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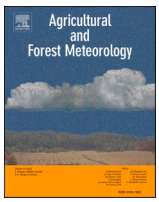

Acute ozone exposure decreases terpene emissions from Canary Island pines

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ABSTRACT

Biogenic volatile organic compounds (BVOCs) play an important role in ecological and atmospheric processes, including production of secondary air pollutants in urban areas. BVOC emission rates and composition are highly context-dependent and can change dramatically under conditions that elicit plant stress. This is particularly concerning given proposed expansion of urban forests where plants could regularly be exposed to spikes of atmospheric pollution stress, such as tropospheric ozone. However, few studies have investigated the effects of ozone exposure on plants commonly found in urban forests. This study characterized the effect of acute ozone exposure on BVOC emission rates in Canary Island pines (*Pinus canariensis*), which are often used in urban landscaping in Southern California. Pine saplings were exposed to four different ozone doses (control, 200 ppb, 300 ppb, 400 ppb) for 2 h. BVOC emission measurements were collected both pre-treatment and 24 h after exposure. Emission rates of total monoterpenes and several individual monoterpene and sesquiterpene compounds decreased when treated with 200 ppb of ozone. Total sesquiterpene emissions decreased significantly only after exposure to 300 ppb ozone, an effect that was largely driven by reduced emissions of the dominant sesquiterpene, α-farnesene. These findings indicate that landscaping with the pines used in this study would not exacerbate urban air quality degradation due to acute ozone stress. Future studies should investigate the effect of other major stressors and combinations of stressors for this and other common urban trees.

1. Introduction

Volatile organic compounds (VOCs) are molecules that easily vaporize at ambient temperature and pressure and are thus readily emitted to the atmosphere. Although atmospheric VOCs have both biogenic and anthropogenic sources, biogenic VOC (BVOC) emissions from terrestrial vegetation are of special interest because they are the largest source globally, exceeding anthropogenic sources by over an order of magnitude (Guenther et al., 1995). BVOCs released from vegetation influence both ecological and atmospheric processes. With regard to ecological function, BVOCs are used in plant defense against biotic and abiotic stressors via inter- and intra-species plant signaling, acting as antioxidants, and/or increasing thermal tolerance of plant cell membranes (Holopainen and Gershenzon, 2010; Loreto and Schnitzler, 2010; Peñuelas and Staudt, 2010; Unsicker et al., 2009). Their high reactivity contributes to important atmospheric chemistry processes that contribute to photochemical pollution, such as the generation or breakdown of tropospheric ozone (depending on atmospheric conditions), and the formation and growth of secondary organic aerosol (SOA) (Shilling et al., 2013; Wang et al., 2020). Previous studies have primarily focused on characterizing BVOC emissions and their atmospheric chemistry in forests, but there is new interest over the last decade in understanding BVOC emissions, transport, and atmospheric fate in urban settings (Calfapietra et al., 2013; Fitzky et al., 2019; Ma et al., 2019). Urban settings were historically dominated by anthropogenic VOCs, but regulation has successfully reduced anthropogenic sources to such an extent that biogenic sources could now drive secondary pollutant production (Churkina et al., 2017; Gu et al., 2021; Nussbaumer and Cohen, 2021). Furthermore, biogenic sources of VOCs in these areas are expected to increase with the rapid expansion of urban forests via implementation of greening programs in large cities. Therefore, we urgently need to improve understanding of the environmental controls on BVOC emissions in urban settings.

There are several major classes of BVOCs, including acetone, methanol, terpenoids, green leaf volatiles, and plant hormones, with terpenoids playing a particularly important role in atmospheric chemistry

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processes (Guenther, 2013). Terpenoids are compounds that generally have the formula $(C_5H_8)_n$, which includes isoprene (C_5H_8), monoterpenes (MT; $C_{10}H_{16}$), like limonene and α-pinene, and sesquiterpenes (SQT; $C_{15}H_{24}$), like farnesene and β-caryophyllene. BVOC emission rate and composition varies between different plant species, different genotypes of the same plant species, and are also influenced by biotic and abiotic environmental conditions, such as temperature, light, soil salinity, herbivory, pathogens, and pollution. One abiotic factor that can induce plant stress is the secondary pollutant, tropospheric ozone, which is formed via photochemical reactions in the atmosphere from VOCs and nitrogen oxides (NOx). Thus, BVOCs can contribute to photochemical production of ozone in urban areas and further exacerbate ozone-induced stress. Ozone is a criteria air pollutant regulated by the U. S. Environmental Protection Agency (EPA) primarily due to its deleterious impacts on human health, but ozone can also greatly reduce plant health and growth (Krupa et al., 2001). Currently, the 8-hour standard for ozone, set by the EPA in 2015, is 70 ppb. Climate change is increasing background ozone levels in polluted areas with a projected increase of 0.2–2 ppb per °C of warming (Masson-Delmotte et al., 2021). Along with chronic exposure to increasing ozone levels in a warmer climate, plants also experience acute ozone exposure in and downwind of urban environments during pollution episodes (Lei et al., 2012). In the Los Angeles basin, strict regulation of NOx and anthropogenic VOCs have drastically reduced the number of ozone exceedance days since the 1960s, but that reduction has hit a plateau in recent years and ozone still frequently exceeds the EPA 8-hour standard (EPA Air Data – Ozone Exceedances). For example, every year between 2010 and 2021 experienced at least 80 ozone exceedance days with five of those years exceeding the EPA standard more than 100 days. Furthermore, maximum hourly ozone typically ranges between 140 and 185 ppb currently and has ranged from 140 to 490 ppb in the Los Angeles basin between 1980 and 2020 (http://www.aqmd.gov/home/air-quality/historical-air-quality-data/historic-ozone-air-quality-trends). Thus, ozone-induced stress due to acute exposure in Los Angeles could be an important environmental control on BVOC emissions from urban forests that remains uncharacterized in emission inventories.

