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Date palm responses to chronic, realistic ozone exposure in a FACE experiment

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Abstract:	Date palms are highly economically important species in hot arid regions, which may suffer ozone (O 3) pollution equivalently to heat and water stress. However, little is known about date palm sensitivity to O 3 . Therefore, to identify their resistance mechanisms against elevated O 3 , physiological parameters (leaf gas exchange, chlorophyll fluorescence and leaf pigments) and biomass growth responses to realistic O 3 exposure were tested in an isoprene-emitting date palm (Phoenix dactylifera L. cv. Nabut Saif) by a Free-Air Controlled Exposure (FACE) facility with three levels of O 3 (ambient [AA, 45 ppb as 24-h average], 1.5 x AA and 2 x AA). We found a reduction of photosynthesis only at 2 x AA although some foliar traits known as early indicators of O 3 stress responded already at 1.5 x AA, such as increased dark respiration, reduced leaf pigment content, reduced maximum quantum yield of PSII, inactivation of the oxygen evolving complex of PSII and reduced performance index PI TOT . As a result, O 3 did not affect most of the growth parameters although significant declines of root biomass occurred only at 2 x AA, suggesting that this date palm cultivar showed an intermediate susceptibility to O 3 . The major mechanism in date palm for reducing the severity of O 3 impacts was a restriction of stomatal O 3 uptake due to low stomatal conductance and O 3 -induced stomatal closure. In addition, an increased respiration in elevated O 3 may indicate a raised capacity of catabolizing metabolites for detoxification and repair. Interestingly, date palm produced low amounts of monoterpenes, whose emission was stimulated in 2 x AA, although isoprene emission declined at both 1.5 and 2 x AA. Our results warrant more research on a biological significance of terpenoids in plant resistance against O 3 stress.

Date palm responses to chronic, realistic ozone exposure in a FACE experiment

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Abstract

Date palms are highly economically important species in hot arid regions, which may suffer ozone (O_3) pollution equivalently to heat and water stress. However, little is known about date palm sensitivity to O_3.

Therefore, to identify their resistance mechanisms against elevated O_3, physiological parameters (leaf gas exchange, chlorophyll fluorescence and leaf pigments) and biomass growth responses to realistic O_3 exposure were tested in an isoprene-emitting date palm (*Phoenix dactylifera* L. cv. Nabut Saif) by a Free-Air Controlled Exposure (FACE) facility with three levels of O_3 (ambient [AA, 45 ppb as 24-h average], 1.5 x AA and 2 x AA). We found a reduction of photosynthesis only at 2 x AA although some foliar traits known as early indicators of O_3 stress responded already at 1.5 x AA, such as increased dark respiration, reduced leaf pigment content, reduced maximum quantum yield of PSII, inactivation of the oxygen evolving complex of PSII and reduced performance index PI_{TOT}. As a result, O_3 did not affect most of the growth parameters although significant declines of root biomass occurred only at 2 x AA, suggesting that this date palm cultivar showed an intermediate susceptibility to O_3. The major mechanism in date palm for reducing the severity of O_3 impacts was a restriction of stomatal O_3 uptake due to low stomatal conductance and O_3-induced stomatal closure. In addition, an increased respiration in elevated O_3 may indicate a raised capacity of catabolizing metabolites for detoxification and repair. Interestingly, date palm produced low amounts of monoterpenes, whose emission was stimulated in 2 x AA, although isoprene emission declined at both 1.5 and 2 x AA. Our results warrant more research on a biological significance of terpenoids in plant resistance against O_3 stress.

Keywords: tropospheric ozone; date palm; volatile organic compounds; VOC; stomatal ozone flux; photosynthetic electron transport; chlorophyll *a* fluorescence

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Introduction

Surface ozone (O_3) is a secondary pollutant formed via reactions from precursors, e.g. nitrogen oxides, carbon monoxide, methane and volatile organic compounds (VOC) in the presence of sunlight. It is an ubiquitous air pollutant, which at present reaches potentially phytotoxic levels in many regions of the world (Mills et al., 2018). Ozone impacts on vegetation have been largely investigated (Paoletti, 2007; Li et al., 2017; Grulke and Heath, 2020), but there is still a lack of knowledge on species from under-investigated areas of the world, e.g. on palms.

Palms are perennial flowering plants in the monocot order Arecales, mostly restricted to tropical and subtropical climates. Among the > 2600 species of palm, only date palm (*Phoenix dactylifera* L.) has been investigated for O_3 responses so far and showed relatively high sensitivity following short-term exposure (8 h) to a spike of O_3 (200 ppb) (Du et al., 2018). Date palm is appreciated both as ornamental tree and as food source, and is widely cultivated wherever the temperature is optimal for ripening of its edible sweet fruits (ab. 40 °C), especially in Northern Africa, the Middle East and South Asia. In these areas, high temperature, intense solar radiation and clear sky favour O_3 formation (Smoydzin et al., 2012; Radaideh, 2016). In fact, elevated O_3 precursor emissions and high O_3 pollution have been documented over the Middle East (Smoydzin et al., 2012) and South Asia (Fry et al., 2012), because of urban development and industrialization (Ohara et al., 2007; Radaideh, 2016) as well as long-range transport of precursors (Lelieveld et al., 2009; Kulshrestha and Kumar, 2014). Although date palm requires good soil water availability for optimal growth, it can tolerate drought (Arab et al., 2016). It presents thick leaves (Doaygei et al., 2013) and low gas exchanges (Arab et al., 2016) that are considered xeromorphic adaptations able to induce cross-tolerance to O_3 (Paoletti, 2006). Date palm sensitivity to O_3 is thus worth of further investigations.

Ozone may reduce plant growth. According to a meta-analysis by Li et al. (2017), an experimentally enhanced O_3 exposure (mean concentrations = 116 ppb) reduced 14% of total biomass compared with the control (mean concentrations = 21 ppb). The negative effect was highlighted in below-ground rather than above-ground growth (Agathokleous et al., 2016). A reduction in plant growth by O_3 is generally related to a damage to photosynthetic systems in leaves (Hoshika et al., 2020a, b). Ozone-induced negative effects on photosynthesis may be associated with a reduced performance of chlorophyll fluorescence and a decline of photosynthetic pigments (Li et al., 2017; Cotrozzi et al., 2018a).

Elevated O_3 exposure also impacts on isoprene emission from leaves. In addition to the pivotal role of VOC on O_3 formation in the atmosphere, biogenic isoprene biosynthesis and emission is postulated to contribute to scavenge O_3–induced reactive oxygen species (ROS) (Loreto and Velikova, 2001; Vickers et al., 2009), maintaining photochemical efficiency and photosynthetic stability (Pollastri et al., 2019) and acting as a signal molecule to alter gene expression for abiotic stress (Harvey and Sharkey, 2016; Zuo et al., 2019). Palms usually emit isoprene from the leaves. For example, Parra (2008) and Hewitt et al. (2009) evaluated the contribution of the high-isoprene-emitting oil palm in tropical plantations to the production of surface O_3 pollution. Arab et al. (2016) found that heat but not drought stimulated the biosynthesis of isoprene in date palm, with photosynthesis only weakly affected by both stressors.

The aim of this study was to clarify the mechanisms of date palm sensitivity to O_3 exposure under realistic ambient conditions in a last-generation O_3 Free-Air Controlled Exposure (O_3 FACE) experiment. The questions addressed here are: (i) does O_3 affect the response of biomass and leaf gas exchange (photosynthetic parameters and VOC emission) in date palm? (ii) is date palm sensitivity explained by avoidance of O_3 stress (restriction of O_3 uptake due to stomatal closure)? (ii) is date palm sensitivity affected by isoprene emission from leaves?

Materials and methods

Experimental design and conditions

The experiment was carried out from May 20th to August 20th, 2019, in a free-air controlled exposure (FACE) facility located in Mediterranean Italy (43°48'59" N, 11°12'01" E, 55 m a.s.l.), where ambient summer conditions allow the growth of tropical plant species (Moura et al., 2018; Fernandes et al., 2019). Three levels of O_3 were applied: ambient (AA), 1.5 times ambient (1.5 x) and twice ambient O_3 concentrations (2 x), with three replicated plots per each O_3 level. A detailed description of the ozone FACE facility is in Paoletti et al. (2017).

Environmental conditions were continuously monitored by recording hourly values of soil moisture by ECH2O EC-5 sensors (Decagon Devices, Pullman WA, USA) and of air temperature, photosynthetic active radiation, relative humidity of the air and precipitation by a Watchdog station (Mod. 2000; Spectrum Technology, Inc., Aurora, IL, USA). Fig. 1 shows environmental conditions (Fig. 1a) and O_3 levels (Fig. 1b) during the experiment. AOT40 (Accumulated dose of ozone Over a Threshold of 40 ppb) values at the end of the 92 days of the experiment were 20,071 ppb·h in AA, 46,297 ppb·h in 1.5 x AA and 61,959 ppb·h in 2 x AA. POD1 (Phytotoxic Ozone Dose above a threshold of 1 nmol m^{-2} s^{-1}) values were 2.43 mmol m^{-2} in AA, 3.93 mmol m^{-2} in 1.5 x AA and 4.64 mmol m^{-2} in 2 x AA. The details of POD1 calculation are described in Suppl. Fig. S1 and Table S1.

Fourty-five micro-propagated 1-year-old plants of date palm (ab. 1 m high) of the cultivar Nabut Saif, raised in a soil-less peat-based potting mix in a plastic "torpedo" pot, were transferred into 4.5 l pots filled with 70% gravel (diameter 3-6 mm) and 30% commercial planting peat-rich soil in December 2018. Pots were kept to overwinter in a phytotron on plastic tablets continuously filled with tap water at 1-2 cm height and watered daily with 50 ml tap water per pot. Conditions included artificial illumination at ca. 200 µmol m^{-2} s^{-1}, 25°C temperature, with a 16/8h light/dark cycle. Plants were transferred to 20 l pots and to shaded

tunnels in the open on 1st May, 2019, and moved to full light after one week. Each pot was fertilized once a month with NPK 20:10:20 with micronutrients (Soluplant 20.10.20, Haifa, Israel). Five potted plants were placed in each plot, for a total of 45 plants. Each pot was watered daily by a drip irrigation system with 800 ml of tap water, i.e. 90% of field capacity.

