funnel (Markus Müller, IONICON Analytic GmbH, personal communication 2019). The drift tube temperature was 100°C. Full PTR-Tof-MS mass spectra were collected with a time resolution of 1 s and up to a mass to charge ratio m/z 547 amu.

Dynamic calibrations of VOCs using a standard gas mixture (Apel Riemer Environmental Inc., Broomfield, CO, USA),

155   containing 15 compounds with different functionality distributed over a mass range of 33-137 amu were performed daily. Compound specific sensitivities varied on the order of 8-20 % for different calibration compounds, which lies within the combined calibration uncertainties; a compound specific average experiment sensitivity was applied to all data. Limits of detection were compound dependent and on the order of 40-800 pptv for a 60 s average period. Humidity dependent calibrations were performed to account for varying humidity in the enclosures depending on plant size and plant

160   transpiration. The PTR-Tof-MS data was analyzed using the PTRTOF Data Analyzer v4 software (Müller et al., 2013) and customized Matlab scripts to obtain volume mixing ratios in the enclosures. Isoprene, with PTR-ToF-MS in H_3O^+ mode, can be detected at m/z 69.070, the sum of monoterpenes at m/z 137.133 and a major fragment at m/z 81.070, the sum of sesquiterpenes at m/z 205.195. GLVs presented in this study were reported as the sum of the dominant compounds with m/z of 83.085, 85.101, 99.080, 101.096 and 143.107, representing 2-hexenal and 3-hexenal $(C_6H_{10}O)H^+$, 3-hexenol $(C_6H_{12}O)H^+$,

165   1-hexanol $(C_6H_{14}O)H^+$, 3-hexenol $(C_6H_{12}O)H^+$ and hexenyl acetate $(C_8H_{14}O_2)H^+$, respectively (Beauchamp et al., 2005; Giacomuzzi et al., 2016; Portillo-Estrada et al., 2017). Shikimate BVOCs were represented by benzene m/z 79.054, phenol m/z 95.050, methyl salicylate m/z 153.055 (MeSa) and eugenol m/z 165.092 (Brilli et al., 2011; Tasin et al., 2012; Maja et al., 2014; Brilli et al., 2016; Giacomuzzi et al., 2016; Portillo-Estrada et al., 2017; Yener et al., 2016; Misztal et al., 2015). Emissions of IS, MT and SQT were standardized to 1000 µmol m^{-2} s^{-1} PAR and 30°C (IS_S, MT_S, SQT_S) using the Guenther

170   et al. (1993) algorithm for IS_S, and Geron et al. (1994) for MT_S and SQT_S.

$$IS_S = \frac{IS}{C_L \times C_T} \tag{1}$$

$$C_L = \frac{\alpha c_{L1} L}{\sqrt{1 + \alpha^2 \times L^2}} \tag{2}$$

$$C_T = \frac{exp \frac{c_{T1}(T - T_S)}{R T_S T}}{1 + exp \frac{c_{T2}(T - T_M)}{R T_S T}} \tag{3}$$

$$MT_S = \frac{MT}{exp(\beta(T - T_S))} \tag{4}$$

175    $$SQT_S = \frac{SQT}{exp(\beta(T - T_S))} \tag{5}$$

Where IS, MT and SQT are emission rates normalized by leaf area at sampling temperature T(K) and sampling PAR flux L (µmol m^{-2} s^{-1}) at half plant height; α (=0.0027), c_{L1}(=1.066), R (= 8.314 J K^{-1} mol^{-1}), c_{T1} (=95,000 J mol^{-1}), c_{T2} (=230,000 J mol^{-1}), T_M (=314 K), β (=0.1) and T_S (=303.15 K) (Guenther et al., 1993; Geron et al., 1994).

Mass flow of air W, transpiration rate E, net photosynthesis A and stomatal conductance g_S were calculated accordingly

180   (CIRAS-3 Operation Manual V. 2-01, PP-Systems, Amesbury, MA, USA):

$$W = \left(\frac{V_0}{60 \times 10^3}\right) \times \left(\frac{1}{22.414}\right) \times \left(\frac{10^4}{a}\right) \quad [mol\ m^{-2} s^{-1}] \tag{6}$$

$$E = \left[\frac{W \times (e_{out} - e_{in})}{(P - e_{out})}\right] \quad [mol\ m^{-2} s^{-1}] \tag{7}$$

$$A = -\left[((C_{out} - C_{in}) \times W) + (C_{out} \times E)\right] \quad [\mu mol\ m^{-2} s^{-1}] \tag{8}$$

$$e_{leaf} = 6.1365 \times exp\left[\frac{T_{leaf} \times (17.502)}{T_{leaf} + 240.97}\right] \tag{9}$$

185    $$r_s = \left[\frac{(e_{leaf} - e_{out})}{\left(E \times (P - (e_{leaf} + e_{out})/2)\right)}\right] - r_b \quad [m^2 s\ mol^{-1}] \tag{10}$$

$$g_S = \frac{1}{r_s} \times 10^3 \quad [mmol\ m^{-2} s^{-1}] \tag{11}$$

where V_0 is the volume air flow, a is the leaf area, e_{in} is the partial water vapor pressure of the air entering the enclosures, e_{out} is the partial water vapor pressure inside the enclosure, P is the atmospheric pressure, C_{in} concentration of CO_2 entering and C_{out} exiting the enclosure, e_{leaf} is the saturation vapor pressure at leaf temperature (T_{leaf}), r_s is the stomatal resistance and r_b is

190   the boundary layer resistance to water vapor transfer, which was assumed zero according to the recommendations of the manufacturer (CIRAS-3 Operation Manual V. 2-01, PP-Systems, Amesbury, MA, USA).

The ratio of the sum of carbon lost in form of BVOC (C_{BVOCs}) vs. the uptake of carbon from net photosynthesis (C_A) was calculated according to Pegoraro et al. (2004), with the BVOCs used to calculate C_{BVOCs} given in Table A3.

After seven days, finishing the emission measurements, all leaves were harvested immediately, imaged with a flatbed

195   scanner (Epson Expression 10000XL, Epson, Japan) and analyzed with the PC program WinFOLIA 2013 Pro (Regent Instruments Inc., Qúebec, Canada) to determine the leave surface area. About 80 % of the leaves' fresh mass was shock-frozen and crushed in liquid nitrogen for biochemical assays (see below). About 20 % of the leaves per plant were dried for three days in a drying room at 40°C to determine dry weight to an accuracy of ±0.001 g for the calculation of enzyme activity and specific leaf area (SLA) (Table A4).

Biogeosciences
Discussions
Open Access
EGU

200   **2.3 Biochemical assay**

For the interpretation of the emissions of GLVs and Shikimate volatiles, enzymatic activities were analyzed additionally to better understand the effect of ozone fumigation during a situation of severe drought. Using foliar materials collected after the seven day period of emission measurements (see above) and stored at -80°C until analysis, peroxidase and antioxidant capacity, and phenol content (TPhe) were measured. Values from plants after seven days of increasing drought (DS×OS, DS)

205   were compared to well-watered reference plants (C) and a well-watered set of plants that received ozone fumigation once (OS).

For measurements of peroxidase activities, 0.5 g plant material, 0.25 g Polyclar AT (Serva Electrophoresis, Heidelberg, Germany) and 0.25 g quartz sand (Sigma-Aldrich, Steinheim, Germany), were homogenized in a mortar with 3 ml 0.1 M potassium phosphate buffer (pH 6.0). After removal of solid compounds by centrifugation at 4°C and $10000 \times g$ for 10

210   minutes, 400 µL of the supernatant were subjected to gel chromatography with Sephadex G25 medium (GE Healthcare, Chicago, IL, USA) to remove low molecular weight compounds. Peroxidase activity was determined according to the Worthington Manual (1972). Briefly, the enzyme assay contained in a final volume of 1110 µL, 1095 µL buffer 0.1 M potassium phosphate buffer + 0.003 % (v/v) H_2O_2 (pH 6.0), 5 µL enzyme preparation, and 10 µL 1 % (w/v) o-dianisidin (Sigma-Aldrich-Aldrich, Vienna Austria) in MeOH.

215   The activity was determined by measuring the extinction at 460 nm on a DU-65 spectrophotometer (Beckman Instruments, Brea, CA, USA) in intervals of 30 s for a period of 6 minutes. The activity was calculated for the linear range of $\Delta E\ min^{-1}$, using an extinction coefficient of $1.13 \times 10^4\,M^{-1}\,cm^{-1}$ for oxidized o-dianisidine (Worthington manual, 1972). The protein content was determined by a modified Lowry procedure (Sandermann and Strominger, 1972) using bovine serum albumin as a standard. All measurements were performed in two technical replicates.

220   For the determination of the antioxidant capacity and the TPhen, the material was lyophilised and homogenized by grinding to fine powder in a mortar. 0.25 g of the lyophilised powder was extracted with 3 ml distilled water for 1 hour in a cooled water bath during sonication. After centrifugation for 5 minutes at 4°C and $10000 \times g$, the supernatant was filtered through a Chromafil AO-20/25 polyamide filter (Roth, Karlsruhe, Germany).

The TPhen was determined as described (Wootton-Beard et al., 2011) with some modifications. Briefly, 100 µL of the

225   aqueous solution was mixed with 6 mL distilled water and 500 µL Folin Ciocalteu Reagent (Sigma-Aldrich, Vienna, Austria) (1:1 v/v with distilled water). After equilibration for 8 minutes, 1.5 ml 20 % Na_2CO_3 (w/v) and 1.9 ml distilled water were added and the mixture was incubated at 40°C for 30 minutes. The TPhen was obtained by measuring the absorbance of the mixture at 765 nm using a freshly prepared standard curve obtained with gallic acid. The results were expressed as µg gallic acid equivalents per g sample. All measurements were performed in technical triplicates.

230   The in vivo antioxidant activity was determined with *Saccharomyces cerevisiae* ZIM 2155 as model system following the procedures described in Slatnar et al. (2012), which estimates intracellular oxidation by fluorometrical measurements using the ROS-sensitive dye 2',7'-dichlorofluorescin (H_2DCF). 100 µl of the aqueous samples were incubated with 10 mL yeast suspension at their stationary phase in phosphate buffered saline (PBS, Merck KGaA, Darmstadt, Germany) at a density of 10^8 cells/suspension at 28°C and 220 rpm for 2 h. After a centrifugation step at room temperature for 5 minutes at $14000 \times g$,

235   the pellet was washed three times with 50 mM potassium phosphate buffer (pH 7.8) and was finally resuspended in 9 volumes of 500 µL 50 mM potassium phosphate buffer (pH 7.8) and incubated for ten minutes at 28°C and 220 rpm in the dark. After addition of 10 µL H_2DCF (1 mM stock solution in 96 % ethanol), the mixture was incubated for further 30 minutes at 28°C and 220 rpm. The fluorescence of the yeast cell suspensions was measured at a GloMax® Multi Microplate Reader (Promega, Walldorf, Germany) using excitation and emission wavelengths of 490 and 520 nm, respectively. Values

240   of fluorescence intensity were measured against a blank, in which the sample was replaced with water. Data are expressed as relative fluorescence intensity, where the values obtained with the blank are defined as 1. Values lower than 1 indicate a

Biogeosciences
Discussions
EGU
Open Access

higher antioxidant activity than the blank (Slatnar et al., 2012). All measurements were performed in two technical replicates.

2.4 Statistical analyses

245 Emission rates, physiological parameters, means and standard deviation were calculated with Matlab (MATLAB and Statistics Toolbox Release 2017a, The MatWorks, Inc., Natick, MA, United States). All leaf gas exchange and BVOC flux measurements collected over the seven-day period for the set DS and DS×OS were aggregated into four ranges of SWP (R1: 0.00 to -1.40 MPa; R2: -1.45 to -2.85 MPa; R3: -2.90 to -4.30 MPa, R4: -4.35 to -6.00 MPa) to perform statistical analysis using the Wilcoxon rank sum test. To test for significant differences in the biochemical markers a one-way ANOVA test was

250 used. For both tests p-values below 0.05 were considered significant.