Both chronic and acute ozone exposure can induce plant stress and alter BVOC emissions and plant photosynthesis. Ozone mainly enters plants via the stomata, which can trigger oxidation processes that cause foliar damage (Fares et al., 2010). The most effective way for the plant to limit ozone damage is through stomatal closure, reducing stomatal conductance and assimilation rate (Kanagendran et al., 2018; Li et al., 2017). With regard to BVOC emissions, ozone exposure often increases emission rates of terpenes, but both positive and negative effects on emissions have been reported (Peñuelas and Staudt, 2010; Agathokleous et al., 2020; da Silva Pedrosa et al., 2020; Kanagendran et al., 2018). Some of the variation in reported effects of ozone exposure could be due to a dose-dependence of the plant stress response. This dose-dependent stress emission response has been demonstrated most clearly for biotic stress. For example, the degree of herbivory damage is associated with stress-induced emission rates of monoterpenes and green leaf volatiles (Copolovici et al., 2011; Faiola and Taipale, 2020; Kari et al., 2019; Niinemets et al., 2013; Yli-Pirilä et al., 2016). A dose-dependent effect on BVOC emissions has also been observed following thermal stress (Copolovici et al., 2012) and acute ozone exposure in tomato plants (Beauchamp et al., 2005), but it is a non-linear effect that sometimes exhibits a "threshold" effect. To our knowledge, no study has investigated a systematic dose-dependent response of BVOC emissions to acute ozone exposure in pines. This is an important gap because pines are commonly used in urban landscaping, placing them in an environment where they will likely be subjected to acute ozone pollution, and where their stress-induced BVOC emissions could further degrade urban air quality.

The effects of ozone stress on plant health and BVOC emissions have largely focused on agricultural crops. These studies were motivated by the significant economic losses associated with ozone damage, which reduces the yields and quality of harvested crops (Chuwah et al., 2015; Hong et al., 2020; McGrath et al., 2015; Mills et al., 2018; Mukherjee et al., 2021). Just a handful of studies have investigated the effect of ozone exposure on BVOC emissions from conifers, but the impact varies by species making it difficult to generalize results to species that have not been studied (Beyers et al., 1992). Scots pines, one of the most broadly distributed conifers globally, exhibit elevated emissions of several sesquiterpenes, monoterpenes, and other volatiles after chronic exposure of 40+ ppb ozone (Ghimire et al., 2017; Heiden et al., 1999; Kivimäenpää et al., 2016), suggesting there is a possibility that conifers in urban areas could exacerbate urban air quality under ozone stress. However, the effects of chronic and acute ozone exposure on conifers that are commonly planted in urban areas have not been studied previously.

The objective of this study was to investigate the dose-dependent effect of acute ozone exposure on BVOC emissions in Canary Island pines (*Pinus canariensis*). Canary Island pines are found throughout California and western United States and are common urban/suburban landscaping plants grown in Mediterranean climates, including the Los Angeles basin, where they are regularly exposed to ozone pollution episodes.

2. Materials and methods

2.1. Plant material

Pinus canariensis trees were obtained from Shadetree Nursery (Irvine, CA) in September 2019 and transported to the University of California, Irvine (UCI) greenhouse, where they were subsequently grown under ambient light and temperature conditions. The trees were donated to the nursery by a local developer in 3.8 liter pots when they were approximately 1.5–1.8 m in height and 3 years old. They were extra trees from a recent development project, and thus the trees were of relevant size given the motivating question of the study to evaluate trees used in urban landscaping. Each pine tree was kept in a 3.8 liter pot at the UCI greenhouse, watered at least weekly, and received no fertilizer supplements within the timeframe of this study. The greenhouse manager checked the soil moisture daily and would provide additional water if the soil was dry, which could happen during particularly warm weeks. At the time of the ozone treatment, all new shoots from the current growing season had fully matured into long needles and no new cones were present on the trees.