Measurements of gas exchange

Daily profiles of net photosynthesis (A) and stomatal conductance (g_s) were measured in clear sky days at 8-9 CET (morning) and 14-15 CET (afternoon) by a portable infrared gas analyser (Li-Cor 6400 instruments, Lincoln, NE, USA) on July 23rd and July 30th in 2019. The concentration of CO_2 in the chamber (Ca) was set to 400 ppm. According to Hoshika et al. (2020a), the leaf cuvette was positioned so as to be fully exposed to the direct solar irradiance (mean photosynthetic photon flux density [PPFD], morning: 1282 and 1277 µmol m^{-2} s^{-1} on 23rd and 30th July, respectively; afternoon: 2122 and 2037 µmol m^{-2} s^{-1} on 23rd and 30th July, respectively). Temperature and relative humidity (RH) in the cuvette were manually set in order to track the ambient values (leaf temperature: 25 and 38 °C in the morning and afternoon, respectively, on July 23rd, 28 and 35 °C in the morning and afternoon, respectively, on July 30th; RH: 46 and 35% in the morning and afternoon, respectively, on July 23rd, 45 and 38% in the morning and afternoon, respectively, on July 30th). In addition, on July 30th, dark respiration (R_n) was measured by switching off the LED light source after plants were kept in the dark for 30 min. All gas exchange measurements were carried out on one fully-expanded leaf per plant (2nd fully expanded leaves), on 1-2 plants in each replicated plot.

Measurements of the kinetics of chlorophyll (Chl) a fluorescence

On 20th August, Chl *a* fluorescence was measured on attached leaves dark adapted with Hansatech leaf clips (30 min) (from all the 5 plants per plot) with three leaves per plant, by a Plant Efficiency Analyser

(Pocket PEA fluorimeter, Hansatech Instruments Ltd., King's Lynn, UK). The emitter wavelength of a non-modulated light source was 625 nm for the actinic light LED. High quality optical band pass filters were used for the detector (Chl *a* fluorescence 730±15 nm). Measurements were performed on circular areas of the leaves of 2 mm diameter, using Hansatech leaf clips homogeneously illuminated by actinic light LEDs set to a saturating light intensity of 3500 µmol photons m^{-2} s^{-1}. Chl *a* fluorescence was recorded within five time intervals; every 10 µs for the initial fluorescence (0 - 300 µs), every 100 µs (0.3 - 3 ms), 1 ms (3 – 30 ms), 10 ms (0.03 – 0.3 s), 100 ms (0.3 – 1 s). Raw data were transferred and processed using PEA Plus software (Hansatech Instruments Ltd.). The primary photochemistry of PSII was further evaluated using well established parameters described in Suppl. Table S2, Suppl. Fig. S2 and Suppl. Fig. S3a-c, according to formulations previously published (Papageorgiou and Govindjee, 2004; Strasser et al., 2010; Bussotti et al., 2011; Chen et al., 2016). The changes in these parameters are associated with various stressors as well as overall plant vitality (Kalaji et al., 2017). All parameters were optimized for a high throughput workflow of Chl *a* fluorescence raw data using the open source software "Libre Office 6" (The Document Foundation, Berlin, Germany). The data were plotted graphically and statistically analyzed by using "Prism 8.4 for Mac OS-X" software (GraphPad Software Inc., La Jolla, USA).

Measurement of synthesis and emission of volatile organic compounds

On August 1^{st}-2^{nd}, from 10 to 12 CET, one leaf (2^{nd} fully expanded leaves) from two plants per replicated plot was sampled for emission of VOC following the methodology by Yuan et al. (2016). In detail, the central part of the leaf was included into the 6 cm^2 cuvette of a LI-6400 system (Li-Cor 6400 instruments, Lincoln, NE, USA). Measurements were performed at standard conditions of 30°C and 1000 µmol m^{-2} s^{-1} PPFD. When photosynthesis reached a steady state, 2-l air samples were collected through a purified Tenax-TA glass tube (Thermal Desorption Tubes, filled with 100 mg Tenax-TA adsorbent (Mesh 60/80), Gerstel, Germany) with a vacuum sampler pump (VSS-1, AP Buck, USA). Blank (no leaf) samples were collected at the beginning and end of each day of sampling. The traps were sealed with Teflon-coated brass

caps immediately after collection and stored at -20°C until analysis to avoid any chemical alteration and/or artefacts. Then, the samples were processed and analyzed with a thermal-desorber (Markes International, Series 2 Unity) connected to a 7890 A gas chromatograph coupled with a 5975C mass detector (GC–MS, Agilent Technologies, Wilmington, USA) as described in Baraldi et al. (2019). Identification and quantification of the sampled isoprenoids were carried out according to Rapparini et al. (2004).

All pinnae of the 2nd fully expanded leaf of each plant were cut into small pieces, shock frozen in liquid nitrogen and ground to fine powder. RNA was isolated using the NucleoSpin RNA Plant Kit (Macherey-Nagel) according to the manufacturers advice with the following exceptions. Leaf tissues were frozen in liquid nitrogen and ground to powder. For each sample, 50 mg of leaf tissue powder were first mixed with 500 µl Fruit-mate^{TM} (Taraka Bio Inc.) and centrifuged for 5 min at 4 °C and 12000 g to remove polysaccharides and polyphenols. The supernatant was mixed with 500 µl RA1 buffer (containing 1:1000 TCEP) from the NucleoSpin RNA Plant Kit. The mixture was transferred to the filter columns in two steps and the filtrate was collected in a new collection tube and mixed well with the same volume of 70% ethanol. The mixture was added again in two steps to the NucleoSpin RNA plant Colum and centrifuged for 1 min at 11,000 g. The washing steps were performed following the manufacturer's instructions. RNA was eluted in 33 µL RNase free water that was incubated twice on the membrane for 1 min. The concentration was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, U.S.A.). Removal of contaminating DNA, cDNA synthesis and qPCR was performed as described previously (Böhm et al., 2018). The following primers were used for qPCR and transcript numbers were normalized to 10,000 transcripts of the housekeeping gene, the growth elongation factor EF1a (Patankar et al., 2016): PdEF1afwd (5'-CTGTTGCAACAAGATGGA-3'), PdEF1arev (5'-CCGAAGGTGACAACCATA-3'), Pd_ISPSfwd (5'-CGTCCTATTAGTCCATGCT-3'), Pd_ISPSrev (5'-GATGGATGTTGGAGTATC-3'), PdMTPS1fwd (5'-TTCCCAAGAATCATAAAGGCTA-3'), PdMTPS1rev (5'-AGTCATATTAAGACACTC-3'), Pd-LevSynfwd (5'-TGCCTTCCCATATTCAAGCAT-3'), Pd-LevSynrev (5'-AGGTACATGAAGCGTGAG-3'), where PdEF1 is growth elongation factor EF1a (NCBI accession XM_008785500.3), Pd_ISPS is a putative isoprene synthase (NCBI

accession XM_008781287.2) and Pd_MTPS1 is a putative monoterpene synthase (NCBI accession XM_026807289.1). Pd_ISPS primers were designed for the putative date palm isoprene synthase (TRINITY_GG_87144_c19_g1_i1.p1 from Helmholz II experiment, unpublished). ISPS transcripts were normalized to 10000 molecules of HKG EF1α using standard curves calculated for individual PCR products.

Measurement of foliar traits and pigments

After the leaf gas exchange measurements, five leaf discs of 0.8 cm diameter per the same target leaf (one leaf, 2nd fully expanded leaves, one to two plants per plot) were collected by using a leaf punch (Fujiwara Scientific Company Co., Ltd., Tokyo, Japan), and weighted by a scale (Model Bp110, Sartorius weighing technology, Germany) in order to calculate fresh weight of the samples (FW). They were then dried at 70°C for at least 72 h in the oven. Leaf mass per area (LMA) was calculated as a ratio of dry mass (DW) and area (LA) of each leaf. Leaf water content was calculated as LWC (%) = (FW−DW)/FW×100.

Other leaf samples (ca. 3 g) of each target leaf (one leaf, 2nd fully expanded leaves, one plant per plot) were harvested, immediately flash-frozen with liquid nitrogen and stored at -80°C until leaf pigment analysis. Leaf pigments were determined by ultra high performance liquid chromatography (UHPLC) using a Dionex UltiMate 3000 system equipped with an Acclaim 120 C18 column (5-µm particle size, 4.6-mm internal diameter × 150-mm length) maintained into a Dionex TCC-100 column oven at 30 °C, and a Dionex UVD 170U detector (Thermo Scientific, Waltham MA, USA; Cotrozzi et al., 2018b). Leaf material (50 mg fresh weight, FW) was homogenized in 1 mL of 100% HPLC-grade methanol and incubated overnight at 5 °C in the dark. The sample supernatants were filtered through 0.2 µm Minisart® SRT 15 aseptic filters. The pigments were eluted using 100% solvent A (acetonitrile/methanol, 75/25, v/v) for the first 14 min to elute xanthophylls (neoxanthin, Neo; violaxanthin, Vio; antheraxanthin, Ant; lutein, Lut; zeaxanthin, Zea; in order of elution), followed by a 1.5-min linear gradient to 100% solvent B (methanol/ethylacetate, 68/32, v/v), which was pumped for 14.5 min to elute chlorophyll b (Chl b) and chlorophyll a (Chl a) and β-carotene (β-

car), followed by 2-min linear gradient to 100% solvent A. The flow rate was 1 ml min^{-1}. The column was allowed to re-equilibrate in 100% solvent A for 1 min before the next injection. The pigments were detected by their absorbance at 445 nm. To quantify the pigment content, known amounts of pure standards were injected into the UHPLC system and an equation correlating the peak area to pigment concentration was formulated. The data were processed using the Thermo Scientific Dionex Chromeleon 7 Chromatography Data System software. Total chlorophyll content (Chl_{TOT}) was calculated as Chl a + Chl b. Total carotenoid content (Car_{TOT}) was calculated as Neo + Vio + Ant + Lut + Zea + β-car, while the xanthophyll cycle pigment content (VAZ) was calculated as Vaz + Ant + Zea. The de-epoxidation state (DEPS) was calculated as (Ant + Zea)/VAZ.