3 Results and Discussions

3.1 Stomatal closure and net photosynthesis

SWP was measured daily and used as a drought stress indicator to study the evolution of *Q. robur* under continuously increasing drought condition. All six trees began the experiment with a high to moderate mean SWP of -0.9 MPa and

255 reached low values in the order of -5.5 MPa after seven days of continuously increasing drought stress. Mean and standard deviation of stomatal conductance (g_S), net photosynthesis (A), leaf temperature (T_{leaf}) and SWP as well as notes for statistically significant differences are summarized in Table 1 for the four drought stress ranges defined in 2.4. The mean stomatal conductance (g_S) of DS×OS was 20.2 mmol m^{-2} s^{-1} in R1 and decreased to 6.8 mmol m^{-2} s^{-1} in R2 (Table 1). For DS it was 42.4 mmol m^{-2} s^{-1} in R1 and decreased to 6.6 mmol m^{-2} s^{-1} in R2. For both sets the reduction of g_S and SWP between

260 R1 and R4 was significant. R1, shown in Fig. 1(a), includes values of trees fumigated with ozone (DS×OS) from the first and the second day of analysis because SWP did not change much. Differently, R1 includes only measurements of the first day for DS. This shows that trees of DS×OS closed their stoma quickly at higher stem water potential after the first ozone fumigation session, and confirms what was reported in other studies that moderate ozone concentrations can induce partially closed stomata (Khatamian et al., 1973; Farage et al., 1991; Wittig et al., 2007). A partial stomatal closure prevented

265 excessive water loss through stomatal openings (Pinheiro and Chaves, 2011; Mc Dowell et al., 2008; Allen et al., 2008) during drought stress, and enhanced the closure with ozone allowing DS×OS plants to better survive the increased drought. Kobayashi et al. (1993) considers the interactive effects of O_3 and drought stress using a growth model of soybean, finding that ozone fumigation reduces or postpones drought stress, similar to the findings of this experiment.

Figure 1 (b) shows a decrease of net photosynthesis (*A*) with the increase of the stress for both set, especially between R1

270 and R2, whereas the values in R3 and R4 are close to zero. In R1, *A* presented the same differences exposed for g_S between the sets. Our results are different from the finding of Tjoelker et al. (1995) and Paoletti (2005), where stomatal conductance and photosynthesis are shown to decouple at moderate ozone exposure due to direct damage to biochemical carboxylation, caused by chronic ozone exposure.

The ratio of sum of carbon lost in form of BVOC (C_{BVOCs}) and the uptake of carbon from net photosynthesis (C_A) is shown in

275 Fig. 2. Initially, at low drought stress (R1), 3-7 % of the assimilated carbon was lost as emitted BVOC, which matches findings in other studies (Sharkey et al., 1991; Baldocchi et al., 1995; Monson and Fall, 1989; Fang et al., 1996), showing that ~2 % of carbon assimilated is lost as IS (C_{IS}/C_A) under unstressed conditions and at 30°C. As CO_2 assimilation rate decreased quickly, and BVOC emission (especially isoprene emission) stayed elevated the ratio of lost vs. fixed carbon increased to 20 % for DS and 16 % for DS×OS in R2. Pegoraro et al. (2004) reported a carbon loss in the order of 50 % for

280 SWP of -2 MPa, in a drought experiment with *Quercus virginiana*. In R3, the increasing stress corresponded to ratios of 0.7 and 1.03 for DS and DS×OS respectively. Alternative carbon sources for isoprene biosynthesis under drought stress are thus

proposed for DS×OS. For example, extra-chloroplastic origin or chloroplastic starch (Karl et al., 2002; Kreuzwieser et al., 2002; Funk et al., 2004; Affek and Yakir, 2003; Schnitzler et al., 2004; Rosenstiel et al., 2003) can sustain carbon sources for isoprene production. The increasing ratio was driven by the decline of fixed carbon with increasing SWP; for IS, the
285   dominant BVOC (averagely 96 % of the total emissions), mean standardized IS emissions of DS×OS treated plants were consistently higher in all SWP ranges compared to DS alone (Figure 3), thus showing the difference between DS and DS×OS in carbon loss ratio in the highest SWP ratio range.

 At very high drought stress this ratio decreased again to 0.4 in DS and 0.8 in DS×OS.

3.2 BVOCs emissions

290   To give a general overview on BVOC emissions for both sets Fig. 4 (a) and (b) show the total mass spectra ranging from 40-220 amu for the first and last day of measurement for DS and DS×OS respectively. Figure 4 (c) shows relative changes of the mass spectra between the first and last day of measurements. The mass range 80–110 amu, hosting many mass to charge ratios associated with GLVs, showed the strongest difference between the two sets. Plants exposed to ozone and drought stress (DS×OS) exhibited smaller increases in this mass range compared to drought stressed (DS) plants. Changes in
295   emissions or lack thereof for IS, MT, SQT and stress related BVOCs are investigated in further detail below and are summarized in Table 2.

3.2.1 Isoprene emissions

Q. robur is generally classified as a high IS emitting (Benjamin and Winer, 1998; Lehning et al., 2002) and medium to low MT and SQT emitting species (Owen et al., 1997; Karl et al., 2009; Steinbrecher et al., 2009). Figure 3 shows standardized
300   isoprene emissions (IS_S) as a function of drought stress for all investigated trees. In the range of SWP R1 the plants were in a low- to no-water-stress condition (Brüggemann and Schnitzler, 2002). Whereas g_S and A (Fig. 1 (a),(b)) decreased rapidly with increasing drought stress and bottom out at -3 MPa, isoprene emissions decreased much slower reaching close to zero emissions at SWP -6 MPa. IS_S in R1 was 12.8 nmol m^{-2} s^{-1} and 18.0 nmol m^{-2} s^{-1} for DS and DS×OS respectively. In R4 the mean IS_S was 1.7 nmol m^{-2} s^{-1} for DS and 3.9 nmol m^{-2} s^{-1} for DS×OS

305

Given that IS_S emissions remain higher in DS×OS for R1 and R2, compared to DS suggests that overall isoprene production within the leaves must have remained high in response to ozone. High IS fluxes due to ozone treatment are also reported in other studies (Fares et al., 2006; Velikova et al., 2005; Kanagendran et al., 2018).

An increase in IS with moderate stress was observed by Pegoraro et al. (2004) and Beckett et al. (2012), who related this
310   finding to an increase in leaf temperatures as a consequence of stomatal closure. In contrast, leaf temperatures did not change significantly in our study, suggesting IS emissions of DS×OS in R2 being a result of a temperature-independent isoprene production.

The decrease of A with decreasing SWP, particularly at mild drought stress (Fig 1b), is much more pronounced than the
315   decrease of IS_S emission rates (Fig 3). Similar results are found for leaf level measurements of *Q. robur* (Brüggemann and Schnitzler, 2002), *Populus alba* (Brilli et al., 2007) and *Quercus virginia* (Pegoraro et al., 2004) as well as on the ecosystem scale in the Ozark region in the central U.S. (Seco et al., 2015).

Even though the rate of photosynthetic carbon assimilation declined much faster under drought than IS, a substantial decline
320   of IS was also seen as drought progressed. Drought stress has been found to be one of the stronger influencing factors affecting photosynthesis but had often only limited influence on IS emission rates (Tingey et al., 1981; Sharkey and Loreto, 1993; Fang et al., 1996).

In young hybrid poplars (*Populus deltoides* cv. 55/56 x P. *deltoides* cv. Imperial), the combined application of elevated ozone and drought decreases isoprene emission, whereas drought alone increases the emission, and ozone alone decreases it

325   (Yuan et al., 2016).

Studies report that volatile isoprenoids strengthen cellular membranes, thus maintaining the integrity of the thylakoid-embedded photosynthetic apparatus and have a generic antioxidant action by deactivating ROS around and inside leaves and thus indirectly reduce the oxidation of membrane structures and macromolecules (Singsaas et al., 1997; Loreto and Velikova, 2001; Affek and Yakir, 2002; Loreto and Schnitzler, 2010; Velikova et al., 2012).

330   ### 3.2.2 Terpenoid emissions

Figure 5 shows MT_S (a) and SQT_S (b) as a function of SWP. Mean MT_S for DS and DS×OS were 1.0×10^{-2} nmol m^{-2} s^{-1} and 3.6×10^{-2} nmol m^{-2} s^{-1} respectively at R1. With the increase of drought stress (R3) DS×OS decreased to 1.5×10^{-2} nmol m^{-2} s^{-1} while DS emissions remained stable (1.0×10^{-2} nmol m^{-2} s^{-1}). For higher drought stress (R4) both sets showed an increase in MT emissions reaching 3.3×10^{-2} nmol m^{-2} s^{-1} for DS and 4.7×10^{-2} nmol m^{-2} s^{-1} for DS×OS.

335   Loreto et al. (2004), demonstrated that ozone can stimulate the emission of monoterpenes in *Q. ilex*, but that ozone has no effect on photosynthesis nor on any other physiological parameter, when Mediterranean oak plants are exposed to mild and repeated, as well as acute ozone stress.

In this experiment MT emissions from *Q. robur*, increased in DS and DS×OS trees. In the case of DS, there was a positive effect of drought, with an increase in MT emissions, although there was a drastic decrease of IS emissions when the water

340   deficit was severe. These observations contrast those by Lluisá and Peñuelas (1998) for *Q. coccifera* reporting a decrease of MT emissions. This could be due to the fact that in the case of *Q. coccifera* no specific terpene storage structures are present in leaves, while they are present in *Q. robur* (Karl et al., 2009).

In both sets SQT_S emissions remained close to zero down to a SWP of -3 MPa. SQT_S emissions increase with increasing

345   drought stress reaching a mean value of 1.4×10^{-2} nmol m^{-2} s^{-1} for DS and 3.5×10^{-2} nmol m^{-2} s^{-1} for DS×OS in R4. The increase of SQT_S in the set with ozone began one day later than in the set without ozone fumigation.

Stress on plants can induce SQT emissions (Toome et al., 2010; Maes and Debergh, 2003; Ibrahim et al., 2006). Ormeño et al. (2007) observe a reduction of sesquiterpenes with drought stress for a variety of plant species including *Q. coccifera*. For

350   *Q. robur* we see an increase of SQT emissions under conditions of severe drought.

The release of SQT from leaves can be triggered when plants face stress due to oxidative processes in leaves, indicating that damaging effects inside the plants start to occur (Beauchamp et al., 2005; Bourtsoukidis et al., 2012). Unlike MT, SQT don't provide an additional barrier to plant damage during severe water stress (Palmer-Young et al., 2015). This is due to their different physico-chemical characteristics and the different pathways that produce them (Niinemets et al., 2004; Umlauf et

355   al., 2004). In the case of SQT emissions, the parallel occurrence of two stresses (ozone and increased drought) generally led to an increase in emissions. In fact, the higher SQT emissions in DS×OS compared to DS may have been due to ozone, similar to those reported in Beauchamp et al. (2005).

3.2.3 GLV and SHIKIMATE emissions

The ΣGLV increased for both sets in R4 (Fig. 6 (a)). Within ΣGLV m/z 99.080, attributable to hexenal isomers, showed the

360   strongest increase in DS (mean value of m/z 99.080 in R4 was 68 % of the Σ GLV emission). Within the cascade of GLV production, (E)-2-hexenal and (Z)-3-hexenal are typically the ones appearing first (Fall et al., 1999).

DS×OS, on the other hand, showed an increase in Shikimate compounds (Fig. 6 (b)) at SWP < -3 MPa, DS showed similar but less pronounced trend. The ΣShikimate was dominated by Methyl Salicylate (MeSa) across the entire SWP range for DS

and in R1-R3 for DS×OS. R4 of DS×OS was dominated by m/z 95.050 (matching the exact mass of protonated phenol,

365    $C_6H_7O^+$).