2.2. Experimental design

All measurements and stress exposure treatments were conducted in the UCI greenhouse. BVOC emissions were measured pre- and post-treatment from each pine tree. Four replicate pines were randomly selected for 2-hour acute ozone exposure at one of the following ozone levels: 0 ppb (control), 200 ppb, 300 ppb, and 400 ppb. Pre-treatment measurements were collected in August 2020, between 11:00 and 16:30 PST. Ozone treatments, and the subsequent sample collection, occurred in September 2020, between 10:30 and 17:00 PST. All measurements were collected at least one week before and 24 h after ozone treatment. BVOC measurements and acute ozone treatment occurred at the whole branch level using a dynamic branch enclosure system similar to that described in Ortega and Helmig (2008). Briefly, the enclosure was constructed around a single branch with a pre-cleaned (150 °C for 30 min) 55 × 60 cm PET oven bag (WRAPOK, China) and secured at the base with a cable tie. A HOBO U12 data logger (Onset, USA) was placed in each enclosure to continuously record air temperature and relative humidity. Inlet air was pumped and filtered through a desiccant dryer and charcoal trap (to scrub background VOCs and ozone) before entering the enclosure at a rate of 3 liters/min (Fig. 1). Enclosures were removed between cartridge sampling and ozone treatments to prevent additional stress on the plants. After each enclosure set-up, branches

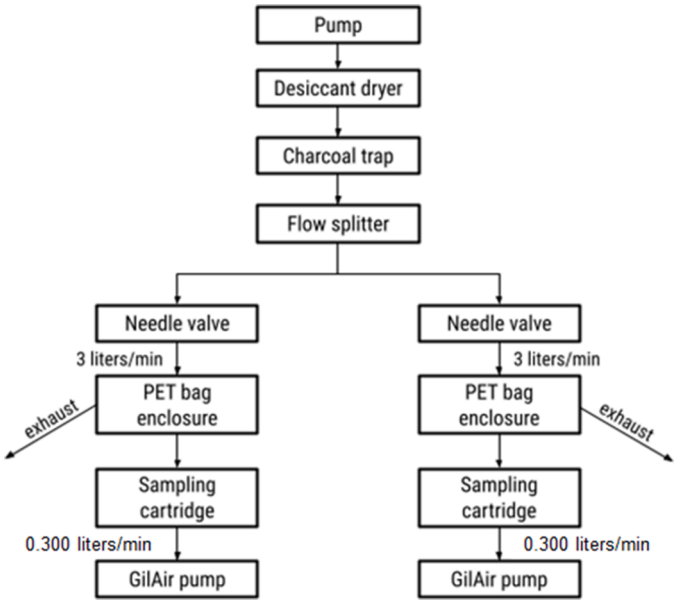

Fig. 1. The schematic of the BVOC sampling setup. The setup collected a sample from two trees concurrently to maximize the number of samples that could be collected in one day. "GilAir pump" refers to the sampling pump (Sensidyne, USA).

were allowed to acclimate to the environment for 60 min before any further sampling or treatment. Each branch enclosure contained needle growth across multiple years, representing an integrated average of needle ages. This is an important consideration since needle age influences stress response more than plant age (Grote et al., 2013), so this approach reduces potential variability by inadvertently sampling different aged needles on different plants. The branches selected for the enclosure placement on each tree were also located at similar positions near the bottom of the trees. This strategy was employed to minimize variability due to differential sampling of shade and sun needles from plant to plant, which can also influence BVOC emission rates and stress response (Esposito et al., 2016; Harley et al., 1999). A complete schedule of the BVOC sample collection and ozone treatments is summarized in Table S1.

2.3. BVOC sampling

BVOC samples were collected from the dynamic enclosure using a Gilian GilAir Plus pump (Sensidyne, USA), which pulled air from the enclosure through a multi-bed sampling cartridge containing Tenax TA and Carbograph adsorbents, at a rate of 0.300 liters per minute. The sampling lines were pre-conditioned for 50 min prior to cartridge installation and sample collection. Samples were collected for 20 min after the conditioning period ended. Excess air from the 3 liters/min being pumped into the enclosure (see Section 2.2) exited at the base of the enclosure (labeled "exhaust" in Fig. 1), meaning the enclosure was held under positive pressure throughout the sampling and conditioning interval to minimize possible contamination of room air entering the enclosure. Enclosures on two trees were installed simultaneously to allow us to collect more samples in a single day as shown in the figure. One branch was sampled from each tree and the average value from the 4 replicate trees was calculated for subsequent analysis. The sample cartridges were analyzed with a thermo-desorption gas chromatograph mass spectrometer (TD: Markes TD-100, GC: Agilent 7890,MS: Agilent 5973). Details regarding the TD-GC–MS settings are provided in the Supplementary Material (Section 1). Samples of empty PET bags with the same flow set-up were also collected and showed no background contamination of BVOCs, confirming that the cleaning process used for the enclosure bags adequately removed potential contaminants.