Assessment of growth and biomass

Plant height, number of leaves and base diameter were recorded at the beginning and the end of the experiment with a ruler and a caliper with 1-mm accuracy. Total above- and below-ground biomass of all plants was harvested at the end of the experiment and put in the oven at 70 °C until constant weight was reached (ab. 72 hours). After that, DW of each plant organ was determined by a scale (Model Bp110, Sartorius weighing technology, Germany).

Statistical analysis

Data from the plants in each plot were averaged and the average was used as statistical unit, i.e. N = 3 plots. Data were tested for normal distribution and homogeneity of variance by Smirnoff and Levene tests, respectively. The effects of O_3 on the linear relationship between g_s and VPD were tested by an analysis of covariance (ANCOVA). To assess the effect of O_3 and measurement time on A and g_s, we applied a two-way analysis of variance (ANOVA). For the other parameters, the effect of O_3 was tested by one-way ANOVA

followed by Tukey's multiple comparison test. All statistical tests were carried out by R software (R 3.5.1: R Core Team, 2018).

Results

Foliar traits and pigments

Date palms showed LMA values in the range of 119 to 133 g m^{-2} (Tab. 1). There were no significant effects of O_3 on LMA, while LWC was increased by O_3. In fact, LWC values were significantly higher in 2 × AA than in AA plants (+19%).

Contents of Chl_{TOT} and Car_{TOT} significantly decreased only under 1.5 × AA, compared with AA (-22 and -32%, respectively), while VAZ decreased under both 1.5 × AA and 2 × AA (ca. -30%; Tab. 1). Lut values were 26% lower under 1.5 x AA than under 2 x AA, but there were no significant difference comparing these enriched O_3 concentrations with AA. No significant O_3 effects were found on Chl a/b ratio, β-car, and DEPS.

Leaf gas exchange

The daily measurements of leaf gas exchange indicate a significant effect of O_3 on A and g_s (Tab. 2). Only the highest 2 × AA O_3 treatment induced a significant decline of A (-51% on July 23rd and -48% on July 30th) and g_s (-57% on July 23rd and -59% on July 30th) relative to AA, which occurred only in the morning, although the interaction of daytime and O_3 treatment was not significant. The responses of g_s to VPD are shown in Fig. 2. Stomatal conductance decreased with increasing VPD at AA and 1.5 x AA treatments. An ANCOVA test revealed that the y-intercept of the relationship between g_s and VPD was lower in 1.5 x AA compared to the control (AA). There was no statistically significant relationship between g_s and VPD at the highest

level of O_3 treatment (2.0 x AA). Dark respiration rate (R_n) was significantly lower in AA than in 1.5 AA and 2 × AA plants (-28% and -36%, respectively) (Fig. 3).

Parameters of the stomatal conductance model are shown in Table S1 and Fig. S1. The maximum stomatal conductance to O_3 (g_{max}) value was set to 73 mmol O_3 m^{-2} PLA s^{-1} as 95th percentile of g_{sto} measurements (Table S1, Fig. S1). The minimum stomatal conductance (f_{min}) value was set to 0.06 (fraction) corresponding to the 5th percentile values of the stomatal conductance recorded throughout the measurements. Stomatal response to light (f_{light}) showed a typical saturation curve with a light saturation point above 2000 µmol m^{-2} s^{-1} (Fig. S1a). The f_{O3} indicates that O_3 decreased stomatal conductance by 0.31% per unit O_3 concentration (ppb) in date palm leaves (Fig. S1b). The optimal temperature for stomatal opening was 27 °C (Fig. S1c). VPD higher than 2.6 kPa induced stomatal closure (Fig. S1d).

Chlorophyll *a* fluorescence

At ambient O_3 level, the induction curve was significantly higher than in the palm leaves exposed to elevated O_3 levels after 300 µs (Fig. S2). The curves of leaves exposed to 1.5 x AA and 2 x AA O_3 exposure exhibited a similar pattern. The relative variable fluorescence ΔV showed marked differences between the AA controls and the two elevated O_3 treatments (Fig. S3a), with the biggest differences within the O-J-phase and the J-I-phase and a lower difference within the I-P-phase, but without a distinct peak at the J-Step and the I-Step (marked as ΔV_J and ΔV_I). This presentation of fluorescence kinetics showed only a small difference between both groups of O_3 treated leaves at 150 µs compared to the AA group (Fig. S3b). On the other hand, marked differences between the control group at ambient O_3 and the two groups exposed to 1.5 and 2 times enhanced O_3 concentrations were identified in ΔW_{OJ} (Fig. S3c) with a peak marked as ΔW_K at an average time of 500 µs. At the beginning of the fluorescence kinetics after 150 µs of illumination, values at the L-Step were similar between AA and O_3 treatments (Fig. 4a). In addition, marked differences in the extent and range of the resulting values at the K-Step (t = 300 to 600 µs) were observed between O_3

treatments and AA, with mostly positive values indicating that the fluorescence level of O_3 treated date palm leaves at this step was higher than in AA (Fig. 4b). The variable fluorescence levels at step J (t = 2 ms) and I (t = 30 ms) also showed marked differences between O_3 treated plants and the AA group (Fig. 4c,d).

The quantum yield of photosystem II (φ_{Po}) showed statistically significant differences between AA and leaves of plants growing at 1.5 x AA and 2 x AA, but no differences were found between 1.5 x AA and 2 x AA (Fig. 5a). The same findings were observed for the performance index PI total (Fig. 5b) and for the number of reaction centers per absorption (10RC/ABS, Fig. 5d). One-way ANOVA revealed a significant difference ($p < 0.05$) between AA and 1.5 x AA for the energy dissipation per active reaction center chlorophyll DI_0/RC (Fig. 5c). However, there were no statistically significant differences between AA and 2 x AA as well as between 1.5 x AA and 2 x AA for this parameter.

Volatile organic compounds

Isoprene emission was significantly impaired by both elevated O_3 treatments (-58% at 1.5 x AA and -50% at 2 x AA), while the relative expression of isoprene synthase transcripts showed a large variability and no clear response to O_3 (Fig. 6a,b). Date palm emitted also small amounts of monoterpenes such as α-pinene, β-pinene, octanal, nonanal, camphor, iso-borneol (data not shown), whose emission was stimulated in 2 x AA plants, so that the emission of total VOCs decreased only at 1.5 x AA (-57% relative to AA) (Fig. 6c,e). Also the relative expression of monoterpene synthases was very variable and the increases at 2 x AA were not statistically significant (Fig. 6d). Isoprene emission was correlated positively with net photosynthesis (Fig. 7a) and negatively with intercellular CO_2 concentration (Ci) (Fig. 7b). However, such correlations were not found in monoterpene emission.

Growth and biomass

Plant height, number of leaves and base diameter were not affected by O_3 treatments (data not shown). However, during the 3-month experiment, the plants significantly increased in diameter from the beginning of the experiment (2.06 ± 0.31 cm) to the end (2.80 ± 0.46 cm), while plant height (107 ± 8.9 cm vs. 111 ± 9.4 cm) and number of leaves (5.5 ± 1.0 vs. 6.0 ± 0.8) were unaltered. Above- and below-ground biomass decreased with increasing O_3 treatments, but the effect was significant only for the 2 x AA roots relative to AA roots (-36%) (Fig. 8a,b).

Discussion

The growth conditions at the study site were suitable for date palm as suggested by the increase in base diameter over the experiment. For instance, the average diurnal air temperature was 19.5/27.8/29.2/30.1 °C in May/June/July/August, i.e. in the range of values in Riyadh, Saudi Arabia (31.1/33.7/34.8/32.7 °C), that is one of the major area of date palm distribution and cultivation (Aleid et al., 2015). The stomatal conductance model showed that the optimal temperature for stomatal opening was 27 °C in this date palm cultivar. The O_3 levels were realistic as they well simulated the conditions in Riyadh. For instance, the O_3 peaks in May/June/July/August were 72/110/105/92 ppb at the ozone FACE and 66/95/98/90 ppb in Riyadh (Butenhof et al., 2015). Such realistic O_3 levels translated into very high values of AOT40. At the end of the experiment, in fact, the date palms were exposed to AOT40 levels that were 4 (AA), 9.2 (1.5 x AA) and 12.4 (2 x AA) times higher than the critical level recommended to protect plants from O_3 injury, i.e. 5 ppm h (CLRTAP, 2014).

Photosynthetic responses to ozone exposure

Elevated O_3 exposure induced significant negative effects on date palm photosynthesis in the morning, especially at the highest 2 x AA O_3 level. Stomatal resistance to CO_2 transport may be considered as a factor to limit photosynthetic activity in elevated O_3 (Kitao et al., 2009; Hoshika et al., 2020b). In fact, stomatal

closure due to elevated O_3 exposure was observed in the morning for date palm plants. However, such stomatal closure cannot be found in the afternoon, suggesting that the closing response induced by atmospheric humidity deficit may over-rule stomatal sensitivity to O_3. Interestingly, the highest O_3 treatment (2 x AA) induced a loss of stomatal response to VPD. It has been shown that O_3 exposure causes an impairment of efficient stomatal control of gas exchange, i.e. stomatal sluggishness (Paoletti, 2005; Hoshika et al., 2019, 2020a). Hoshika et al. (2019) demonstrated that the sluggish response of stomata was attributed to ethylene emission. However, the mechanisms are still under investigated.