MeSa is considered as a volatile stress signaling molecule from plants (Karl et al., 2008). High emissions of MeSa are also found in the case of the tobacco plant (*Nicotiana tabacum* L. cultivars) in both O_3 sensitive and O_3 tolerant, exposed to ozone at high concentrations (Heiden et al., 1999; Beauchamp et al., 2005).

370   Observing the increase in GLV emissions in DS and Shikimate emissions in DS×OS was important to understand how ozone affected the *Q. robur* trees exposed to drought stress. The impact of exposure to high ozone concentrations on ROS production was not significant and not associated with membrane lesions in Pellegrini et al. (2019). In this experiment, GLV emissions in R4 were low in ozone treated plants (DS×OS), while plants that were exposed to drought only (DS) exhibited higher emissions. The observations of this experiment can be interpreted such that plants did not suffer from detrimental

375   effects due to acute ozone exposure yet (e.g. Beauchamp et al., 2005), but that mild ozone exposure can potentially delay effects of drought stress and help maintain membrane structure and integrity.

The activation of an efficient free radical scavenging system can minimize the adverse effects of a general peroxidation (Miller et al., 1999). This was not the case in DS, where exposure to severe water stress alone led to an increase of GLV emissions suggesting the onset of physical membrane damage, as the enhancement of the lipoxygenase activity, in

380   accordance with other studies (Ebel et al., 1995; Wenda-Piesik, 2011). In addition to the lipoxygenase and hydroperoxide lyase systems producing GLVs, the phenylpropanoid pathway signals plant responses to stimuli induced by abiotic factors (Dixon and Paiva, 1995; Baier et al., 2005; Heath, 2008; Vogt, 2010), but drought stress alone does not induce the phenylpropanoid pathway in *Q. robur* (Pellegrini et al., 2019).

On the other hand, DS×OS, showed a small increase of GLV only at the highest stress level. We take this to indicate that

385   ozone has the potential to inhibit drought stress damage and therefore the emissions of GLV, by stimulating the phenylpropanoid pathway to form an antioxidant protection for chloroplasts (Pellegrini et al., 2019). Cabané et al. (2004) report that, in poplar leaves, ozone exposure not only stimulate the enzymes of the phenylpropanoid pathway, but also the activity of the enzyme SHDH of the shikimate pathway that yield TPhe in fully developed leaves.

To better understand the emissions of GLVs and Shikimate volatiles, we looked at antioxidant capacity, total phenol content

390   and peroxidase activity summarized in Table 3. No significant differences were found for antioxidant capacity between the sets DS, DS×OS and their corresponding references C, and OS. However, it appeared that the OS had the highest oxidizing capacity. TPhen in the fully developed leaves was significantly higher in the two groups experiencing drought stress (DS, DS×OS) than in those with no drought stress (C, OS). Pellegrini et al. (2019) found, a significant difference in TPhen content in well-watered plants with the increase of ozone, and a decrease at moderate drought and no significant influence of ozone

395   on TPhen during severe drought in *Q. robur*. The results of our study showed no significant decrease in TPhen due to ozone fumigation both in well-watered and severe drought condition (OS, DS×OS). Peroxidase activity analysis did not show significant differences between the four sets. This is in accordance with the finding of Schwanz and Polle (2001) who found that unspecific peroxidase activities are not affected by drought stress in *Q. robur*.

4. Conclusions

400   The changes in BVOC emissions of *Q. robur* subject to continuously increasing drought were investigated and differences in the drought progression were observed in plants with and without ozone fumigation. Stomatal conductance and net photosynthesis showed a fast reaction to increasing drought closing stomata and reducing CO_2 uptake strongly. IS_S emissions, on the other hand, stayed high down to a SWP of -3 MPa and then decreased gradually. We believe that leaves must have maintained a high production of IS to sustain similar emissions at a SWP of -2 MPa. MT_S and SQT_S emissions

405 increased under high drought stress. Plants that were subject to one hour of ozone fumigation (~100 ppbv) every day in addition to reduced watering showed lower stomatal conductance at mild drought stress compared to those with no ozone fumigation, and consecutively the effect of drought was slowed down. The Shikimate pathway, producing antioxidants, was stimulated earlier in the set with ozone. The combination of (i) sustained isoprene emissions, (ii) increase of antioxidants due to the higher stimulation of the two pathways (phenylpropanoid and shikimate) and (iii) early closure of the stomata resulted

410 in a longer endurance of drought stress in the set exposed to ozone. Therefore, it was concluded that fumigation with ozone (~100 ppbv) decelerated the effect of drought in *Q. robur*. Overall *Q. robur* leaves appeared very resistant to drought stress. Consequently GLVs indicating cell damage were only emitted at SWP < -5 MPa.

As seasonal drought events and elevated ozone concentrations often occur in parallel in mid latitudes (Löw et al., 2006; Panek et al., 2002) it is important to study their combined stress effects. In this study we observe that a combination of

415 stresses can lead to opposing feedbacks that alter BVOC emissions. These effects are compound specific and reflect biochemical changes in the plant.

Author contribution

AP, LK, TGK, GW, HH drafted the manuscript, which was commented by all co-authors. Laboratory work was performed by AP, LK, ACF, MG, TGK, HS and JG. AP, LK, ACF and HH analyzed and interpreted the data.

420 **Competing interests**

The authors declare that they have no conflict of interest.

Acknowledgments

430 **References**

Affek, H.P., and Yakir, D.: Protection by isoprene against singlet oxygen in leaves, Plant Physiol., 129, 269–277, https://doi.org/10.1104/pp.010909, 2002.

Affek, H.P., and Yakir, D.: Natural abundance carbon isotopes composition of isoprene reflects incomplete coupling between isoprene synthesis and photosynthesis carbon flow, Plant Physiol. 131, 1727–1736,

435 https://doi.org/10.1104/pp.102.012294, 2003.

Allen, C.D., Macalady, A., Chenchouni, H., Bachelet, D., McDowell, N.,Vennetier, M., Kitzberger, T., Gonzales, P., Hogg, T., Rigling, A., Breshears, D., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A., and Cobb, N.: A global overview of drought and heat-induced tree mortalityreveals emerging climate change risks for forests, For Ecol. Manag., 259, 660–684, https://doi.org/10.1016/j.foreco.2009.09.001, 2010.

440   Amin, H.S., Atkins, P.T., Russo, R., Brown, A.W., Sive B., Hallar, A.G., and Huff Hartz, K.E.: Effect of bark beetle infestation on secondary organic aerosol precursor emissions, Environ. Sci. Technol., 46, 5696–5703, https://doi.org/10.1021/es204205m, 2012.

Amin, H.S., Russo, R.S., Sive, B., Hoebeke, E.R., Dodson, C., McCubbin I.B., Hallar, A.G., and Huff Hartz, K.E.: Monoterpene emissions from bark beetle infested Engelmann spruce trees, Atmos. Environ., 72, 130–133,
445   https://doi.org/10.1016/j.atmosenv.2013.02.025, 2013.

Baier, M., Kandlbinder, A., Golldack, D., and Dietz, K. J.: Oxidative stress and ozone: perception, signaling and response, Plant Cell Environ., 28, 1012–1020, https://doi.org/10.1111/j.1365-3040.2005.01326.x, 2005.

Baldocchi, D., Guenther, A.B., Harley, P.C., Klinger, L., Zimmerman, P., Lamb, B., and Westberg, H.: The fluxes and air chemistry of isoprene above a deciduous hardwood forest, Philos. Trans. A Math. Phys. Eng. Sci., 351, 279–296,
450   https://doi.org/10.1098/rsta.1995.0034, 1995.

Baldwin, I.T., Halitschke, R., Paschold, A., von Dahl, C.C., and Preston, C.A.: Volatile signaling in plant-plant interactions: "Talking trees" in the genomics era, Science, 311, 812–815, https://doi.org/10.1126/science.1118446, 2006.

Barstow, M., and Khela, S.: *Quercus robur*. The IUCN Red List of Threatened Species 2017: e.T63532A3126467, https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T63532A3126467, 2017.

455   Beauchamp, J., Wisthaler, A., Hansel, A., Kleist, E., Miebach, M., Niinemets, Ü., and Wildt, J.: Ozone induced emissions of biogenic VOC from tobacco: relationships between ozone uptake and emission of LOX products, Plant Cell Environ., 28, 1334–1343, https://doi.org/10.1111/j.1365-3040.2005.01383.x, 2005.

Beckett, M., Loreto, F., Velikova, V., Brunetti, C., Di Ferdinando, M., Tattini, M., and Farrant, J.M.: Photosynthetic limitations and volatile and non-volatile isoprenoids in the poikilochlorophyllous resurrection plant *Xerophyta humilis*
460   during dehydration and rehydration, Plant Cell Environ., 35, 2061–2074, https://doi.org/10.1111/j.1365-3040.2012.02536.x, 2012.

Behnke, K., Kleist, E., Uerlings, R., Wildt, J., Rennenberg, H., and Schnitzler, J. P.: RNAi-mediated suppression of isoprene biosynthesis in hybrid poplar impacts ozone tolerance, Tree Physiol., 29, 725–736, https://doi.org/10.1093/treephys/tpp009, 2009.

465   Benjamin, M. T., and Winer, A. M.: Estimating the ozone-forming potential of urban trees and shrubs, Atmos. Environ., 32, 53–68, https://doi.org/10.1016/S1352-2310(97)00176-3, 1998.

Bentley, R.: The shikimate pathway: a metabolic tree with many branches, Crit. Rev. Biochem. Mol. Biol., 25, 307–84, https://doi.org/10.3109/10409239009090615, 1999.

Berg, A.R., Heald, C.L., Huff Hartz, K.E., Hallar, A.G., Meddens, A.J.H., Hicke, J.A., Lamarque, J.-F.,and Tilmes, S.: The
470   impact of bark beetle infestation on monoterpene emissions and secondary organic aerosol formation in Western North America, Atmos. Chem. Phys., 13, 3149–3161, https://doi.org/10.5194/acp-13-3149-2013, 2013.

Betz, G.A., Knappe, C., Lapierre, C., Olbrich, M., Welzl, G., Langebartels, C., Heller, W., Sandermann, H., and Ernst, D.: Ozone affects shikimate pathway transcripts and monomeric lignin composition in European beech (*Fagus sylvatica* L.), Eur. J. Forest Res., 128, 109–116, https://doi.org/10.1007/s10342-008-0216-8, 2009.

475   Bourtsoukidis, E., Bonn, B., Dittmann, A., Hakola, H., Hellén, H., and Jacobi, S.: Ozone stress as a driving force of sesquiterpene emissions: a suggested parameterization, Biogeosciences, 9, 4337–4352, https://doi.org/ 10.5194/bg-9-4337-2012, 2012.

Bowen, I. S.: The ratio of heat losses by conduction and by evaporation from any water surface, Phys. Rev. E, 27, 779–787, https://doi.org/ 10.1103/PhysRev.27.779, 1926.

480   Brilli, F., Barta, C., Fortunati, A., Lerdau, M., Loreto, F., and Centritto, M.: Response of isoprene emission and carbon metabolism to drought in white poplar (*Populus alba*) saplings, New Phytol., 175, 244–254, https://doi.org/10.1111/j.1469-8137.2007.02094.x, 2007.

Brilli, F., Ruuskanen, T.M., Schnitzhofer, R., Müller, M., Breitenlechner, M., Bittner, V., and Hansel, A.: Detection of plant volatiles after leaf wounding and darkening by Proton Transfer Reaction "Time-of-Flight" Mass Spectrometry (PTR-TOF),
485   PloS One6, e20419, https://doi.org/10.1371/journal.pone.0020419, 2011.

Brilli, F., Gioli, B., Fares, S., Terenzio, Z., Zona, D., Gielen, B., and Ceulemans, R.: Rapid leaf development drives the seasonal pattern of volatile organic compound (VOC) fluxes in a 'coppiced' bioenergy poplar plantation, Plant Cell Environ., 39, 539–555, https://doi.org/10.1111/pce.12638, 2016.