2.4. Ozone treatment

Ozone was introduced into the enclosure by pumping cleaned inlet air through a low-pressure mercury lamp with fused quartz (Jelight Company Inc., USA). The lamp emits UV light (185 nm) and generates ozone via photolysis of atmospheric oxygen (O_2). Exhaust air was pulled from the enclosure with a vacuum pump and filtered with a HEPA filter and a charcoal trap to remove ozone and prevent it from exhausting to the greenhouse room. The pines were treated with elevated ozone for two hours to simulate acute ozone exposure (200 ppb, 300 ppb, or 400 ppb). The flow rates and lamp settings required to establish each mixing ratio were determined using empty enclosures using an ozone monitor (Part # 106-M, 2B Technologies, Inc.). This approach was used because plants actively uptake ozone, and that uptake can vary resulting in variation in ozone mixing ratios in the enclosure even under the same ozone input. This approach ensured that each replicate was exposed to the same ozone mixing ratio regardless of how much ozone was being actively taken up by the plants during each treatment. Mixing ratios of 200–400 ppb fall within the range - 100–800 ppb for 1–6 h - seen in other acute ozone studies (Chen and Gallie, 2005; Puckette et al., 2008; Vanzo et al., 2014) and reflect the maximum hourly ozone observed in the Los Angeles basin between 1980 and 2020 (South Coast Air Quality Management District). Similar to the ozone-exposed groups, the control group was also treated in the same set-up for two hours, but with an inactive ozone-generating lamp. The control group was set at 0 ppb to act as a null exposure treatment, as is typical with studies that measure dose-dependent stress response.

2.5. BVOC analysis and emission rate calculations

The process used for BVOC compound identification and quantitation is detailed in the Supplementary Material (Section 2). Briefly, compounds were identified with the NIST 129 K library with a match value greater than 80%. Quantitation of the peak area was conducted using an external limonene standard cartridge that was run with each set of samples. Limonene was calibrated to the following terpene standards to generate relative response factors for each compound: α-pinene, 3-carene, camphene, β-pinene, β-myrcene, α-phellandrene, β-farnesene, β-caryophyllene, and valencene. For analytes where an authentic standard was not available, the analyte was assigned to one of the 10 standards based on similarity in structure (primarily based on cyclic structure being bicyclic, monocyclic or acyclic). Unidentified monoterpenes were assigned α-pinene as the proxy standard and unidentified sesquiterpenes were assigned β-caryophyllene as the proxy standard. Needle surface area in each enclosure was estimated following the approach described in Kivimäenpää et al. (2016). BVOC emission rate for each analyte was calculated using Eq. (1):

$$E = \frac{A * F}{RF_l * \ RRF_s * \ V_s * S} \quad (1)$$

Where E is emission rate in ng m^{-2} h^{-1}, A = integrated area from MS, F is enclosure flow rate in L h^{-1}, RF_l = response factor for limonene in area ng^{-1}, RRF_s = relative response factor for the terpene analyte authentic standard or proxy standard (this value was set to 1 for limonene, by definition), V_s = volume of enclosure air sample in L, and S = needle surface area in m^2. Emission rates were normalized for temperature using Eq. (2), which was adapted from Guenther et al. (1993) and Guenther et al. (2012):

$$B = \frac{E}{e^{\beta(T-303)}} \quad (2)$$

Where B is the basal emission rate in ng m^{-2} h^{-1}, E = emission rate in ng m^{-2} h^{-1}, β is the beta-coefficient = 0.1 for monoterpenes and 0.17 for

sesquiterpenes, and T = temperature in K.

2.6. Statistical analysis

Outliers for the basal emission rates of each volatile were identified with a two-tailed Dixon's Q test, using R package *outliers* (Komsta, 2011), and were not included in subsequent statistical analyses. The Tukey test was used to test pairwise comparisons of the means of the pre- and post-treatment emission rates of each treatment group, as well as comparisons of pre-treatment emission rates. Statistical tests were conducted with the R package *rstatix* (Kassambara, 2021). We employed principal component analysis (PCA), using *vegan* (Oksanen et al., 2020), *FactoMineR* (Lê et al., 2008), *factoextra* (Kassambara and Mundt, 2020), and *missMDA* (Josse and Husson, 2016), to compare and visualize the differences in the emission composition of all groups before and after ozone treatment, both separately and combined. Permutational multivariate analysis of variance (PERMANOVA) was conducted with *vegan* (Oksanen et al., 2020) to test for statistical significance of differences between emission profiles. All statistical analyses were visualized in R 4.0.0 (RStudio, 2020) using packages *ggpubr* (Kassambara, 2020) and *ggplot2* (Wickham et al., 2021).