A reduction of photosynthesis due to elevated O_3 exposure may be caused by not only stomatal but also biochemical limitation (Hoshika et al., 2020b). In fact, an impairment of the gas exchange was in accordance with the overall reduction of photosynthetic pigments (i.e. Chl_{TOT} and Car_{TOT}) which play a crucial role in light harvesting for photosynthesis. The degradation of chlorophyll and carotenoids was similarly found in O_3 exposed leaves due to oxidative-stress destruction (Watanabe et al., 2013; Cotrozzi et al., 2018a). Fast chlorophyll fluorescence kinetics of dark-adapted samples indicated that O_3 exposure strongly affected photosynthetic electron transport in date palm leaves. Ozone exposure decreased the maximum quantum efficiency of PSII (φ_{Po}; F_V/F_M), the average performance (PI_{TOT}), and the activity of PSII RCs as reported before (Contran et al., 2009; Bussotti et al., 2011; Pellegrini et al., 2011; Salvatori et al., 2013; Zhang et al., 2018b). Recently, we developed a new spectroscopic model to predict these fluorescence parameters in date palm (Cotrozzi et al., 2020). For a detailed analysis of hyperspectral parameters *see* Cotrozzi et al. (2020). In addition, our results show clear differences within the OJ-phase between the control AA group and the two groups growing at elevated O_3. The differences in the functionality of PSII of date palms exposed to elevated O_3 were not caused by a lesser connectivity between PSII units, since there was no significant difference of the fast fluorescence kinetics at 150 µs between plants exposed to either elevated O_3 treatment or AA. Many studies demonstrated that inactivation of the oxygen evolving complex (OEC) is a typical reaction of plants to abiotic stress like heat stress or drought (Oukarroum et al., 2007; Oukarroum et al., 2009; Hu et al., 2020). This inactivation leads to an inhibition of

electron transport by releasing the manganese cluster, the main component of OEC, thereby causing an imbalance between the electron flow from the OEC to the RCs (Chen et al., 2016). Studies on the effect of elevated O_3 on the photosynthetic performance of several woody species such as beech, oak and poplars showed that inactivation or breakdown of OECs represent an early response to O_3 stress (Bussotti et al., 2011; Desotgiu et al., 2013), as also observed in our experiment.

Differences were not only found within the OJ-phase, but also the JI-phase and to a lesser extent the IP-phase. The JI-phase is related to the redox state of the plastoquinone pool (Tóth et al., 2007). Its redox is affected by oxidative stress caused by O_3, probably via the plastidic NADH-plastoquinone oxidoreductase (Ndh) complex (Guéra et al., 2005). Thus, the present findings suggest that oxidative stress due to O_3 exposure led to disturbances in the plastoquinone pool of the photosynthetic electron transport chain. The IP-phase of the fluorescence transient is related to photosystem-I (PSI) activity (Schansker et al. 2005), indicates the rate of reduction of ferredoxin (Cascio et al., 2010) and is thought to constitute a measure of PSI electron acceptors (Tsimilli-Michael and Strasser, 2008; Živcák et al., 2014). Experiments with *Quercus ilex* L. and *Arbutus unedo* L. showed that the activity of PSI has a key role in O_3-mediated oxidative stress (Mereu et al., 2011). Bussotti et al. (2011) reported that the main impact of O_3-mediated oxidative stress was in and beyond PSI for poplar hybrids. However, our results suggest that date palms were less sensitive to O_3 in the last part of the photosynthetic electron transport chain, with only a minor effect on PSI activity.

Isoprene and monoterpene responses to ozone exposure

Volatile isoprenoids such as isoprene and monoterpenes are among the most abundant and reactive biogenic VOCs produced by plants (Guenther et al., 2012). Isoprene-emitting species occur in many plant taxa across many functional types, but they are more often found in woody plant species including palms (Kesselmeier and Staudt, 1999). Our date palms emitted low amounts of isoprene (on average 8.4 ± 0.8 ng m^{-2} s^{-1} in AA) relative to literature data for this species (~27 ng m^{-2} s^{-1} in Arab et al., 2016, still at 30 °C but

under 1200 µmol m^{-2} s^{-1} PAR). Tree species on average emit 22-79 ng m^{-2} s^{-1} isoprene at or near standard conditions, but different leaf environments and measurement techniques may significant affect such values (Geron et al., 2001). Isoprene emission from date palm leaves declined at both 1.5 and 2 x AA relative to AA, while the relative expression of isoprene synthase transcripts did not respond to O_3. Photosynthesis and isoprene emission declined in tandem, which is supported by a meta-analysis of isoprenoid responses to abiotic factors including O_3 (Feng et al., 2019).

Interestingly, date palm also produced low amounts of total monoterpenes (on average 1.8 ± 0.3 ng m^{-2} s^{-1} in AA), whose emission was stimulated in 2 x AA leaves, although an increase in the relative expression of monoterpene synthase transcripts in 2 x AA leaves was not statistically significant. The stimulation of monoterpene emission by O_3 exposure was similarly reported mainly in evergreen species by the meta-analysis (Feng et al., 2019). The monoterpene emission from date palm leaves in elevated O_3 was not dependent on photosynthesis. The uncoupling of monoterpene emission from photosynthesis may be a hormetic response to the initial stage of stress (Agathokleous et al., 2018).

Biomass responses to ozone exposure

A leaf-level photosynthetic activity may be closely related to plant growth. As a result of O_3-induced damage to photosynthetic activity, biomass accumulations are usually limited (Li et al., 2017; Gao et al., 2017). In date palm, however, plant height, number of leaves, base diameter and above-ground biomass were not significantly affected by elevated O_3 exposure, while root biomass was reduced only at the highest 2 x AA level. It is well known that a reduction of photosynthate allocation to roots is one of the first steps of O_3 injury to plants (Carriero et al., 2015; Mrak et al., 2019). Decrease in root biomass due to O_3 was reported in approx. 40% of studies on trees according to a meta-analytic review (Agathokleous et al., 2016).

Reasons of intermediate susceptibility to ozone

Ozone exposure did not significantly affect most of the growth parameters, although 2 x AA O_3 exposure reduced below-ground biomass. These results indicate that this date palm cultivar may be intermediately susceptible to O_3. In fact, evergreen species such as date palm is more resistant to O_3 than deciduous species (Feng et al., 2018), because larger availability of cell walls implies larger apoplastic substrate for O_3 detoxification (Moldau, 1998). However, plant resistance to O_3 may depend not only on leaf habit (deciduous and evergreen) but also various physiological factors (Feng et al., 2018).

A well-known factor mitigating O_3 susceptibility of plant species is low stomatal conductance and, thus, restricted stomatal O_3 uptake (Reich, 1987). Here we present a Jarvis-type stomatal conductance model for estimating the maximum g_s in this species and estimated a g_{max} value (73 mmol O_3 m^{-2} s^{-1}) that is in line with the values of desert shrubs and in the lower range of global values found in a meta-analysis of g_s in woody plants (Hoshika et al., 2018). Although our g_{max} value was slightly higher than previous observations of g_s in the same species (40 to 50 mmol O_3 m^{-2} s^{-1}, Du et al., 2018; Kruse et al., 2017, 2019), a previous Jarvis-type g_s modeling study on *P. dactylifera* found a much higher g_{max} in a different cultivar (ab. 200 mmol O_3 m^{-2} s^{-1} in cv. Medjool, Sperling et al 2014). Stomatal conductance of different date palm cultivars may differ considerably (Al-Jabr et al., 2007), thus suggesting that further cultivar-specific studies are also needed. Such a low capacity of stomatal O_3 uptake implied an uncoupling of AOT40 and POD1 values. In fact, AOT40 values at the end of the experiment were 2.3 and 3.1 times higher than AA, while POD1 values were only 1.6 and 1.9 times higher in 1.5 x AA and 2 x AA, respectively, which well synthesizes the elevated stomatal defense capacity of date palm from elevated atmospheric concentrations of O_3. As a matter of fact, the function f_{O3} indicates that O_3 decreased stomatal conductance by 0.31% per unit O_3 concentration (ppb) in date palm. As a result, 2 x AA O_3 exposure reduced g_s by 46%. Ozone-induced stomatal closure may act as an avoidance response to reduce a possible O_3 damage to plants (Hoshika et al., 2020a).

Stomatal closure, however, may also cause excess light energy and production of reactive oxygen species (ROS), which raises susceptibility to photoinhibitory damage (Takagi et al., 2017; Giudi et al., 2019). It seems that date palm plants adopted a major protective mechanism to reduce the absorption of excitation energy and preserve photosystems from photoinhibition through a reduction in the number of light-harvesting antennae rather than in the chlorophyll antenna size (as confirmed by the unchanged values of Chl a/b ratio; Cotrozzi et al., 2018b). Indeed, no other differential responses in leaf pigment parameters were observed between plants exposed to 1.5 and 2 x AA levels. Although a reduction of VAZ was observed, other well-known photo-protective mechanisms (i.e. changing chlorophyll composition, increasing β-car levels and DEPS; Esteban et al., 2015) were not activated, indicating that a re-organization of the photosynthetic apparatus did not occur and confirming the intermediate susceptibility to O_3 of date palm.

The ability of increasing dark respiration under O_3 stress may have also contributed to mitigate the susceptibility of date palm. While the respiratory CO_2 loss is a major cause for the reduction of carbon gain due to elevated O_3 (Zhang et al., 2018a; Podda et al., 2019), dark respiration is important in the biosynthetic processes of growth and maintenance, which raises a metabolic capacity for repair of damaged tissues and detoxification especially under abiotic and/or biotic stress (Weraduwage et al., 2011). In fact, no oxidative damage (assessed in terms of lipid peroxidation) was observed under both increased O_3 concentrations, with a slight decrease of hydrogen peroxide reported only under 2 x AA (Arab et al. in prep). This response was due to an ability of date palm to regulate its major enzymatic and non-enzymatic antioxidants. In particular, the decreased Car_{tot} (only under 1.5 x AA) and VAZ values indicate that these compounds could be consumed by the cell in order to counteract the possible reactive oxygen species generation by preventing their peroxidation action. Is it known that they are involved in non-photochemical quenching mechanisms, thus reducing the risk of photo-oxidative stress (Niinemets et al., 2003). Based on relative physical-chemical features and intra-cellular distribution, these antioxidant compounds may serve distinct and complementary functions (Close and Beadle, 2003).