Brüggemann, N., and Schnitzler, J.P.: Comparison of isoprene emission, intercellular isoprene concentration and
490   photosynthetic performance in water-limited oak (*Quercus pubescens* Willd. and *Quercus robur* L.) saplings, Plant Biol., 4, 456–463, https://doi.org/10.1055/s-2002-34128, 2002.

Cabané, M., Pireaux, J.C., Leger, E., Weber, E., Dizengremel, P., Pollet, B., and Lapierre, C.: Condensed lignins are synthesized in poplar leaves exposed to ozone, Plant Physiol., 134, 586–594, https://doi.org/10.1104/pp.103.031765, 2004.

Chaves, M.M., Maroco, J.P., and Pereira, J.S.: Understanding plant responses to drought: from genes to the whole plant,
495   Funct. Plant Biol., 30, 239–264, https://doi.org/10.1071/FP02076, 2003.

Cheng, A. X., Lou, Y. G., Mao, Y. B., Lu, S., Wang, L. J., and Chen, X.Y.: Plant terpenoids: biosynthesis and ecological functions, J. Integr. Plant Biol., 49, 179–186, https://doi.org/10.1111/j.1744-7909.2007.00395.x, 2007.

CIRAS-3 Operation Manual V. 2-01, PP-Systems, Amesbury, MA, USA, 2018.

Cotrozzi, L., Remorini, D., Pellegrini, E., Guidi, L., Lorenzini, G., Massai, R., Nali, C., and Landi, M.: Cross-talk between
500   physiological and metabolic adjustments adopted by *Quercus cerris* to mitigate the effects of severe drought and realistic future ozone concentrations, Forests, 8, 148, https://doi.org/10.3390/f8050148, 2017.

Croft, K. P. C., Jüttner, F., and Slusarenko, A. J.: Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv. *phaseolicola*, Plant Physiol., 101, 13–24, https://doi.org/10.1104/pp.101.1.13, 1993.

505   Dai, A.: Increasing drought under global warming in observations and models, Nat. Clim. Change, 3, 52–58, https://doi.org/10.1038/nclimate1633, 2013.

Dixon, R.A., and Paiva, N.L.: Stress-induced phenylpropanoid metabolism, Plant Cell, 7, 1085–1097, https://doi.org/10.1105/tpc.7.7.1085, 1995.

Ebel, R.C., Mattheis, J.P., and Buchanan, D.A.: Drought stress of apple trees alters leaf emissions of volatile compounds,
510   Physiol. Plant., 93, 709, https://doi.org/10.1111/j.1399-3054.1995.tb05120.x, 1995.

EEA: Air quality in Europe – 2017 report. EEA Report No17/20217. European Environment Agency (https://www.eea.europa.eu/publications/air-quality-in-europe-2017), 2017.

Ellenberg, H. H.: Vegetation ecology of central Europe, fourth ed, Cambridge University Press, Cambridge, 1988.

Fall, R., Karl, T., Hansel, A., Jordan, A., and Lindinger, W.: Volatile organic compounds emitted after leaf wounding: On-
515   line analysis by proton-transfer-reaction mass spectrometry, J. Geophys. Res., 104, 15963–15974, https://doi.org/10.1029/1999JD900144, 1999.

Fang, C., Monson, R. K., and Cowling, E. B.: Isoprene emission, photosynthesis, and growth in sweetgum (*Liquidambar styraciflua*) seedlings exposed to short- and long-term drying cycles, Tree Physiol., 16, 441–446, https://doi.org/10.1093/treephys/16.4.441, 1996.

520   Farage, P., Long, S., Lechner, E., and Baker, N.: The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone, Plant Physiol., 95, 529–535, https://doi.org/10.1104/pp.95.2.529, 1991.

Fares, S., Barta, C., Brilli, F., Centritto, M., Ederli, L., Ferranti, F., Pasqualini, S., Reale, L., Tricoli, D., and Loreto, F.: Impact of high ozone on isoprene emission, photosynthesis and histology of developing *Populus alba* leaves directly or indirectly exposed to the pollutant, Physiol. Plant., 128, 456–465, https://doi.org/10.1111/j.1399-3054.2006.00750.x, 2006.

525   Filella, I., Primante, C., Llusiá, J., Martín González, A.M., Farré-Armengol, G., Rodrigo, A., Bosch, J., and Peñuelas, J.:
      Floral advertisement scent in a changing plant-pollinators market, Sci. Rep., 3, 3434, doi.org/10.1038/srep03434, 2013.

      Filella, I., Wilkinson, M.J., Llusiá, J., Herwitt, C.N., and Peñuelas, J.: Volatile organic compounds emissions in Norway
      spruce (*Picea abies*) in response to temperature changes, Physiol. Plant., 130, 58–66, https://doi.org/10.1111/j.1399-
      3054.2007.00881.x, 2007.

530   Fitzky, A. C., Sandén, H., Karl, T., Fares, S., Calfapietra, C., Grote, R., Saunier, A., and Rewald, B.: The interplay between
      ozone and urban vegetation BVOC emissions, ozone deposition, and tree ecophysiology, Front. For. Glob. Change, 2, 50,
      https://doi.org/10.3389/ffgc.2019.00050, 2019.

      Funk, J.L., Mak, J.E., and Lerdau, M.T.: Stress-induced changes in carbon sources for isoprene production in *Populus
      deltoids*, Plant Cell Environ., 27, 747–755, https://doi.org/10.1111/j.1365-3040.2004.01177.x, 2004.

535   Giacomuzzi, V., Cappellin, L., Khomenko, I., Biasioli, F., Schütz, S., Tasin, M., Knight, A.L., and Angeli, S.: Emission of
      volatile compounds from apple plants infested with *Pandemis heparana larvae*, antennal response of conspecific adults, and
      preliminary field trial, J. Chem. Ecol., 42, 1265–1280, https://doi.org/10.1007/s10886-016-0794-8, 2016.

      Geron, C., Guenther, A., and Pierce, T.: An improved model for estimating emissions of volatile organic compounds from
      forests in the eastern United States, J. Geophys. Res., 99, 773–12, https://doi.org/10.1029/94JD00246, 1994.

540   Graus, M., Müller, M., and Hansel, A.: High resolution PTR-TOF: quantification and formula confirmation of VOC in real
      time, J. Am. Soc. Mass Spectr., 21, 1037–1044, https://doi.org/10.1016/j.jasms.2010.02.006, 2010.

      Guenther, A.B., Zimmerman, P.R., Harley, P.C., Monson, R.K., and Fall, R.: Isoprene and monoterpene emission rate
      variability: Model evaluation and sensitivity analysis, J. Geophys. Res., 98, 609–617, https://doi.org/10.1029/93JD00527,
      1993.

545   Guenther, A.B., Hewitt, C.N., Erickson, D., Fall, R., Geron, C., Graedel, T., Harley, P., Klinger, L., Lerdau, M., McKay,
      W.A., Pierce, T., Scholes, B., Steinbrecher, R., Tallamraju, R., Taylor, J., and Zimmermann, P.: A global model of natural
      volatile organic compounds emissions, J. Geophys. Res., 100, 8873–8892, https://doi.org/10.1029/94JD02950, 1995.

      Guenther, A.B., Jiang, X., Heald, C.L., Sakulyanontvittaya, T., Duhl, T., Emmons, L.K., and Wang, X.: The model of
      emissions of gases and aerosols from nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling
550   biogenic emissions, Geosci. Model Dev., 5, 1471–1492, https://doi.org/10.5194/gmd-5-1471-2012, 2012.

      Hatanaka, A.: The biogeneration of green odor by green leaves, Phytochemistry, 34, 1201–1218,
      https://doi.org/10.1016/0031-9422(91)80003-J, 1993.

      Heath, R.L.: Modification of the biochemical pathways of plants induced by ozone: what are the varied routs to change?,
      Environ. Pollut., 155, 453–463, https://doi.org/10.1016/j.envpol.2008.03.010, 2008.

555   Heiden, A.C., Hoffmann, T., Kahl, J., Kley, D., Klockow, D., Langebartels, C.,Mehlhorn, H., Sandermann, H. Jr.,
      Schraudner, M., Schuh, G., and Wildt, J.: Emission of volatile organic compounds from ozone-exposed plants, Ecol. App.,
      l9, 1160–1167, https://doi.org/10.1890/1051-0761(1999)009[1160:EOVOCF]2.0.CO;2, 1999.

      Heiden, A. C., Kobel, K., Langebartels, C., Schuh-Thomas, G., and Wildt, J.: Emissions of oxygenated volatile organic
      compounds from plants, part I: Emissions from lipoxygenase activity, J. Atmos. Chem., 45, 143–172,
560   https://doi.org/10.1023/A:1024069605420, 2003.

      Herrmann, K.M., and Weaver, L.M.: The shikimate pathway, Annu. Rev. Plant. Physiol. Plant. Mol. Biol., 50, 473–503,
      https://doi.org/10.1146/annurev.arplant.50.1.473, 1999.

      Hollaway, M. J., Arnold, S. R., Challinor, A. J., and Emberson, L. D.: Intercontinental trans-boundary contributions to
      ozone-induced crop yield losses in the Northern Hemisphere, Biogeosciences, 9, 271–292, https://doi.org/10.5194/bg-9-271-
565   2012, 2012.

Ibrahim, M.A., Nissinen, A., Prozherina, N., Oksanen, E.J., and Holopainen, J.K.: The influence of exogenous monoterpene treatment and elevated temperature on growth, physiology, chemical content and headspace volatiles of two carrot cultivars (*Daucus carota L.*), Environ.Exp. Bot., 56, 95–107, https://doi.org/10.1016/j.envexpbot.2005.01.006, 2006.

IPCC: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L.: Climate
570 Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge Univ. Press, Cambridge, UK, and New York, 2007.

Jolivet, Y., Bagard, M., Cabané, M., Vaultier, M.N., Gandin, A., Afif, D., Dizengremel P., and Le Thiec, D.: Deciphering the ozone-induced changes in cellular processes: a prerequisite for ozone risk assessment at the tree and forest levels, Ann. For. Sci., 73, 923–943, https://doi.org/10.1007/s13595-016-0580-3, 2016.

575 Karl, M., Guenther, A., Köble, R., Leip, A., and Seufert, G.: A new European plant-specific emission inventory of biogenic volatile organic compounds for use in atmospheric transport models, Biogeosciences, 6, 1059–1087, https://doi.org/10.5194/bg-6-1059-2009, 2009.

Karl, T., Fall, R., Rosentiel, T.N., Prazeller, P., Larsen, B., Seufert, G., and Lindinger, W.: On-line analysis of the $^{13}CO_2$ labeling of leaf isoprene suggests multiple subcellular origins of isoprene precursors, Planta, 215, 894–905,
580 https://doi.org/10.1007/s00425-002-0825-2, 2002.

Karl, T., Guenther, A., Turnipseed, A., Patton, E.G., and Jardine, K.: Chemical sensing of plant stress at the ecosystem scale, Biogeosciences, 5, 1287–1294, https://doi.org/10.5194/bg-5-1287-2008, 2008.

Kanagendran, A., Pazouki, L., and Niinemets, Ü.: Differential regulation of volatile emission from *Eucalyptus globulus* leaves upon single and combined ozone and wounding treatments through recovery and relationships with ozone uptake,
585 Environ. Exp. Bot., 145, 21–38, https://doi.org/10.1016/j.envexpbot.2017.10.012, 2018.

Kangasjärvi, J., Talvinen, L., Utriainen, M., and Karjalainen, R.: Plant defence systems induced by ozone, Plant Cell Environ., 17, 783–794, https://doi.org/10.1111/j.1365-3040.1994.tb00173.x, 1994.

Kessler, A., and Balwin, I.T.: Defensive function of herbivore-induced plant volatile emissions in nature, Science, 291, 2141–2144, https://doi.org/10.1126/science.291.5511.2141, 2001.