3. Results

3.1. Composition of BVOC emissions

The BVOCs identified in the pine emissions were primarily monoterpenes (MTs) and sesquiterpenes (SQTs), which together comprised 98.0–99.6% of all measured emissions by mass (Fig. 2A). Although oxygenated monoterpenes (OMTs) were detected, their total emission rates were minimal compared to MTs and SQTs, representing only 0.3–0.9% in pre-treatment samples and 0.6–2.0% in post-treatment samples. A summary table of all terpene emission rates is provided in the supplementary information (Table S2). Of the monoterpenes emitted, six compounds made up 84.9% or more of the emissions (Fig. 2B). α-Pinene was the most abundant monoterpene contributing 50.7–73.4% and 61.0–78.6% in pre-treatment and post-treatment monoterpenes, respectively. The other dominant monoterpene emissions were β-pinene, ocimene, camphene, limonene and tricyclene (Fig. 2B and Table S2). Of the sesquiterpenes emitted, 89.8% or more of the emissions were made up of three compounds that were identified with strong NIST library matches (Fig. 2C). α-Farnesene was the most abundant sesquiterpene, with relative contributions to SQT emissions of more than 96.6% for all groups except 300 ppb post-treatment, where α-farnesene had a relative contribution of 85.8%. Other identified sesquiterpenes included δ-cadinene and β-caryophyllene. (Fig. 2C and Table S2).

A principal component analysis (PCA) was conducted to evaluate whether there were clear changes in the BVOC emission profile between groups. Qualitatively, there was more overlap between pre-treatment emission profiles than post-treatment emission profiles (Fig. 3). After ozone treatment, the emissions of pines exposed to 200 ppb of ozone were slightly separated from emissions of the control and 400 ppb groups along the first principal component (PC1) axis (Fig. 3B). The emissions of pines exposed to 300 ppb of ozone were slightly separated from the other treatment groups along the second principal component (PC2) (Fig. 3B). However, there was some overlap along both axes. A PCA plot comparing pre- and post-treatment profiles for each ozone dosage is included in the Supplementary Material (Fig S3). A quantitative comparison was conducted using PERMANOVA with results shown in Table 1. All pre-treatment profiles were similar ($P = 0.958$). Post-treatment profiles were less similar, but the separation was not statistically significant ($P = 0.104$). Of the pre- and post-treatment comparisons at each ozone dose, only the 300 ppb exposure resulted in a significantly different emission profile ($P < 0.05$).

3.2. Ozone effects on BVOC emission rates

The dose-dependent effect of acute ozone exposure on BVOC emission rates was evaluated by focusing on monoterpenes and sesquiterpenes since they comprised >98% of emissions by mass (Fig. 2). Total monoterpene emissions were moderately reduced after a 200 ppb ozone dose. (Fig. 4A; $p = 0.0709$). Total monoterpene emissions were not significantly different after any other ozone dose. The effects of acute ozone exposure on emissions of the major monoterpenes varied by compound and ozone exposure dose. Significant decreases in emission rate after 200 ppb ozone exposure were observed for camphene ($p = 0.0274$), limonene ($p = 0.00231$), tricyclene ($p = 0.0115$), and