If isoprene emission confers protection to date palm against O_3 injury (Loreto and Velikova, 2001; Velikova et al., 2005), we may expect that synthesis and emission of isoprene increased with increasing O_3 exposure. However, our results contrasted with this hypothesis, suggesting that isoprene emission from date palm leaves may have not contributed to the protection against O_3 stress. A recent review questioned the common belief that isoprene has immediate, physical effects on plants such as changing membrane properties or quenching ROS (Lantz et al., 2019), because it is highly volatile and does not dissolve into cellular components in great quantity (Harvey et al., 2015). Interestingly, on the other hand, O_3 increased monoterpene emission, which may be also considered as a factor to protect plants from O_3 damage (Fares et al., 2008). While isoprene and monoterpenes share common biosynthetic pathways, monoterpenes are usually stored after synthesis in special organs, such as resin ducts or glands (Kesselmeier and Staudt, 1999). So far, it is unclear whether O_3 may have a direct effect on the storage pools of monoterpenes, and also the number of O_3 studies on monoterpene-emitting species is limited (e.g. Carriero et al., 2016; Mochizuchi et al., 2017). Hadacek et al. (2011) proposed that non-linear hormetic effects regulate the responses of stored secondary metabolites to abiotic factors, and that the reduction of ROS is a key step of the hormetic effects caused by these compounds. ROS are known to be stimulated by O_3 exposure (Pellegrini et al., 2019; Podda et al., 2019) as also shown in our date palms (data not shown). Overall, our results warrant more research on the redox chemistry of terpenoids under O_3 stress.

Conclusions

This is the first experiment on date palm responses to chronic, realistic O_3 exposure. Significant declines of several gas exchange parameters and root biomass occurred only at the highest 2 x AA O_3 level. Some variables that are usually known as early responses to O_3 stress, responded already at 1.5 x AA. Isoprene emission did not appear to contribute to date palm O_3 tolerance as it declined at both elevated O_3 levels. In contrast, monoterpene emission was stimulated at 2 x AA and its O_3 responses should be further evaluated.

The major defense mechanisms that emerged from this study were avoidance of O_3 stress (i.e. exclusion of O_3 entry by low maximum stomatal conductance to O_3 [73 mmol O_3 m^{-2} s^{-1}] and O_3-induced stomatal stomatal closure) and high capacity of catabolizing metabolites for detoxification and repair as indicated by increased dark respiration. We thus conclude that this date palm cultivar was intermediately susceptible to O_3, but further studies with different cultivars are recommended as date palm cultivars may significantly differ in their physiological traits such as stomatal conductance. These results are needed for a proper O_3 risk assessment in the areas where *P. dactylifera* grows.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contribution

E. Paoletti, Y. Hoshika, L. Arab, and H. Rennenberg conceived the study and methodology, and Y. Hoshika, L. Arab, S. Martini, L. Cotrozzi, D. Weber and P. Ache acquired data from the ozone FACE experiment; Y. Hoshika and S. Martini contributed to the analysis of leaf gas exchange and biomass data; L. Neri and R. Baraldi contributed to the analysis of BVOC emission; L. Cotrozzi and E. Pellegrini contributed to the analysis of leaf pigments; D. Weber contributed to the analysis of chlorophyll fluorescence data; P. Ache, L. Arab, H.M. Müller and R. Hedrich contributed to the analysis of RNA data; E. Paoletti, R. Hedrich, S. Alfarraj and H. Rennenberg contributed to the interpretation of data to understand the date palm defense mechanisms to ozone stress. E. Paoletti prepared the draft of the manuscript and all authors were involved in writing the paper.

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Figure and table legends

Fig. 1. Environmental conditions over the experimental period (from May 20th to August 20th, 2019 = 92 days of exposure). a) Daily averages of temperature (Temp), vapor pressure deficit (VPD), photosynthetic active solar radiation (PAR), and precipitation. b) Daily ozone averages and AOT40 and POD1 values in the three ozone treatments, i.e. ambient air (AA), 1.5 x AA and 2 x AA.

Fig. 2. Relationship between stomatal conductance (g_s) and leaf-to-air vapor pressure deficit (VPD) in date palm leaves grown under different O_3 concentrations (AA, ambient, 1.5 x AA, 2 x AA). Data were obtained under a PPFD > 1000 µmol m^{-2} s^{-1}. Simple linear regression analyses were applied: *** $p < 0.001$, ** $p < 0.01$, ns denotes not significant. Since the regressions were statistically significant for AA and 1.5 x AA, we applied the ANCOVA; * $p < 0.05$, ns denotes not significant.

Fig. 3. Average (± standard error) of dark respiration rate (R_n) on July 30th. Different letters show significant effects of the ozone treatments (AA, ambient, 1.5 x AA, 2 x AA) (Tukey test, $p < 0.05$, N = 3 plots).

Fig. 4. Effect of different ozone levels on date palms, assessed with Chlorophyll-a fluorescence measurements *in vivo*. Boxplots show differences between elevated ozone levels compared to ambient ozone level (AA). Orange boxes, 1.5 x AA; red boxes, 2 x AA. Whiskers: 1,5 x IQR, dots: outliers, cross: average, line: median; green dotted line at y = 0: reference value at ambient ozone level; n = 3 plots. (a) ΔW_L difference of variable fluorescence ΔW_{OK}, at the L-Step at t = 150 µs. (b) difference of variable fluorescence ΔW_{OJ}, at the K-Step t = 300-600 µs. (c) difference of variable fluorescence ΔV, at the J-Step t = 2 ms. (d) difference of variable fluorescence ΔV, at the I-Step t = 30 ms.

Fig. 5. Effect of different ozone levels on date palms on (a) maximum quantum yield for primary photochemistry φ_{Po} (b) the performance index PI_{TOT}, (c) energy dissipation per active reaction centres DI_0/RC, (d) reaction centres per absorption 10RC/ABS. Boxplots show: Green boxes, ambient ozone level (AA); orange boxes, 1.5 x AA; red boxes, 2 x AA. Whiskers: 1,5 x IQR, dots: outliers, cross: average, line: median. Different letters show significant differences among treatments (Tukey test, $p<0.05$, N = 3 plots).

Fig. 6. Average (± standard error) of isoprene emission (a), relative expression of isoprene synthase transcripts (b), monoterpene emission (c), relative expression of monoterpene synthases (d) and total

volatile organic compounds (VOC) emission (e) in date palm leaves on 1-2 August. Different letters show significant effects of the ozone treatments (AA, ambient, 1.5 x AA, 2 x AA) (Tukey test, p<0.05, N=3 plots).

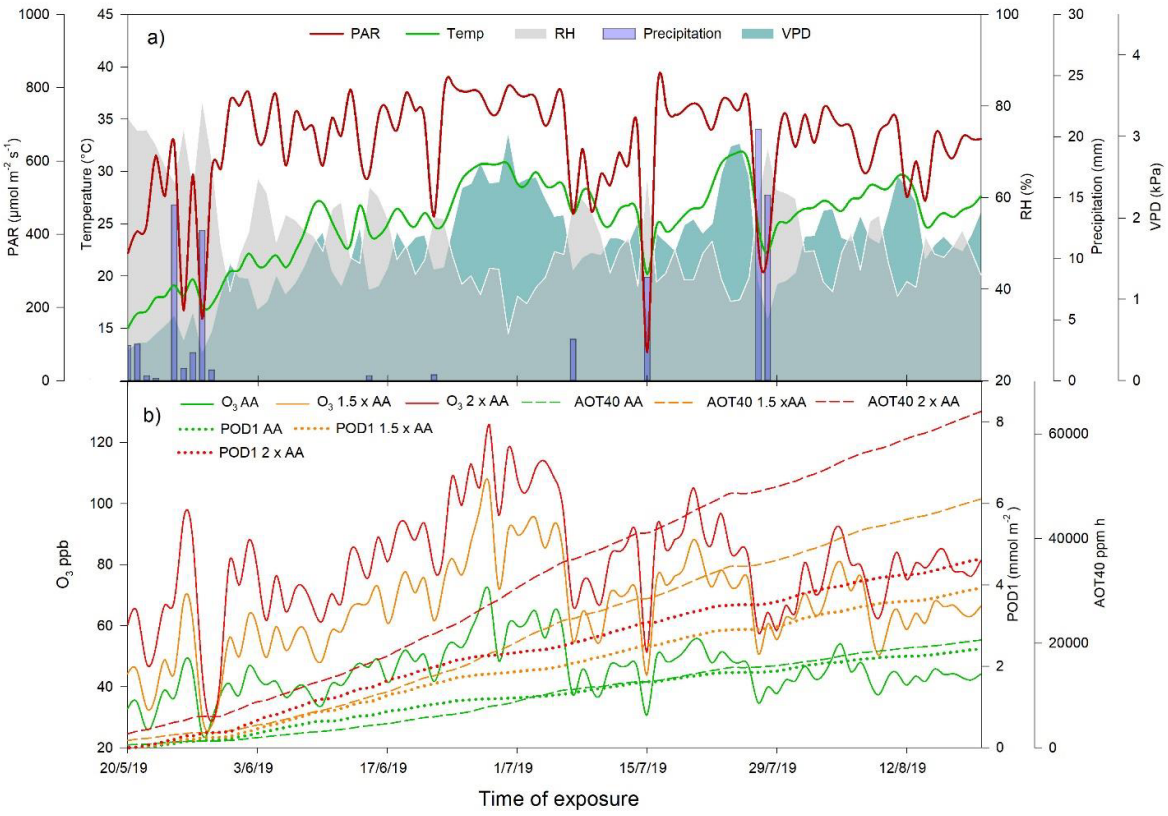
Fig. 7. Relationships between BVOC emission and net photosynthesis (a, isoprene; c, monoterpene), intercellular CO_2 concentration (b, isoprene; d, monoterpene) in date palm leaves grown under different O_3 concentrations (AA, ambient, 1.5 x AA, 2 x AA). Simple linear regression analyses: ** $p < 0.01$, * $p < 0.05$, ns denotes not significant.

Fig. 8 Above- (a) and below-ground (b) biomass of date palms on August 20th, i.e. at the end of 92 days of exposure to ambient ozone (AA), 1.5 x AA and 2 x AA. Different letters show significant differences among treatments (Tukey test, p<0.05, N = 3 plots).