590 Khatamian, H., Adedipe, N.O., and Ormrod, D.P.: Soil-water aspects of ozone phytotoxicity in tomato plants, Plant Soil, 30, 531–541, https://doi.org/10.1007/BF00010693, 1973.

Kiendler-Scharr, A., Wildt, J., Dal Maso, M., Hohaus, T., Kleist, E., Mentel, T.F. Tillmann, R., Uerlings, R., Schurr, U., and Wahner, A.: New particle formation in forests inhibited by isoprene emissions, Nature, 461, 381–384, https://doi.org/10.1038/nature08292, 2009.

595 Kobayashi, K., Miller, J.E., Flagler, R.B., and Heck, W.W.: Model analysis of interactive effects of ozone and water-stress on the yield of soybean, Environ. Pollut., 82, 39–45, https://doi.org/10.1016/0269-7491(93)90160-P, 1993.

Kreuzwieser, J., Graus, M., Wisthaler, A., Hansel, A., Rennenberg, H., and Schnitzler, J.P.: Xylem-transported glucose as additional carbon source for leaf isoprene formation in *Quercus robur*, New Phytol., 156, 171–178, https://doi.org/10.1046/j.1469-8137.2002.00516.x, 2002.

600 Lal, R.: World soils and the greenhouse effect, IGBP Newsletter 37, 4–5, 1999.

Laothawornkitkul, J., Taylor, J.E., Paul, N.D., and Hewitt, C.N.: Biogenic volatile organic compounds in the Earth system. New Phytol., 183, 27–51, https://doi.org/10.1111/j.1469-8137.2009.02859.x, 2009.

Lee, J.D., Lewis, A.C., Monks, P.S., Jacob, M., Hamilton, J.F., Hopkins, J.R., Watson, N.M., Saxton, J.E., Ennis, C., Carpenter, L.J., Carslaw, N., Fleming, Z., Bandy, B.J., Oram, D.E., Penkett, S.A., Slemr, J., Norton, E., Rickard, A.R.,
605 Whalley, L.K., Heard, D.E., Bloss, W.J., Gravestock, T., Smit, S.C., Stanton, J., Pilling, M.J., and Jenkin, M.E.: Ozone photochemistry and elevated isoprene during the UK heatwave of August 2003, Atmos. Environ, 40,7598-7613, https://doi.org/10.1016/j.atmosenv.2006.06.057, 2006.

15

Lehning, A., Zimmer, I., Steinbrecher, R., Hauff, K., Briiggemann. N., and Schnitzler. J. P.: Isoprene synthase activity and its relation to isoprene emission in *Quercus robur* L. leaves, Plant Cell Environ., 22, 495–504,

610   https://doi.org/10.1046/j.1365-3040.1999.00425.x, 2002.

Lichtenthaler, H. K.: The 1-deoxy-D-xylulose-5-phosphatepathway of isoprenoid biosynthesis in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol., 50, 47–66, https://doi.org/10.1146/annurev.arplant.50.1.47, 1999.

Lichtenthaler, H.K., Schwendler, J., Disch, A., and Rohmer, M.: Biosynthesis of isoprenoids in higher plant chloroplasts proceedsvia a mevalonate-independent pathway, FEBS Lett., 400, 271–274, https://doi.org/10.1016/S0014-5793(96)01404-

615   4, 1997.

Llusiá, J., and Peñuelas, J.: Changes in terpene content and emission in potted Mediterranean woody plants under severe drought, Can. J. Bot., 76, 1366–1373, https://doi.org/10.1139/b98-141, 1998.

Loreto, F., and Schnitzler, J.P.: Abiotic stresses and induced BVOCs, Trends Plant Sci., 15, 154–166. https://doi.org/10.1016/j.tplants.2009.12.006, 2010.

620   Loreto, F., and Velikova, V.: 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, https://doi.org/10.1104/pp.010497, 2001.

Loreto, F., Pinelli, P., Manes, F., and Kollist, H.: Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves, Tree Physiol., 24, 361–367,

625   https://doi.org/10.1093/treephys/24.4.361, 2004.

Löw, M., Herbinger, K., Nunn, A. J., Häberle, K. H., Leuchner, M., Heerdt, C., Werner, C., Wipfler, P., Pretzsch, H., Tausz, M., and Matyssek, R.: Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*), Trees, 20, 539–548, https://doi.org/10.1007/s00468-006-0069-z, 2006.

Maes, K., and Debergh, P.C.: Volatiles emitted from in vitro grown tomato shoots during abiotic and biotic stress, Plant Cell

630   Tiss. Org., 75, 73–78, https://doi.org/10.1023/A:1024650006740, 2003.

Maffei, M.E.: Sites of synthesis, biochemistry and functional role of plant volatiles, S. Afr. J. Bot., 76, 612–631, https://doi.org/10.1016/j.sajb.2010.03.003, 2010.

Mahmoud, S. S., and Croteau, R.B.: Strategies for transgenic manipulation of monoterpene biosynthesis in plants, Trends Plant Sci., 7, 366–373, https://doi.org/10.1016/S1360-1385(02)02303-8, 2002.

635   Maja, M.M., Kasurinen, A., Yli-Pirilä, P., Joutsensaari, J., Klemola, T., Holopainen, T., and Holopainen, J.: Contrasting responses of silver birch VOC emissions to short- and long-term herbivory, Tree Physiol., 34, 241–252, https://doi.org/10.1093/treephys/tpt127, 2014.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Sperry, J., West, A., Williams, D., and Yepez, E.A.: Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb

640   to drought?, New Phytol., 178, 719–739, https://doi.org/10.1111/j.1469-8137.2008.02436.x, 2008.

Miller, J.D., Arteca, R.N., and Pell, E.J.: Senescence-associated gene expression during ozone-induced leaf senescence in *Arabidopsis*, Plant Physiol., 120, 1015–1024, https://doi.org/10.1104/pp.120.4.1015, 1999.

Misztal, P.K., Hewitt, C.N., Wildt, J., Blande, J.D., Eller, A.S.D., Fares, S., Gentner, D.R., Gilman, J.B., Graus, M., Greenberg, J., Guenther, A.B., Hansel, A., Harley, P., Huang, M., Jardine, K., Karl, T., Kaser, L., Keutsch, F.N., Kiendler-

645   Scharr, A., Kleist, E., Lerner, B.M., Li, T., Mak, J., Nölscher, A.C., Schnitzhofer, R., Sinha, V., Thornton, B., Warneke, C., Wegener, F., Werner, C., Williams, J., Worton, D.R., Yassaa, N., and Goldstein, A.H..: Atmospheric benzenoid emissions from plants rival those from fossil fuels, Sci. Rep., 5, 12064, https://doi.org/10.1038/srep12064, 2015.

Monson, R.K., and Fall, R.: Isoprene emission from aspen leaves, Plant Physiol., 90, 267–274, https://doi.org/10.1104/pp.90.1.267, 1989.

650 Müller, M., Mikoviny, T., Jud, W., D'Anna, B. and Wisthaler, A.: A new software tool for the analysis of high resolution PTR-TOF mass spectra, Chemometr. Intell. Lab., 127, 158–165, https://doi.org/10.1016/j.chemolab.2013.06.011, 2013.

Müller, J.F., Stavrakou, T., Wallens, S., de Smedt, I., van Roozendael, M., Potosnak, M. J., Rinne, J., Munger, B., Goldstein, A., and Guenther, A. B.: Global isoprene emissions estimated using MEGAN, ECMWF analyses and a detailed canopy environment model, Atmos. Chem. Phys., 8, 1329–1341, https://doi.org/10.5194/acp-8-1329-2008, 2008.

655 Niinemets, Ü.: Mild versus severe stress and BVOCs: thresholds, priming and consequences, Trends Plant Sci., 15, 145–153, https://doi.org/10.1016/j.tplants.2009.11.008, 2010.

Niinemets, Ü., Loreto, F., and Reichstein, M.: Physiological and physicochemical controls on foliar volatile organic compound emissions, Trends Plant Sci., 9, 180–186, https://doi.org/10.1016/j.tplants.2004.02.006, 2004.

Ormeño, E., Mevy, J.P., Vila, B., Bousquet-Melou, A., Greff, S., Bonin, G., and Fernandez, C.: Water deficit stress induces
660 different monoterpene and sesquiterpene emission changes in Mediterranean species. Relationship between terpene emissions and plant water potential, Chemosphere, 67, 276–284, https://doi.org/10.1016/j.chemosphere.2006.10.029, 2007.

Owen, S., Boissard, C., Street, R., Duckham, S., Csiky, O., and Hewitt, C.: Screening of 18 Mediterranean plant species for volatile organic compound emissions, Atmos. Environ., 31, 101–117, https://doi.org/10.1016/S1352-2310(97)00078-2, 1997.

Palmer-Young, E.C., Veit, D., Gershenzon, J., and Schuman, M.C.: The sesquiterpenes (e)-ß-farnesene and (e)-α-
665 bergamotene quench ozone but fail to protect the wild tobacco nicotiana attenuata from ozone, UVB, and drought stresses, PLoS One, 10, e0127296, https://doi.org/10.1371/journal.pone.0127296, 2015.

Panek, J. A., Kurpius, M. R., and Goldstein, A. H.: An evaluation of ozone exposure metrics for a seasonally drought-stressed ponderosa pine ecosystem, Environ. Pollut., 117, 93–100, https://doi.org/10.1016/S0269-7491(01)00155-5, 2002.

Paoletti, E.: Ozone slows stomatal response to light and leaf wounding in a Mediterranean evergreen broadleaf, *Arbutus*
670 *unedo*, Environ. Poll., 134, 439–445, https://doi.org/10.1016/j.envpol.2004.09.011, 2005.

Pegoraro, E., Rey, A., Greenberg, J., Harley, P., Grace, J., Malhi, Y., and Guenther, A.: Effect of drought on isoprene emission rates from leaves of *Quercus virginiana* Mill, Atmos. Environ., 38, 6149–6156, https://doi.org/10.1016/j.atmosenv.2004.07.028, 2004.

Pellegrini, E., Hoshika, Y., Dusart, N., Cotrozzi, L., Gérard, J., Nali, C., Vaultier, M.-N., Jolivet, Y., Lorenzini, G., and
675 Paoletti, E.: Antioxidative responses of three oak species under ozone and water stress conditions, Sci. Total Environ., 647,390–399, https://doi.org/10.1016/j.scitotenv.2018.07.413, 2019.

Peñuelas, J., and Filella, I.: Responses to a warming world, Science, 294, 793–795, https://doi.org/10.1126/science.1066860, 2001.

Peñuelas, J., and Llusiá,, J.: The complexity of factors driving volatile organic compound emissions by plants, Biol. Plant.,
680 44, 481–487, https://doi.org/10.1023/A:1013797129428, 2001.

Peñuelas, J., and Llusiá, J.: BVOCs: plant defense against climate warming?, Trends Plant Sci., 8, 105–109, https://doi.org/10.1016/S1360-1385(03)00008-6, 2003.

Peñuelas, J., and Llusià, J.: Plant VOC emissions: making use of the unavoidable, Trends Ecol. Evol., 19, 402–404, https://doi.org/10.1016/j.tree.2004.06.002, 2004.

685 Peñuelas, J., and Munné-Bosch, S.: Isoprenoids: an evolutionary pool for photoprotection, Trends Plant Sci., 10, 166–169, https://doi.org/10.1016/j.tplants.2005.02.005, 2005.

Peñuelas, J., and Staudt, M.: BVOCs and global change, Trends Plant Sci., 15, 133–144, https://doi.org/10.1016/j.tplants.2009.12.005, 2010.

Peñuelas, J., Llusià, J., and Gimeno, B.S..: Effects of ozone concentrations on biogenic volatile organic compounds emission
690 in the Mediterranean region, Environ. Pollut., 105, 17–23, https://doi.org/10.1016/S0269-7491(98)00214-0, 1999.

Pinheiro, C., and Chaves, M.M.: Photosynthesis and drought: can we make metabolic connections from available data?, J. Exp. Bot., 62, 869–882, https://doi.org/10.1093/jxb/erq340, 2011.