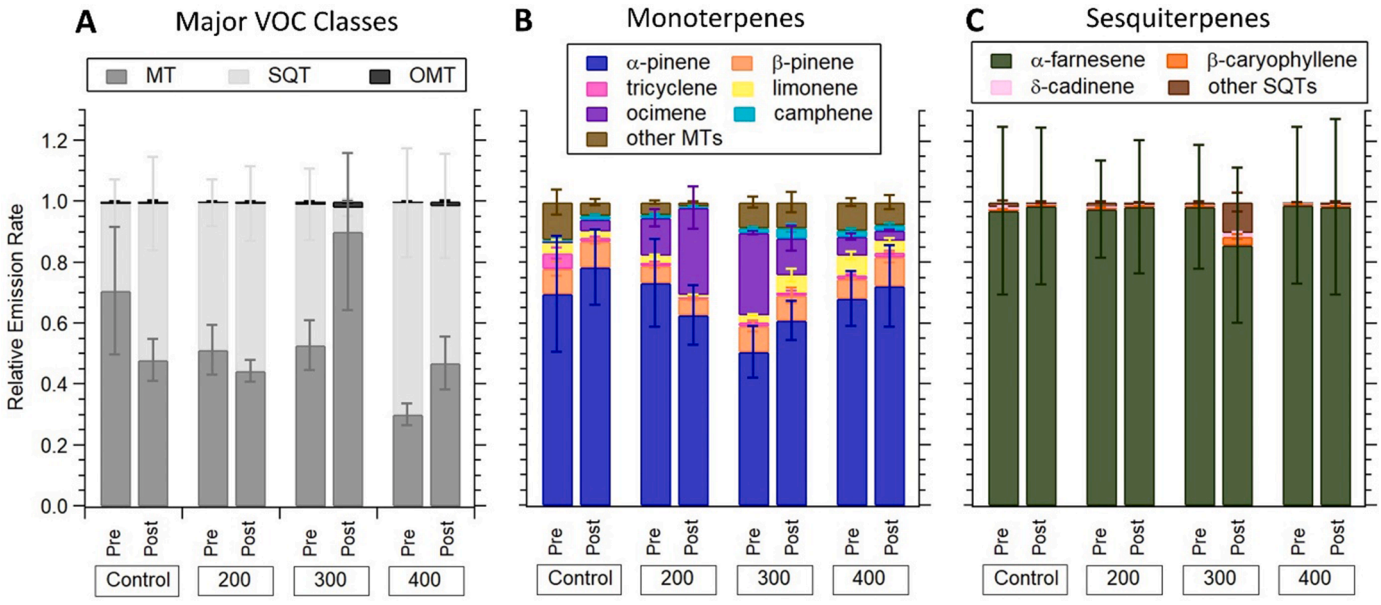

Fig. 2. Relative emission rates (normalized to a sum of 1) of (A) major BVOC classes, (B) major monoterpenes, and (C) major sesquiterpenes for P. canariensis before and after ozone treatment at ozone concentrations of 0 (control), 200, 300, and 400 ppb ($N = 4$ trees for each ozone dose). Error bars denote the standard deviation from the 4 replicates.

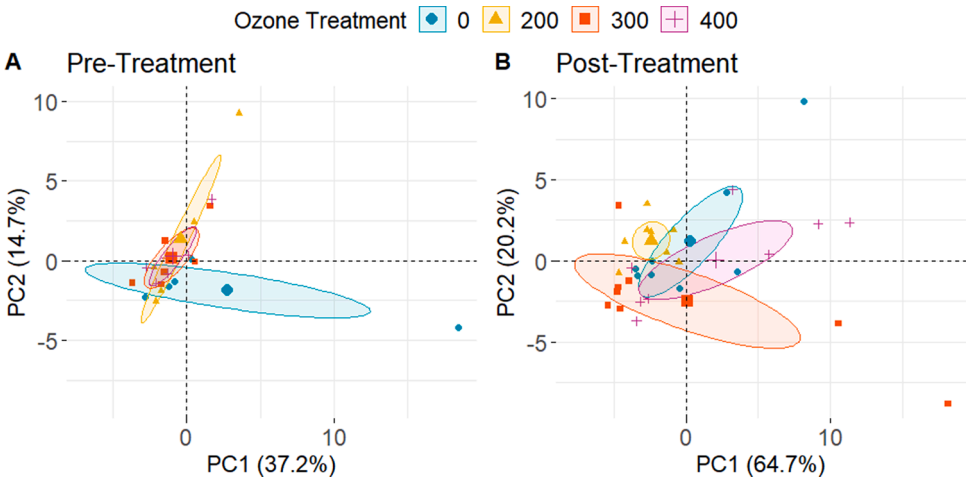

Fig. 3. Principal component analysis of the emission rates of all BVOCs emitted by P.canariensis (A) before and (B) after ozone treatment. Samples from the 0 ppb group are represented by blue circles, 200 ppb by yellow triangles, 300 ppb by red squares, and 400 ppb by purple crosses. The average value for each respective treatment group is represented by the larger shape in the middle of each group's 95% confidence ellipse ($N = 4$ trees for each ozone dose).

Table 1
Results from permutational multivariate analysis of variance (PERMANOVA). Degrees of freedom are indicated with "df" and '*' signifies a statistical significance with p-value 0.05 or lower.

Comparison	DF	Sum of squares	R^2	P value
all pre-treatment	3	0.160	0.053	0.958
all post-treatment	3	0.781	0.154	0.104
all pre- vs post-treatment	1	0.264	0.032	0.167
control, pre- vs post-treatment	1	0.062	0.035	0.764
200 ppb, pre- vs post-treatment	1	0.008	0.005	0.955
300 ppb, pre- vs post-treatment	1	0.481	0.200	0.042*
400 ppb, pre- vs post-treatment	1	0.006	0.003	0.954

unidentified monoterpene, UMT10 ($p = 0.00173$), while moderately significant decreases were observed for β-pinene ($p = 0.0614$) and UMT5 ($p = 0.0552$) (Fig. 4C-E and Table S2). In contrast, ocimene emission rates decreased after 300 ppb ozone exposure (Fig. 4F and Table S2, $p = 0.00606$) and UMT10 emission rates moderately decreased after 400 ppb ozone exposure (Table S2, $p = 0.09370$). No significant differences at any ozone dose were observed for the biggest contributor to MT emissions: α-pinene, which has an average emission rate approximately 5.7–45.7 times higher than that of the other major monoterpenes (Fig. 5B and Table S2). There were no significant differences in pre-treatment emission rates of all monoterpenes for all groups, except for ocimene emission rates of the 300 ppb group (Fig. S1, $p = 0.0136$ between 0 ppb and 300 ppb, $p = 0.00777$ between 200 ppb and 300 ppb, $p = 0.00132$ between 400 ppb and 300 ppb).