Tab. 1. Mass per area (LMA), water content (LWC), total chlorophyll content (Chl_{TOT}), chlorophyll a/b ratio (Chl a/b), total carotenoid content (Car_{TOT}), β-carotene (β-car), lutein (Lut), xanthophyll cycle pigment content (VAZ) and de-epoxidation state (DEPS) in the leaves of date palm plants grown under three levels of O_3 concentration (AA, ambient O_3 concentration, 1.5 × AA, 2 × AA). Each value is the mean ± standard error (N = 3 plots). Asterisks show the significance of ANOVA: ** $p < 0.01$, * $p < 0.05$, ns denotes not significant. Different letters show significant differences among treatments (p<0.05, Tukey test).

Tab 2. Average (± standard error) of the daily profiles of net photosynthesis (*A*) and stomatal conductance (g_s) of date palm leaves on July 23rd and 30th 2019. Asterisks show the significance of two-way ANOVA: ** $p < 0.01$; * $p < 0.05$; ns, not significant. Different capital letters show results of a one-way ANOVA with daytime as a factor, while different lower-case letters show results of a one-way ANOVA with O_3 treatments (AA, ambient, 1.5 x AA, 2 x AA) within each daytime as a factor (Tukey test, p<0.05, N=3 plots).

Figures and Tables



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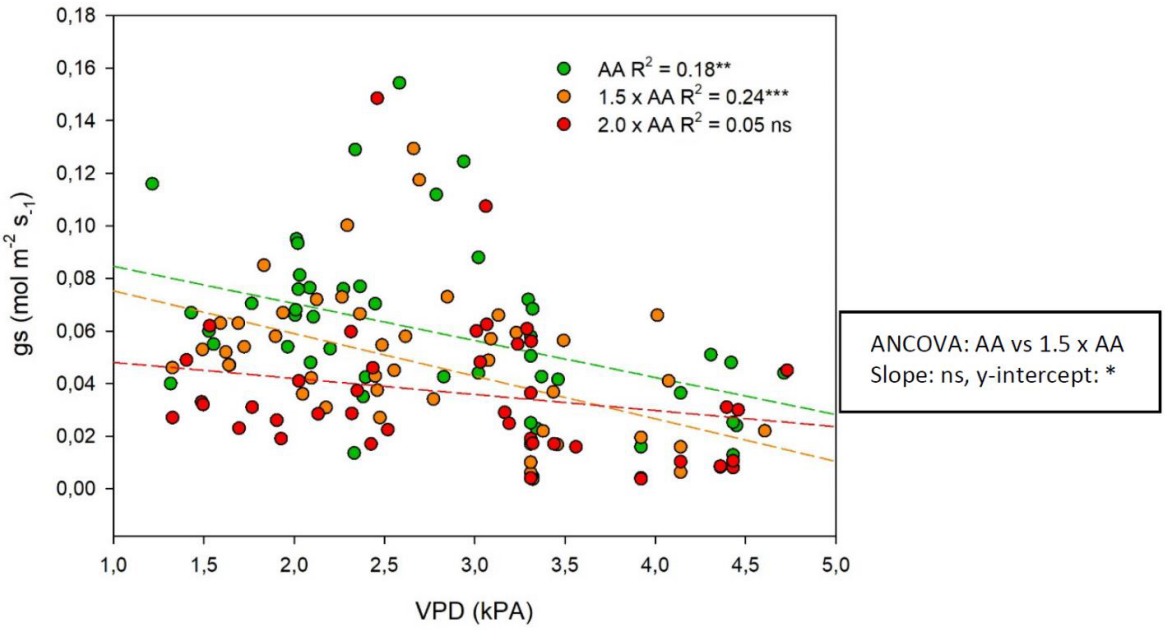

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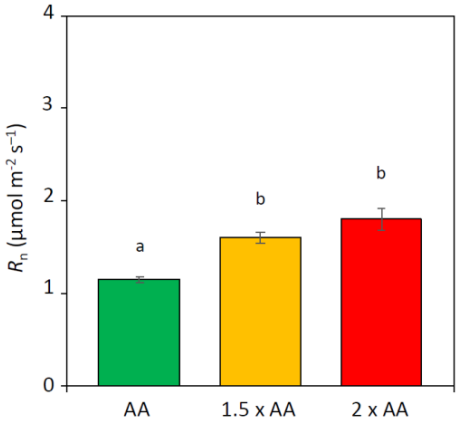

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932 **Fig. 3** Average (± standard error) of dark respiration rate (R_n) on July 30th. Different letters show significant

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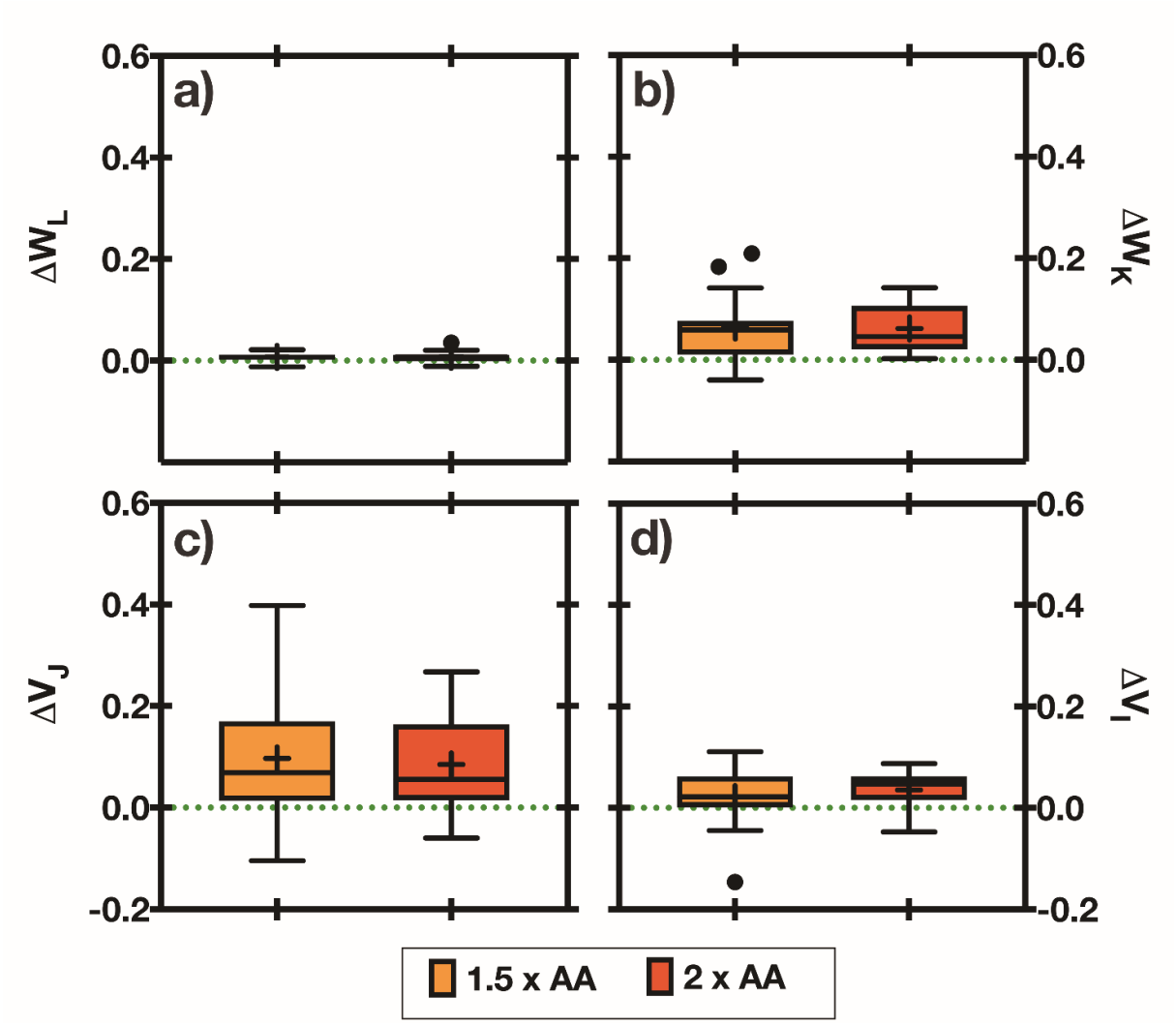


Fig. 4. Effect of different ozone levels on date palms, assessed with Chlorophyll-a fluorescence measurements *in vivo*. Boxplots show differences between elevated ozone levels compared to ambient ozone level (AA). Orange boxes, 1.5 x AA; red boxes, 2 x AA. Whiskers: 1,5 x IQR, dots: outliers, cross: average, line: median; green dotted line at y = 0: reference value at ambient ozone level; n = 3 plots. (a) ΔW_L difference of variable fluorescence ΔW_{OK}, at the L-Step at t = 150 µs. (b) difference of variable fluorescence ΔW_{OJ}, at the K-Step t = 300-600 µs. (c) difference of variable fluorescence ΔV, at the J-Step t = 2 ms. (d) difference of variable fluorescence ΔV, at the I-Step t = 30 ms.