Portillo-Estrada, M., Kazantsev, T., and Niinemets, Ü.: Fading of wound-induced volatile release during Populus tremula leaf expansion, J. Plant Res., 130, 157–165, https://doi.org/10.1007/s10265-016-0880-6, 2017.

695   Rosenstiel, T., Potosnak, M., Griffin, K., Falle, R., and Monson, R.K.: Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem, Nature, 421, 256–259, https://doi.org/10.1038/nature01312, 2003.

Royal Society: Ground-level ozone in the 21st century: future trends, impacts and policy implications, Royal Society Science Policy Report, 15, The Royal Society, London, UK, 2008.

Ruzicka, L.: The isoprene rule and the biogenesis of terpenic compounds, Experientia, 9, 357–367,
700   https://doi.org/10.1007/BF02167631, 1953.

Sandermann, H., and Strominger, J.L.: Purification and properties of C55-isoprenoid alcohol phosphokinase from *Staphylococcus aureus*, J Biol Chem., 247, 5123–5131, 1972.

Schaub, M., Haeni, M., Calatayud, V., Ferretti, M., and Gottardini, E.: ICP Forests. Ozone concentrations are decreasing but exposure remains high in European forests, ICP forests brief, 3, https://doi.org/10.3220/ICP1525258743000, 2018.

705   Schnitzler, J.P., Graus, M., Kreuzwieser, J., Heizmann, U., Rennenberg, H., Wisthaler, A., and Hansel, A.: Contribution of Different Carbon Sources to Isoprene Biosynthesis in Poplar Leaves, Plant Physiol., 135, 152–160, https://doi.org/10.1104/pp.103.037374, 2004.

Schwanz, P., and Polle, A.: Differential stress responses of antioxidative systems to drought in pendunculate oak (*Quercus robur*) and maritime pine (*Pinus pinaster*) grown under high CO_2 concentrations, J. Exp. Bot., 52, 133–143,
710   https://doi.org/10.1093/jexbot/52.354.133, 2001.

Schwender, J., Zeidler, J., Gröner, R., Müller, C., Frocke, M., Braun, S., Lichtenthaler, F. W., and Lichtenthaler, H. K.: Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae, FEBS Lett., 414, 129–134 https://doi.org/10.1016/S0014-5793(97)01002-8, 1997.

Seco, R., Karl, T., Guenther, A., Hosman, K.P., Pallardy, S.G., Gu, L., Geron, C., Harley, P., and Kim, S.: Ecosystem-scale
715   volatile organic compound fluxes during an extreme drought in a broadleaf temperate forest of the Missouri Ozarks (central USA), Glob. Chang. Biol., 21, 3657–3674, https://doi.org/10.1111/gcb.12980, 2015.

Sharkey, T. D., and Loreto, F.: Water stress, temperature and light effects on the capacity for isoprene emission and photo-synthesis of kudzu leaves, Oecologia, 343, 1–6 https://doi.org/10.1007/BF00320984, 1993.

Sharkey T.D., Loreto F., and Delwiche C.F.: High carbon dioxide and sun/shade effects on isoprene emission from oak and
720   aspen tree leaves, Plant Cell Environ., 14, 333–338, https://doi.org/10.1111/j.1365-3040.1991.tb01509.x, 1991.

Siddique, M. R. B., Hamid, A. I. M. S., and Islam, M. S.: Drought stress effects on water relations of wheat, Bot. Bull. Acad. Sin, 41, 35–39, https://doi.org/10.7016/BBAS.200001.0035, 2010.

Silver, G. M., and Fall, R.: Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts, Plant Physiol., 97, 1588–1591, https://doi.org/10.1104/pp.97.4.1588, 1991.

725   Sindelarova, K, Granier, C, Bouarar, I., Guenther, A., Tilmes, S., Stavrakou, T., Müller, J.-F., Kuhn, U., Stefani, P., and Knorr, W.: Global data set of biogenic VOC emissions calculated by the MEGAN model over the last 30 years, Atmos. Chem. Phys., 14, 9317–9341, https://doi.org/10.5194/acp-14-9317-2014, 2014.

Singsaas, E.L., Lerdau, M., Winter, K., and Sharkey, T.D.: Isoprene increases thermotolerance of isoprene-emitting species, Plant Physiol., 115, 1413–1420, https://doi.org/10.1104/pp.115.4.1413, 1997.

730   Slatnar, A., Jakopic, J., Stampar, F., Veberic, R., and Jamnik, P.: The effect of bioactive compounds on in vitro and in vivo antioxidant activity of different berry juices, PLoS One, 7, e47880, https://doi.org/10.1371/journal.pone.0047880, 2012.

Steinbrecher, R., Smiatek, G., Köble, R., Seufert, G., Theloke, J., Hauff, K., Ciccioli, P., Vautard, R., and Curci, G.: Intra- and inter-annual variability of VOC emissions from natural and semi-natural vegetation in Europe and neighboring countries, Atmos. Environ., 43, 1380–1391, https://doi.org/10.1016/j.atmosenv.2008.09.072, 2009.

735   Tasin, M., Cappellin, L., and Biasioli, F.: Fast direct injection mass-spectrometric characterization of stimuli for insect electrophysiology by proton transfer reaction-time of flight mass-spectrometry (PTR-ToF-MS), Sensors, 12, 4091–4104, https://doi.org/10.3390/s120404091, 2012.

Tingey, D.: The effect of environmental factors on the emission of biogenic hydrocarbons from live oak and slash pine. In: Bufalini, J., Arnts, R. (Eds.), Atmospheric Biogenic Hydrocarbons. Butterworth, Stoneham, MA, 53–72, 1981.

740   Tingey, D.T., Evans, R., and Gumpertz, M.: Effects of environmental conditions on isoprene emission from live oak, Planta, 152, 565–570, https://doi.org/10.1007/BF00380829, 1981.

Tingey, D., Turner, D., and Weber, J.: Factors controlling the emissions of monoterpenes and other volatile organic compounds, Trace Gas Emissions by Plants, T. Sharkey, H. Mooney, E. Holland, Academic, San Diego, Calif., 1991.

Tjoelker, M. G., Volin, J. C., Oleksyn, J., and Reich, P.B.: Interaction of ozone pollution and light effects on photosynthesis

745   in a forest canopy experiment, Plant Cell Environ., 18, 895–905, https://doi.org/10.1111/j.1365-3040.1995.tb00598.x, 1995.

Toome, M., Randjärv, P., Copolovici, L. Niinemets, Ü., Heinsoo, K., Luik, A., and Steffen, M.N.: Leaf rust induced volatile organic compounds signalling in willow during the infection, Planta, 232, 235–243, https://doi.org/10.1007/s00425-010-1169-y, 2010.

Umlauf, D., Zapp, J., Becker, H., and Adam, K.P.: Biosynthesis of the irregular monoterpene artemisia ketone, the

750   sesquiterpene germacrene D and other isoprenoids in *Tanacetum vulgare* L. (Asteraceae), Phytochemistry, 65, 2463–2470, https://doi.org/10.1016/j.phytochem.2004.08.019, 2004.

Unger, N., Harper, K., Zheng, Y. Kiang, N. Y., Aleinov, I., Arneth, A., Schurgers, G., Amelynck, C., Goldstein, A., Guenther, A., Heinesch, B., Hewitt, C. N., Karl, T., Laffineur, Q., Langford, B., McKinney, K. A., Misztal, P., Potosnak, M., Rinne, J., Pressley, S., Schoon, N., and Serça, D .: Photosynthesis-dependent isoprene emission from leaf to planet in a

755   global carbon-chemistry-climate model, Atmos. Chem. Phys., 13, 10243–10269, https://doi.org/10.5194/acp-13-10243-2013, 2013.

Ülker, E. D., Tavşanoğlu, Ç., and Perktaş, U.: Ecological niche modelling of pedunculate oak (*Quercus robur*) supports the 'expansion–contraction' model of Pleistocene biogeography, Biol. J. Linn. Soc., 123, 338–347, https://doi.org/10.1093/biolinnean/blx154, 2018.

760   Van Hees, A. F. M.: Growth and morphology of pedunculate oak (*Quercus robur* L) and beech (*Fagus sylvatica* L) seedlings in relation to shading and drought, Ann. sci. for., 54, 9–18, https://doi.org/10.1051/forest:19970102, 1997.

Velikova, V., Tsonev, T., Pinelli, P., Alessio, G.A., and Loreto, F.: Localized ozone fumigation system for studying ozone effects on photosynthesis, respiration, electron transport rate and isoprene emission in field-grown Mediterranean oak species, Tree Physiol., 25, 1523–1532, https://doi.org/10.1093/treephys/25.12.1523, 2005.

765   Velikova, V., Sharkey, T.D., and Loreto, F.: Stabilization of thylakoid membranes in isoprene-emitting plants reduces formation of reactive oxygen species, Plant Signal and Behav., 7, 139–141, https://doi.org/10.4161/psb.7.1.18521, 2012.

Vitale, M., Salvatori, E., Loreto, F., Fares, S., and Manes, F.: Physiological responses of *Quercus ilex* leaves to water stress and acute ozone exposure under controlled conditions, Water Air Soil Pollut., 189, 113–125, https://doi.org/10.1007/s11270-007-9560-4., 2008.

770   Vogt, T.: Phenylpropanoid biosynthesis, Mol. Plant, 3, 2–20, https://doi.org/10.1093/mp/ssp106, 2010.

Volz, A., and Kley, D.: Evaluation of the Montsouris series of ozone measurements made in the 19th-century, Nature, 332, 240–242, https://doi.org/10.1038/332240a0, 1988.

Wenda-Piesik, A.: Volatile organic compound emissions by winter wheat plants (*Triticum aestivum* L.) under *Fusarium spp.* infestation and various abiotic conditions, Pol. J. Environ. Stud., 20, 1335–1342, 2011.

775   Wildermuth, M. C., and Fall, R.: Light-dependent isoprene emission. Characterization of a thylakoid-bound isoprene synthase in *Salix discolorchloroplasts*, Plant Physiol., 112, 171–182, https://doi.org/10.1104/pp.112.1.171, 1996.

Wildermuth, M. C., and Fall, R.: Biochemical characterization of stromal and thylakoid-bound isoforms of isoprene synthase in willow leaves, Plant Physiol., 116, 1111–1123, https://doi.org/10.1104/pp.116.3.1111, 1998.

Williams, L.E., and Araujo, F.J.: Correlations among predawn leaf, midday leaf, and midday stem water potential and their
780 correlations with other measures of soil and plant water status in *Vitis vinifera*, J. Amer. Soc. Hort. Sci., 127, 448–454, https://doi.org/10.21273/JASHS.127.3.448, 2002.

Wittig, V.E., Ainsworth, E.A., and Long, S.P.: To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments, Plant Cell Environ., 30, 1150–1162, https://doi.org/10.1111/j.1365-3040.2007.01717.x, 2007.

785 Worthington Biochemical Corporation: The Worthington Manual, Freehold, New York, p. 1943, 1972.

Wootton-Beard, P. C., Moran, A., and Ryan, L.: Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin– Ciocalteu methods, Food Res. Int., 44, 217–224, https://doi.org/10.1016/j.foodres.2010.10.033, 2011.

Yener, S., Sánchez-López, J.A., Granitto, P.M., Cappellin, L., Märk, T.D.,Zimmermann, R., and Biasioli, F.: Rapid and
790 direct volatile compound profiling of black and green teas (*Camellia sinensis*) from different countries with PTR-ToF-MS, Talanta, 152 ,45–53, https://doi.org/10.1016/j.talanta.2016.01.050, 2016.

Yuan, X., Calatayud, V., Gao, F., Fares, S., Paoletti, E., Tian, Y., and Feng, Z.: Interaction of drought and ozone exposure on isoprene emission from extensively cultivated poplar, Plant Cell Environ., 39, 2276–2287, https://doi.org/10.1111/pce.12798, 2016.