Like monoterpenes, the emission rates of sesquiterpenes were higher prior to ozone exposure than after exposure, but this suppression effect was observed at a different ozone dose than for monoterpenes. A significant decline in total sesquiterpene emission rates was observed only after treatment with 300 ppb of ozone (Fig. 5A; $p = 0.0215$). This effect was primarily driven by the decline in α-farnesene emissions (Fig. 5C and Table S2; $p = 0.0213$) because α-farnesene was the dominant contributor to total sesquiterpene emissions, with an average emission rate approximately 290 and 610 times higher than that of δ-cadinene and β-caryophyllene, respectively (Fig. 2C). Most other individual

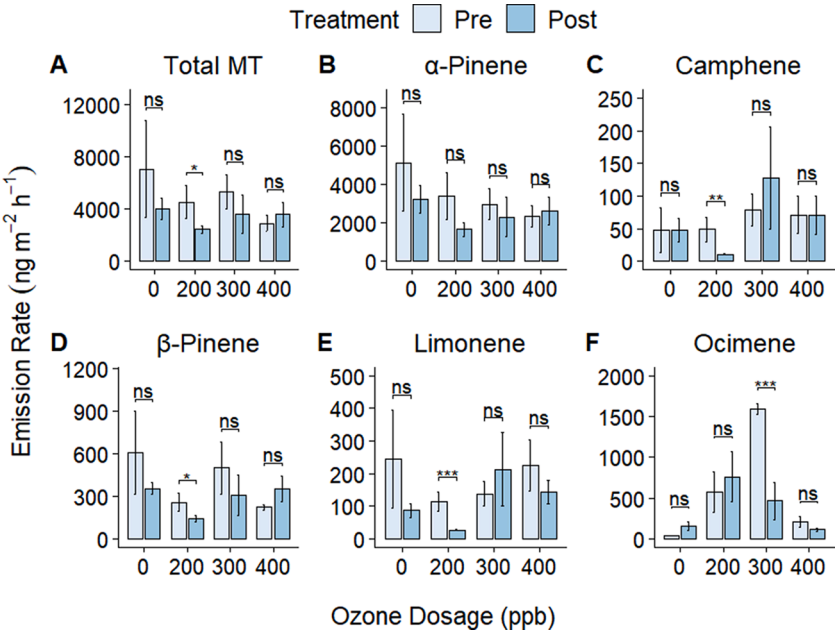

Fig. 4. Average basal emission rates (ng m^{-2} h^{-1}), normalized to 30 °C, of (A) total monoterpenes and individual monoterpenes: (B) α-pinene, (C) camphene, (D) β-pinene, (E) limonene, and (F) ocimene of P. canariensis before and after acute ozone exposure treatment, with a standard error bar ($N = 4$ trees for each ozone dose). Statistical differences in emission rates between pre- and post-treatment at a significance level of $p < 0.1$ is indicated by '*', $p < 0.05$ by '**', and $p < 0.01$ by '***'. 'ns' signifies no significant differences. An authentic standard was used for all shown monoterpenes, except for ocimene, which required a proxy standard, β-myrcene.