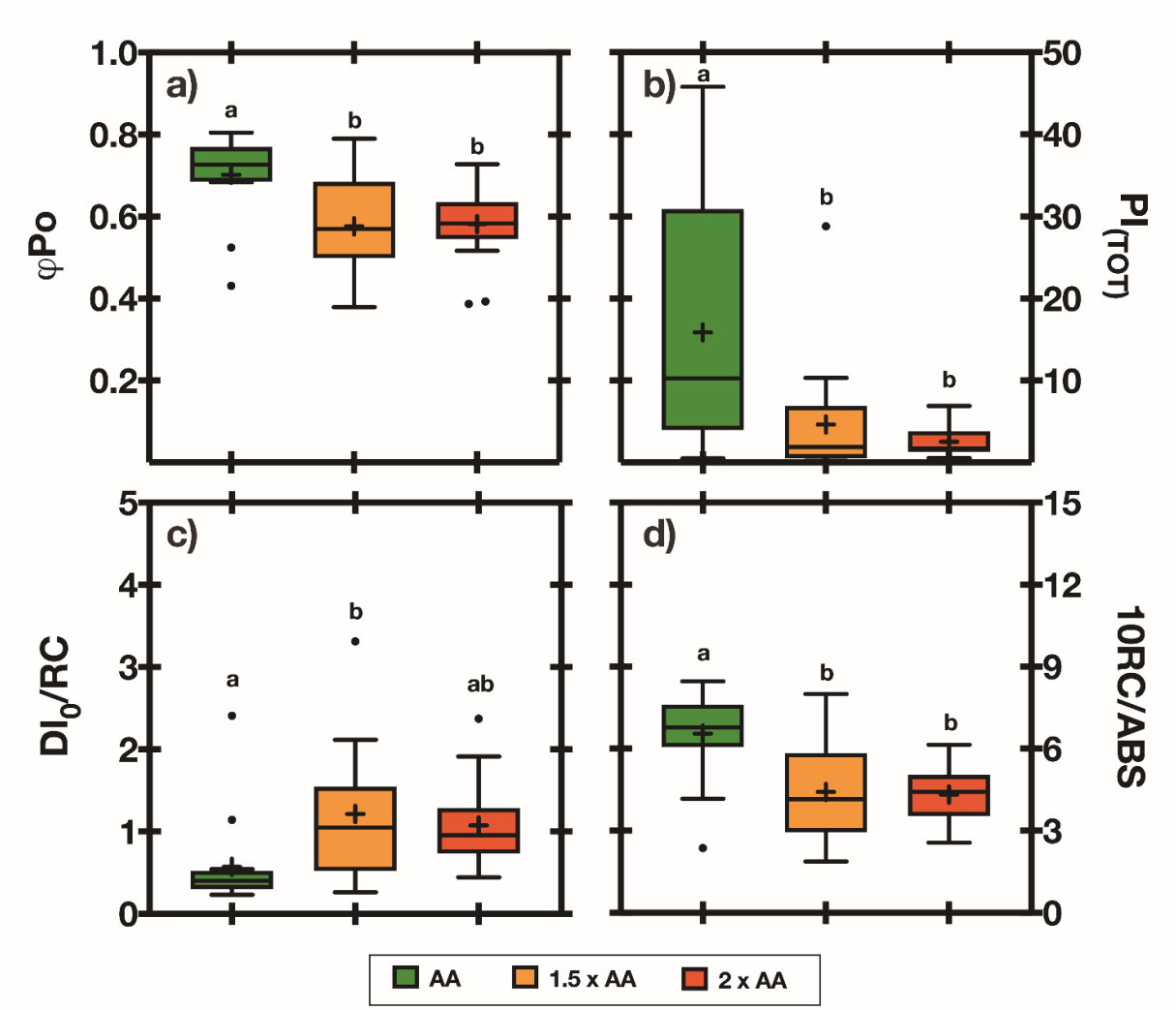


Fig. 5. Effect of different ozone levels on date palms on (a) maximum quantum yield for primary photochemistry φ_{Po} (b) the performance index PI_{TOT}, (c) energy dissipation per active reaction centres DI_0/RC, (d) reaction centres per absorption 10RC/ABS. Boxplots show: Green boxes, ambient ozone level (AA); orange boxes, 1.5 x AA; red boxes, 2 x AA. Whiskers: 1,5 x IQR, dots: outliers, cross: average, line: median. Different letters show significant differences among treatments (Tukey test, $p<0.05$, N = 3 plots).

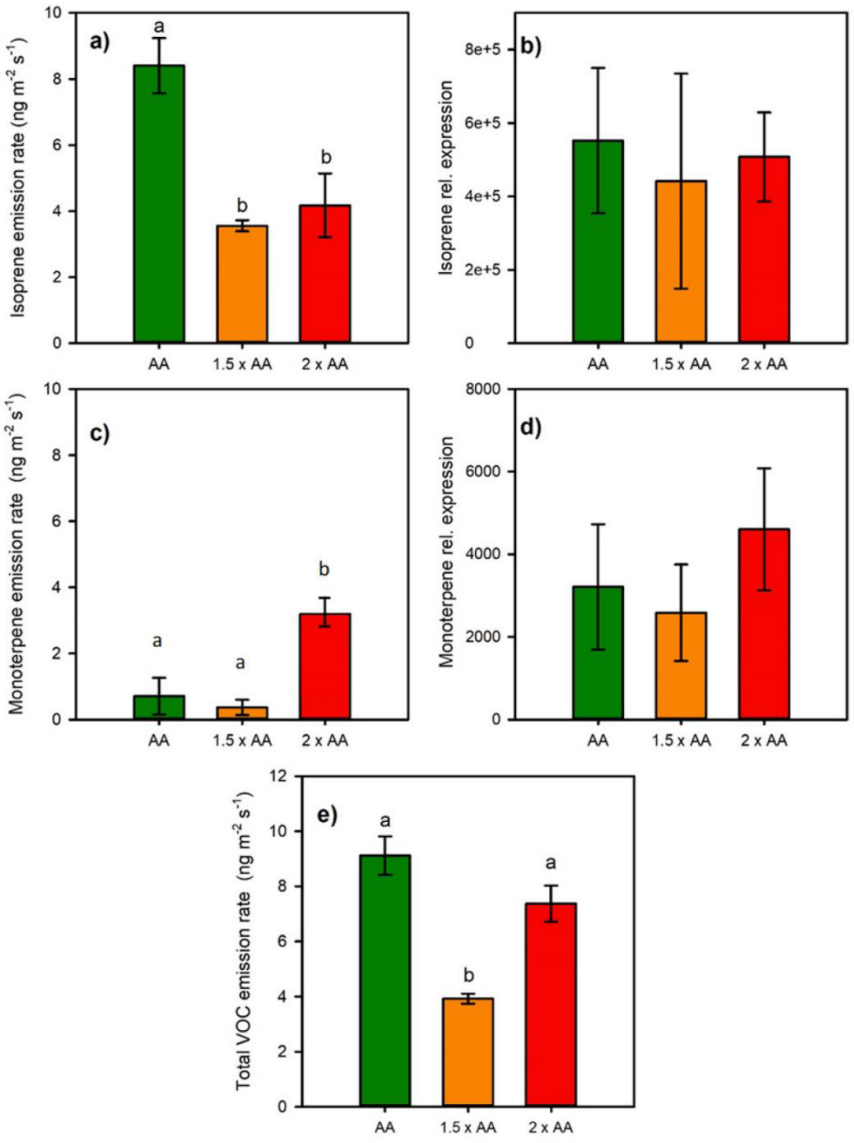


Fig. 6 Average (± standard error) of isoprene emission (a), relative expression of isoprene synthase transcripts (b), monoterpene emission (c), relative expression of monoterpene synthases (d) and total volatile organic compounds (VOC) emission (e) in date palm leaves on 1-2 August. Different letters show significant effects of the ozone treatments (AA, ambient, 1.5 x AA, 2 x AA) (Tukey test, $p<0.05$, N=3 plots).

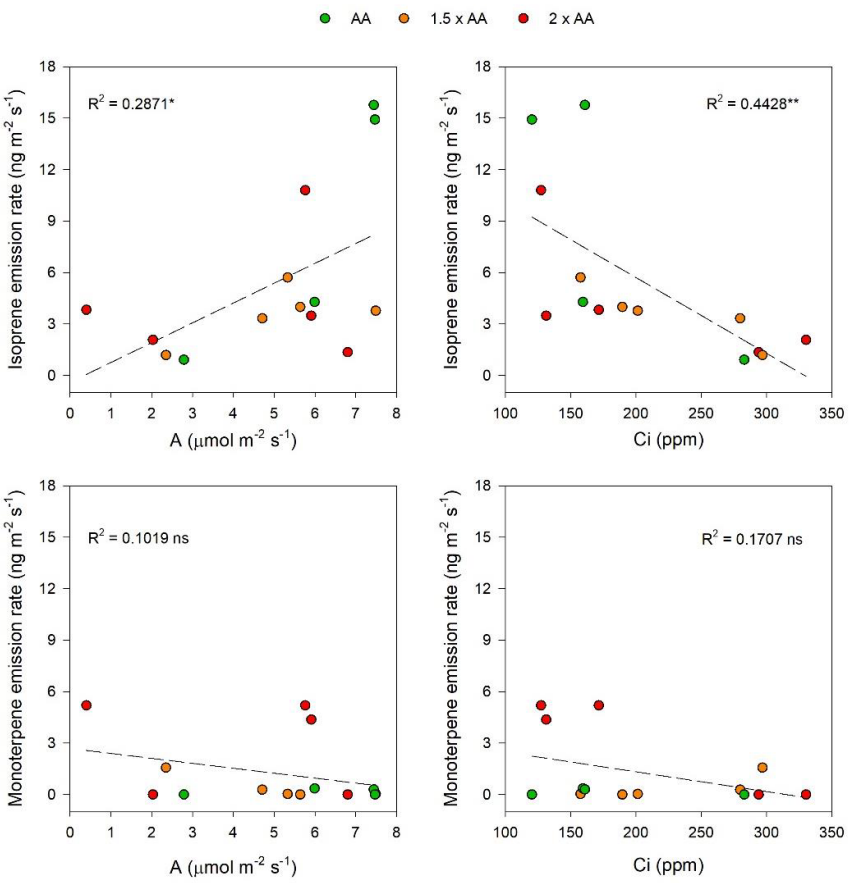


Fig. 7 Relationships between BVOC emission and net photosynthesis (a, isoprene; c, monoterpene), intercellular CO_2 concentration (b, isoprene; d, monoterpene) in date palm leaves grown under different O_3 concentrations (AA, ambient, 1.5 x AA, 2 x AA). Simple linear regression analyses: ** $p < 0.01$, * $p < 0.05$, ns denotes not significant.