795 **Tables**

Table 1: Mean and standard deviation in parenthesis for stomatal conductance (g_s), net photosynthesis (A), leaf temperature (Tl_{eaf}) and stem water potential (SWP) divided into four ranges of SWP (R1: 0.00 to -1.40 MPa; R2: -1.45 to -2.85 MPa; R3: -2.90 to -4.30 MPa, R4: -4.35 to -6.00 MPa). Values in bold marked with ([a]) indicate a significant (p-values<0.05) differences between R1 and R4, ([b]) indicate a significant difference between the set under drought stress (DS) and the set under drought stress with ozone
800 treatment (DS×OS). Values marked with ([c]) indicate close to significant differences with p- values of 0.05-0.06 between R1 and R4.

	R1		R2		R3		R4	
	DS	DS×OS	DS	DS×OS	DS	DS×OS	DS	DS×OS
g_s [mmol m^{-2} s^{-1}]	**42.4 (28.9)**[a]	20.2 (13.8)[c]	6.6 (4.9)	6.8 (2.7)	3.8 (0.8)	3.3 (0.4)	**3.9 (0.7)**[a]	2.9 (0.1)[c]
A [μmol m^{-2} s^{-1}]	**3.38 (2.08)**[a]	1.99 (1.37)[c]	0.58 (0.78)	0.52 (0.36)	0.08 (0.07)	0.05 (0.02)	**0.10 (0.10)**[a]	0.02 (0.004)[c]
T_{leaf} [K]	302.3 (1.9)	303.0 (1.7)	**302.5 (1.0)**[b]	**301.3 (0.6)**[b]	302.1 (0.9)	302.6 (1.2)	301.1 (1.1)	302.7 (0.2)
SWP [MPa]	**-0.9 (0.2)**[a]	-0.9 (0.1)[c]	-2.0 (0.1)	-2.3 (0.2)	-3.3 (0.2)	-3.6 (0.5)	**-5.5 (0.5)**[a]	-5.4 (0.7)[c]

Table 2: Mean and standard deviation for standardize isoprene emissions (IS$_S$), standardized monoterpenes emissions (MT$_S$), standardized sesquiterpenes emissions (SQT$_S$), sum of GLV (ΣGLV) and sum of Shikimate (ΣSHIKIMATE) for each set divided by range of stem water potential (SWP) (R1: 0.00 to -1.40 MPa; R2: -1.45 to -2.85 MPa; R3: -2.90 to -4.30 Mpa, R4: -4.35 to -6.00
805 MPa). Values in bold marked with ([a]) indicate a significant (p-values<0.05) differences between R1 and R4, ([d]) between R2 and R3, ([b]) indicate a significant difference between the set under drought stress (DS) and the set under drought stress with ozone treatment (DS×OS). Values marked with ([c]) indicate close to significant differences with p-values of 0.05-0.06 between R1 and R4.

	R1		R2		R3		R4	
	DS	DS×OS	DS	DS×OS	DS	DS×OS	DS	DS×OS
IS$_S$ [nmol m^{-2} s^{-1}]	**12.8 (2.0)**[a]	18.0 (7.3)[c]	**8.6 (3.8)**[b]	**17.3 (4.1)**[b]	6.9 (2.4)	10.6 (4.4)	**1.7 (0.9)**[a]	3.9 (2.6)[c]
MT$_S$ [nmol m^{-2} s^{-1}]	**0.010 (0.002)**[a]	0.036 (0.026)	0.009 (0.004)	0.023 (0.013)	0.010 (0.002)	0.015 (0.010)	**0.033 (0.014)**[a]	0.047 (0.012)

Biogeosciences
Discussions

SQT_S [nmol m^{-2} s^{-1}]	**0.002 (0.001)[a]**	0.002 (0.001)[c]	0.003 (0.002)	0.001 (0.001)	0.005 (0.003)	0.007 (0.008)	**0.014 (0.005)[a]**	0.035 (0.007)[c]
ΣGLV [nmol m^{-2} s^{-1}]	**0.002 (0.001)[a]**	0.003 (0.004)	**0.004 (0.005)[b]**	**0.001 (0.003)[b]**	0.002 (0.002)	0.001 (0.001)	**0.032 (0.045)[a]**	0.009 (0.010)
$\Sigma SHIKIMATE$ [nmol m^{-2} s^{-1}]	0.001 (0.001)	0.003 (0.001)[c]	0.001 (0.002)	0.003 (0.003)	0.004 (0.002)	0.008 (0.012)	0.003 (0.001)	0.009 (0.001)[c]

Table 3: Mean and standard deviation of antioxidant capacity, total phenol content (TPhen), peroxidase activity for well-watered sets with (OS) and without (C) ozone treatment, and for sets under severe drought stress with (DS×OS) and without (DS) ozone treatment after seven days of measurements. ([e]) represent values with significant (p-value <0.05) differences between C and DS, ([f]) represent values with significant differences between OS and DS×OS.

|	Antioxidant Capacity [(F ODSample^{-1}) (F ODControl^{-1})$^{-1}$]	TPhen [grGAEequiv. kg^{-1} (DW)]	Peroxidase Activity [μmol s^{-1} kg^{-1}(DW)]
C	0.9 (0.1)	**35.6 (11.7)[e]**	0.9 (0.7)
OS	0.8 (0.04)	**25.8 (11.7)[f]**	0.6 (0.3)
DS	1.0 (0.1)	**86.5 (24.1)[e]**	0.9 (0.3)
DS×OS	1.0 (0.1)	**77.1 (9.2)[f]**	0.8 (0.4)

Figures
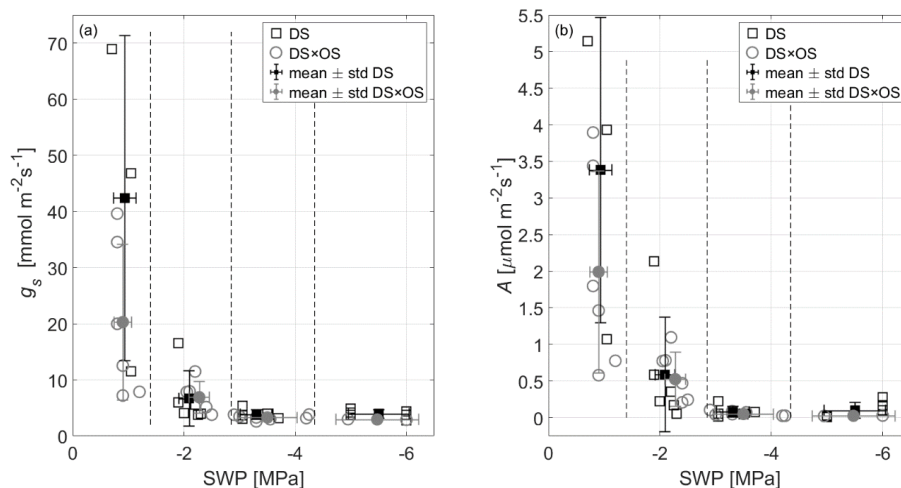
Figure 1: (a) Stomatal conductance (g_s) and (b) net photosynthesis (A) of all trees as a function of stem water potential (SWP). Empty markers represent individual trees where the black squares represent trees out of the set under drought stress (DS) and the gray circles out of the set under drought stress with ozone treatment (DS×OS). Filled squares and circles represent means calculated for each SWP range with the corresponding standard deviation. SWP ranges are separated by vertical dashed lines.
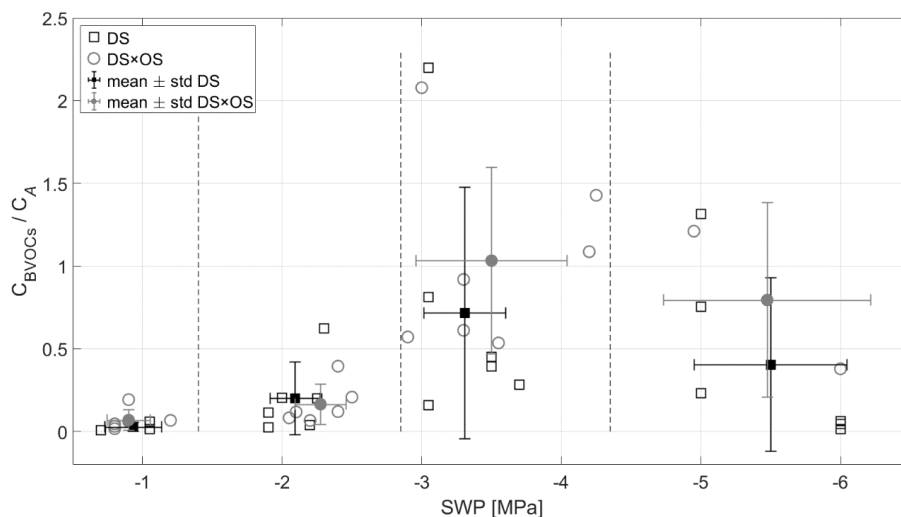
Figure 2: Ratio of sum of carbon emitted by all analyzed BVOCs (C_{BVOCs}) and the sum of carbon uptake via net photosynthesis (C_A) versus the stem water potential (SWP). Empty markers represent individual trees where the black squares represent trees out of the set under drought stress (DS) and the gray circles out of the set under drought stress with ozone treatment (DS×OS). Filled squares and circles represent means calculated for each SWP range with the corresponding standard deviation. SWP ranges are separated by vertical dashed lines.
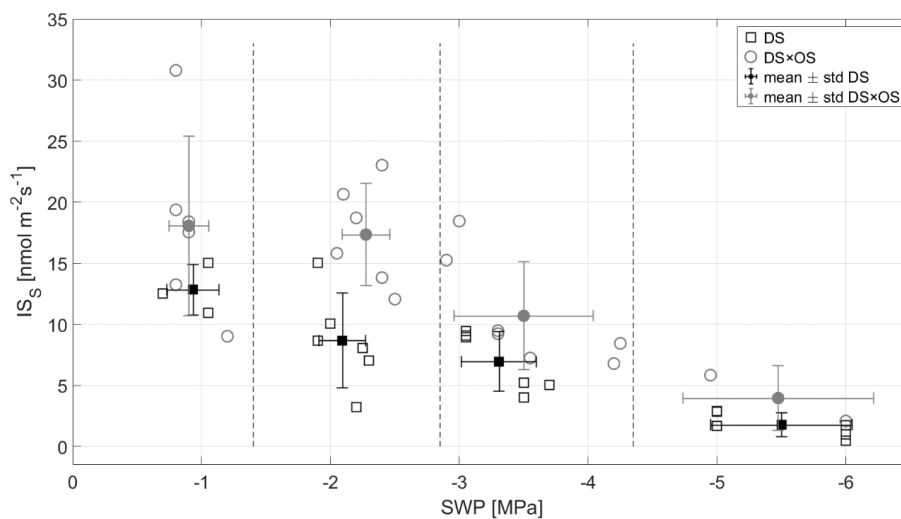
Figure 3: Standardized isoprene emission (IS_S) versus stem water potential (SWP). Empty markers represent individual trees where the black squares represent trees out of the set under drought stress (DS) and the gray circles out of the set under drought stress with ozone treatment (DS×OS). Filled squares and circles represent means calculated for each SWP range with the corresponding standard deviation. SWP ranges are separated by vertical dashed lines.
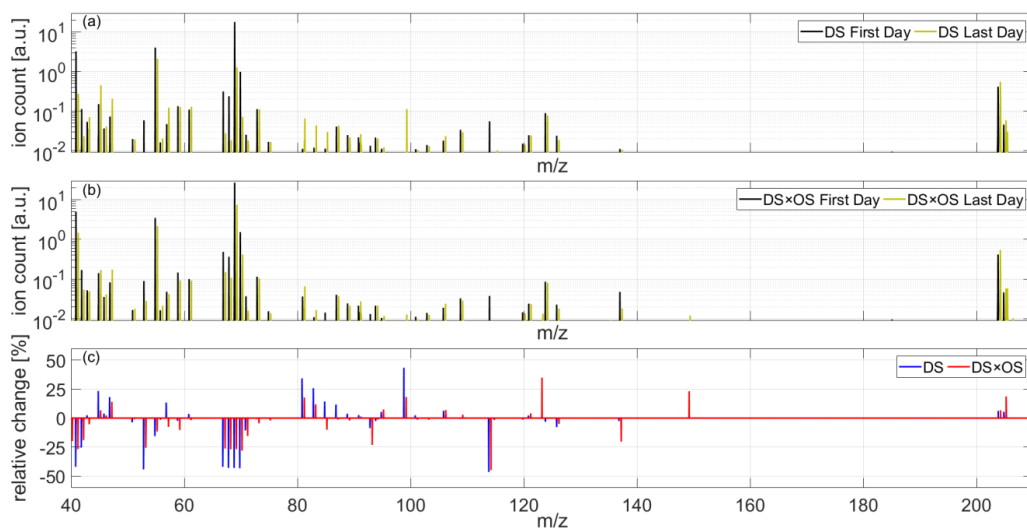
Figure 4: Mean mass spectra of the set under drought stress (DS) (a) and the set under drought stress with ozone fumigation (DS×OS) (b), on the first (black) and last (yellow) day of measurement. (c) Relative change in the mass spectra between the last and the first day of analysis for DS (blue) and DS×OS (red).
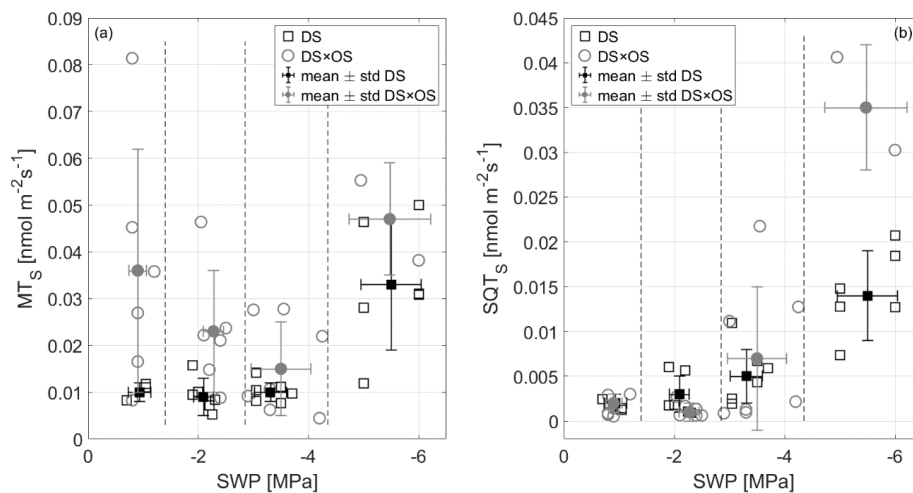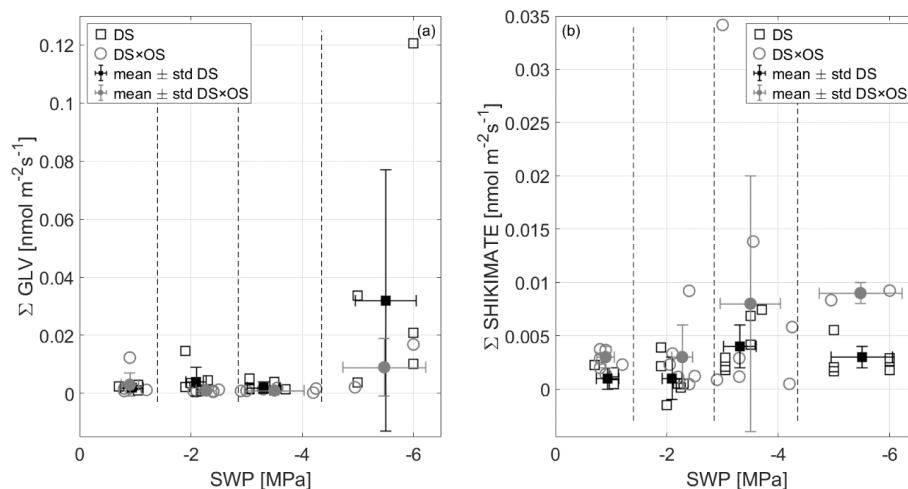

835

Figure 5: Standardized monoterpenes (MT$_S$) (a) and sesquiterpenes (SQT$_S$) (b) emissions versus stem water potential (SWP). Empty markers represent individual trees where the black squares represent trees out of the set under drought stress (DS) and the gray circles out of the set under drought stress with ozone treatment (DS×OS). Filled squares and circles represent means calculated for each SWP range with the corresponding standard deviation. SWP ranges are separated by vertical dashed lines.

840

Figure 6: The sum of green leaf volatiles (ΣGLV) (a) and the sum of Shikimate (ΣShikimate) compound (b) emissions versus stem water potential (SWP). Empty markers represent individual trees where the black squares represent trees out of the set under drought stress (DS) and the gray circles out of the set under drought stress with ozone treatment (DS×OS). Filled squares and circles represent the mean values calculated for each SWP range with the corresponding standard deviation. SWP ranges are
845 **separated by vertical dashed lines.**

Biogeosciences
Discussions

Appendix A
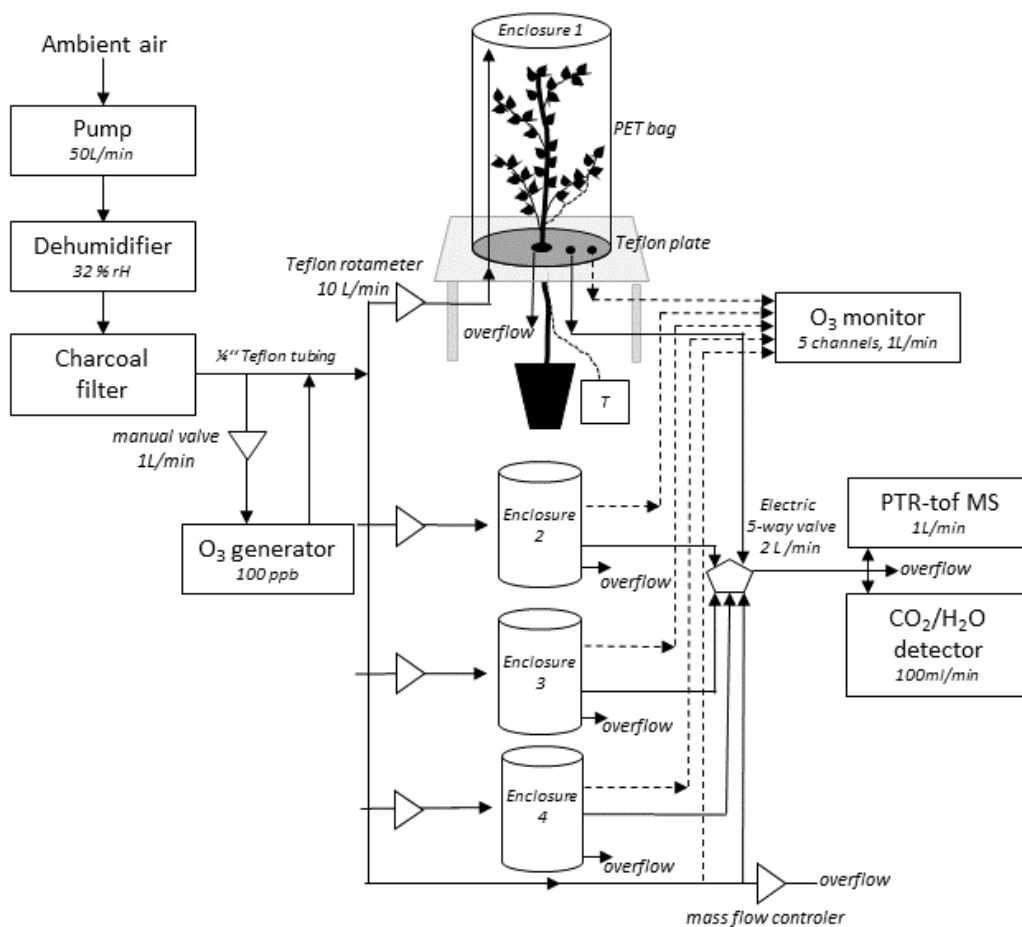
Figure A1: Scheme of a custom-made plant enclosure and set up of the experiment. In brief, the chambers consisted of a PTFE-covered bottom plate with an opening mechanisms to insert and seal the plant stem using PTFE-plugs; furthermore, the bottom plate featured three in- and outlets for gas sampling and ozone exposure; the inlet was raised above the bottom plate to allow for air mixing. The upper part of the chamber consisted of a transparent, 12-liter PET-bag, holding most of the tree crown. The bags were tightly sealed towards the bottom plate.

850

Table A2: Acronyms and experimental conditions used in this experiment.

ACRONYMS	
A	Net photosynthesis (CO_2 assimilation rate)
BVOCs	Biogenetic Volatile Organic Compounds
C	Control samples without ozone treatment
DS	Set under drought stress without ozone treatment
DS×OS	Set under drought stress with ozone treatment
g_S	Stomatal Conductance
GLVs	Green Leaf Volatiles
IS	Isoprene
ISs	Standardized emissions of Isoprene

MeSa	Methyl Salicylate
MT	Sum of Monoterpenes
MT_S	Standardized emissions of MT
O_3	Ozone
OS	Well-watered control samples with ozone treatment
PTR-ToF-MS	Proton Transfer Reaction Time of Flight Mass Spectrometer
Q. robur	*Quercus robur* L.
ROS	Reactive Oxygen Species
SQT	Sum of Sesquiterpenes
SQT_S	Standardized emissions of SQT
std	Standard deviation
SWP	Stem Water Potential
TPhe	Total Phenol content

EXPERIMENTAL CONDITIONS	
Enclosure Pressure	2.386 kPa
Mean leaf temperature	29.06 °C
Mean PAR	1374 μmol m^{-2} s^{-1}
Ozone concentration	100 ppb
Standardized temperature	30 °C
Standardized PAR	1000 μmol m^{-2} s^{-1}

855   **Table A3: m/z ratio and chemical formula of compounds used for the calculation of the sum of BVOCs in C_{BVOCs} / C_A.**

m/z ratio	Chemical formula
33.033	$(CH_4O)H^+$
45.033	$(C_2H_4O)H^+$
47.049	$(C_2H_6O)H^+$
57.033	$(C3H_4O)H^+$
57.069	$(C_4H_8)H^+$
59.049	$(C_3H_6O)H^+$
61.028	$(C_2H_4O_2)H^+$
71.049	$(C_4H_6O)H^+$
73.064	$(C_4H_8O)H^+$
79.054	$(C_6H_6)H^+$
83.085	$(C_6H_{10})H^+$
85.101	$(C_6H12)H^+$
87.080	$(C_5H_{10}O)H^+$
93.069	$(C_7H_8)H^+$
95.050	$(C_6H_5OH)H^+$
99.080	$(C_6H_{10}O)H^+$
101.096	$(C_6H12O)H^+$
107.049	$(C_7H_6O)H^+$
107.073	$(C_8H_{10})H^+$

143.107	$(C_8H_{14}O_2)H^+$
145.122	$(C_8H_{16}O_2)H^+$
153.055	$(C_8H_8O_3)H^+$
165.092	$(C_{10}H_{12}O_2)H^+$
211.133	$(C_{12}H_{18}O_3)H^+$
225.149	$(C_{12}H_{20}O_3)H^+$
265.144	$(C_{15}H_{20}O_4)H^+$
69.070	$(C_5H_8)H^+$
137.133	$(C_{10}H_{16})H^+$
205.195	$(C_{15}H_{24})H^+$

Table A4: Mean dry weight and mean specific leaf area for the 20 % of the total analysed leaves of sets DS and DS×OS.

	Dry weight [g]	Specific leaf area [m^2]
DS	1.16	0.015
DS×OS	0.82	0.011

860