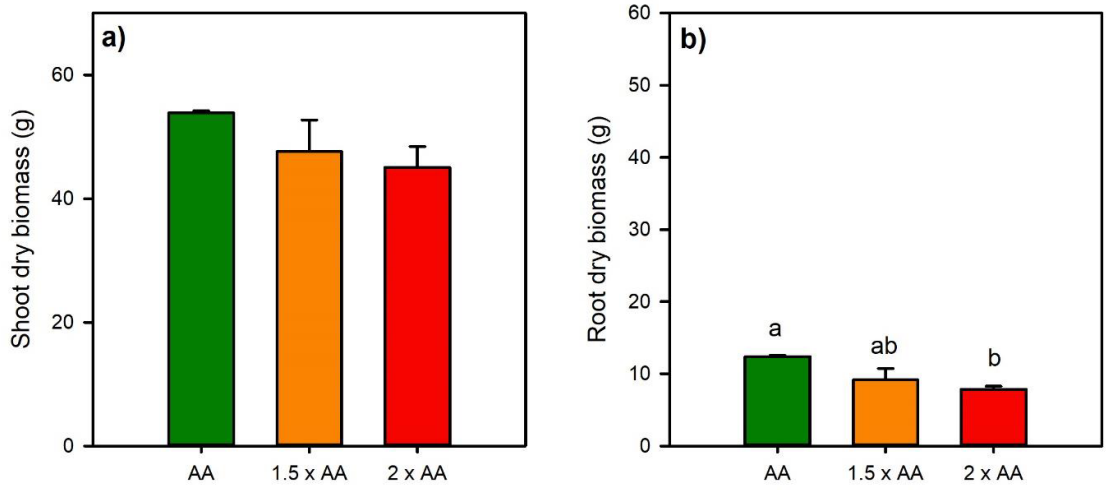


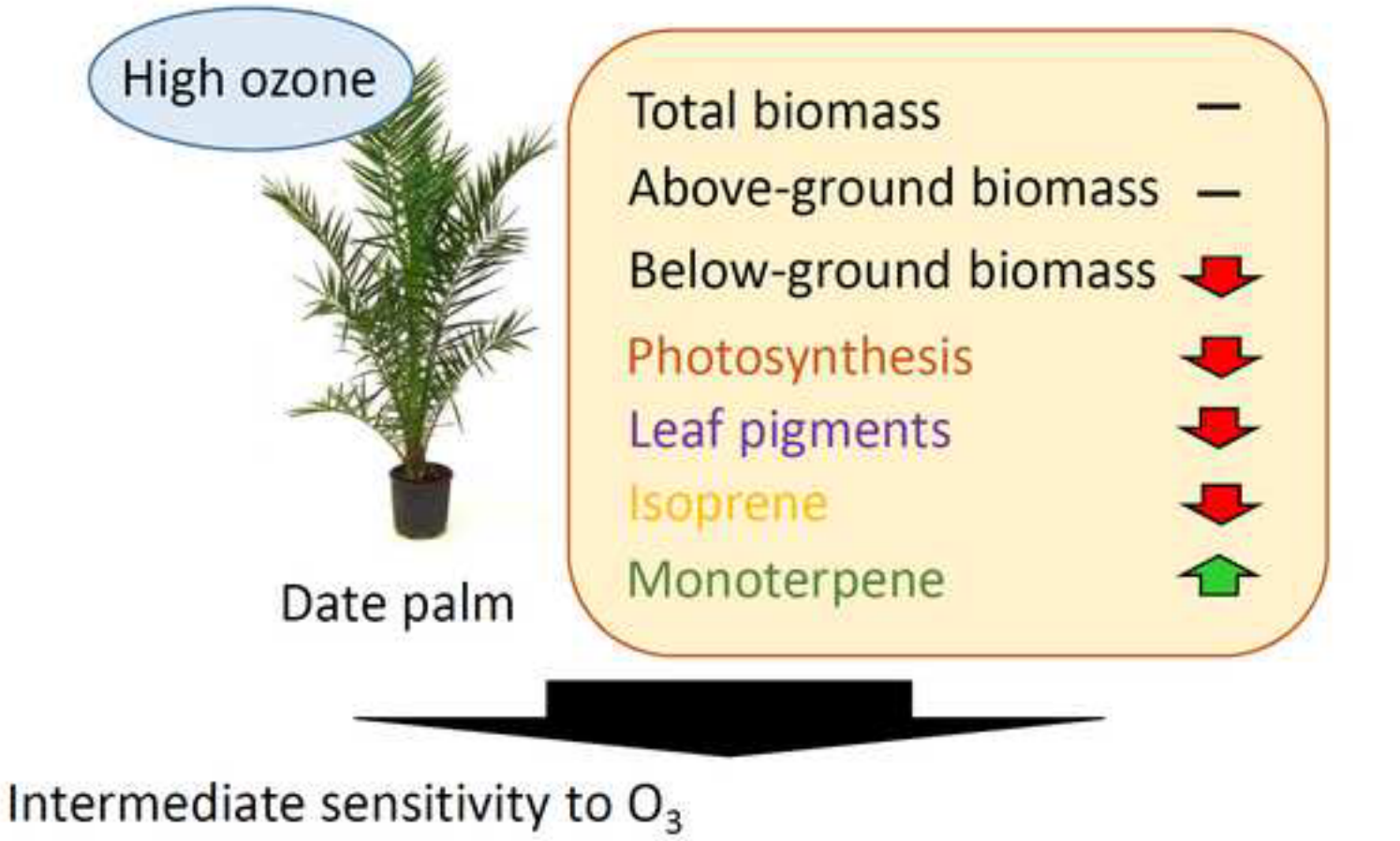
Fig. 8 Above- (a) and below-ground (b) biomass of date palms on August 20th, i.e. at the end of 92 days of exposure to ambient ozone (AA), 1.5 x AA and 2 x AA. Different letters show significant differences among treatments (Tukey test, $p<0.05$, N = 3 plots).

Tab. 1. Mass per area (LMA), water content (LWC), total chlorophyll content (Chl_{TOT}), chlorophyll a/b ratio (Chl a/b), total carotenoid content (Car_{TOT}), β-carotene (β-car), lutein (Lut), xanthophyll cycle pigment content (VAZ) and de-epoxidation state (DEPS) in the leaves of date palm plants grown under three levels of O_3 concentration (AA, ambient O_3 concentration, 1.5 × AA, 2 × AA). Each value is the mean ± standard error (N = 3 plots). Asterisks show the significance of ANOVA: ** $p < 0.01$, * $p < 0.05$, ns denotes not significant. Different letters show significant differences among treatments (p<0.05, Tukey test).

		LMA (g m^{-2})	LWC (%)	Chl_{TOT} (µmol g^{-1} FW)	Chl a/b	Car_{TOT} (µmol g^{-1} FW)	β -car (µmol g^{-1} FW)	Lut (µmol g^{-1} FW)	VAZ (µmol g^{-1} FW)	DEPS (%)
AA		133.4 ± 9.8 a	45.8 ± 2.8 a	2.7 ± 0.1 a	3.5 ± 0.2 a	136 ± 8 a	2.1 ± 0.3 a	0.99 ±0.02 ab	78 ± 3 a	46 ± 3 a
1.5 × AA		118.8 ± 3.3 a	52.2 ± 0.4 ab	2.1 ± 0.1 b	3.7 ± 0.1 a	92 ± 14 b	1.6 ± 0.1 a	0.82 ± 0.08 b	51 ± 9 b	52 ± 2 a
2 × AA		122.1 ± 4.8 a	54.5 ± 1.6 b	2.3 ± 0.3 ab	3.3 ± 0.1 a	114 ± 8 ab	1.8 ± 0.1 a	1.11 ± 0.09 a	60 ± 7 b	48 ± 7 a
ANOVA results (p-values)	*O_3 effect*	*0.329 ns*	***0.040 ****	***0.016 ****	*0.110 ns*	***0.006 *****	*0.078 ns*	***0.007 *****	***0.007 *****	*0.335 ns*

Tab. 2. Average (± standard error) of the daily profiles of net photosynthesis (*A*) and stomatal conductance (g_s) of date palm leaves on July 23rd and 30th 2019. Asterisks show the significance of two-way ANOVA: ** $p < 0.01$; * $p < 0.05$; ns, not significant. Different capital letters show results of a one-way ANOVA with daytime as a factor, while different lower-case letters show results of a one-way ANOVA with O_3 treatments (AA, ambient, 1.5 x AA, 2 x AA) within each daytime as a factor (Tukey test, p<0.05, N=3 plots).

			A (µmol m^{-2} s^{-1})		g_s (mol m^{-2} s^{-1})	
July 23rd	**Morning**					
	AA		6.3 ± 0.8 a		0.07 ± 0.01 a	
	1.5 × AA		5.0 ± 0.3 ab	A	0.06 ± 0.00 a	A
	2 × AA		3.1 ± 0.3 b		0.03 ± 0.00 b	
	Afternoon					
	AA		2.8 ± 0.4		0.04 ± 0.01	
	1.5 × AA		2.6 ± 1.0	B	0.04 ± 0.01	B
	2 × AA		2.6 ± 0.4		0.04 ± 0.01	
	ANOVA results (p-values)	*O_3:*	*0.048 **		*0.036 **	
		Time:	*0.001 ***		*0.041 **	
		O_3 x Time:	*0.081 ns*		*0.125 ns*	
July 30th	**Morning**					
	AA		7.4 ± 0.8 a		0.06 ± 0.01 a	
	1.5 × AA		5.1 ± 0.7 ab	A	0.05 ± 0.00 a	A
	2 × AA		3.8 ± 0.5 b		0.03 ± 0.00 b	
	Afternoon					
	AA		4.3 ± 1.2		0.04 ± 0.01	
	1.5 × AA		3.9 ± 0.9	B	0.04 ± 0.01	A
	2 × AA		2.7 ± 0.2		0.03 ± 0.00	
	ANOVA results (p-values)	*O_3:*	*0.022 **		*0.037 **	
		Time:	*0.016 **		*0.248 ns*	
		O_3 x Time:	*0.383 ns*		*0.206 ns*	



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Supplementary Material

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Conflict of interest

The authors declare that they have no conflicts of interest.

Declaration of interests

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