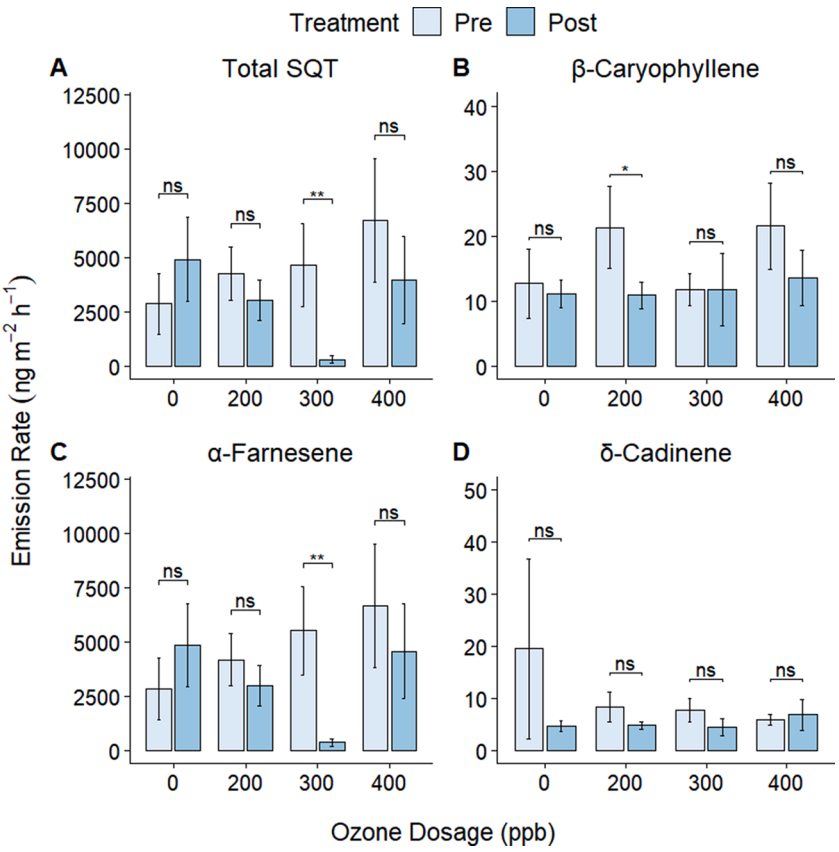

Fig. 5. Average basal emission rates (ng m^{-2} h^{-1}), normalized to 30 °C, of (A) total sesquiterpenes and individual sesquiterpenes: (B) β-caryophyllene, (C) α-farnesene, and (D) δ-cadinene of P. canariensis before and after acute ozone exposure with a standard error bar ($N = 4$ trees for each ozone dose). Statistical differences in emission rates between pre- and post-treatment at a significance level of $p < 0.1$ is indicated by '*' and $p < 0.05$ by '**'. 'ns' signifies no significant differences. An authentic standard was used for all shown sesquiterpenes, except for δ-cadinene, which required a proxy standard, β-caryophyllene.

sesquiterpene compounds that were affected by acute ozone exposure showed a moderate decrease at the 200 ppb dose, similar to most monoterpenes. For example, β-caryophyllene ($p = 0.0869$), UST12 ($p = 0.0826$), UST13 ($p = 0.0786$), UST14 ($p = 0.0764$), and UST 16 ($p = 0.0799$) all decreased after 200 ppb ozone exposure (Fig. 5B and Table S2). No significant differences at any ozone dose were observed for δ-cadinene (Fig. 5D and Table S2). There were no significant differences in pre-treatment emission rates of all sesquiterpenes for all groups (Fig. S2).

4. Discussion

4.1. Ozone effects on gas exchange characteristics and implications for urban air quality

BVOC emission rates from Canary Island Pines decreased significantly after 2 h of 200 ppb or 300 ppb ozone exposure. This result contrasts with many other published studies that show an increase in emission rates following acute ozone exposure in *Nicotiana tabacum* (Heiden et al., 1999; Kanagendran et al., 2018) and chronic ozone exposure in *P. sylvestris* (Ghimire et al., 2017; Heiden et al., 1999; Kivimäenpää et al., 2016). The difference could be due to variation in ozone sensitivity between plants and expected differences between acute versus chronic exposure. However, herbaceous crops (such as *N. tabacum*) and woody trees (such as the pines used in this study) will likely have very different responses to ozone exposure. Plants in the *Pinaceae* family, including conifers like *P. sylvestris* and *P. canariensis*, are generally less responsive to ozone than other woody plants and *N. tabacum* (Agathokleous et al., 2020; Bergmann et al., 2017; Felzer et al., 2007). In particular, Heiden et al. (1999) found that the effects of ozone exposure on BVOC emission rates was smaller in *P. sylvestris* than in *N. tabacum,* although this was a chronic exposure study so it is difficult to compare with our results. Furthermore, Then et al. (2009) reported that *P. canariensis* (the same plant species that was the subject of the current study) did not display significant stress after exposure to elevated ozone (double ambient concentration), while *P. sylvestris* has shown strong stress responses to ozone exposure and is commonly considered a particularly ozone-sensitive conifer species (Bergmann et al., 2017; Huttunen and Manninen, 2013). Thus, our results are consistent with those suggesting that *P. canariensis* has high ozone resistance. An alternate explanation of our results could be related to temporal dependence of the stress response. For example, monoterpene and sesquiterpene emission rates from *N. tabacum* immediately increased after acute ozone exposure, but the emissions did not stay elevated for long (Kanagendran et al., 2018). In that case, the emission rates gradually decreased over the plants' recovery period, and 48 h after treatment, monoterpene emission rates across all ozone fumigation levels were significantly lower in ozone-treated plants than in the control. A similar process could be occurring in *P. canariensis,* and their recovery period could be within the interval between treatment and sampling in this study (e.g. 24 h) due to the lower sensitivity to ozone of conifers in comparison to herbaceous crops. There were no systematic differences in plant stomatal conductance or carbon assimilation between different treatment groups as measured with a Licor 6800 Portable Photosynthesis System (Fig S4) that can explain the decrease in BVOC emissions. However, the measurements were collected under ambient light conditions which varies even between different needles on the same tree, leading to high variance in the measurements within and between treatment groups. Despite the large spread in the data, it is clear that all plants were still photosynthesizing and none of them were stressed to a degree that shut down carbon assimilation.

The composition of BVOC emissions significantly changed only at the 300 ppb dose. This was driven largely by a decrease in relative contribution from sesquiterpenes. Changes in composition can affect atmospheric chemistry processes that can influence urban air quality. For example, an increased proportion of terpenes with an acyclic chemical

structure is associated with reduced formation of atmospheric particles (Faiola et al., 2019; Khalaj et al., 2021). In this case, α-farnesene was the major sesquiterpene affected by the ozone exposure. α-Farnesene is an acyclic sesquiterpene with lower propensity to form atmospheric particles than α-pinene (Ylisirniö et al., 2020). This shift in composition (reduced α-farnesene and increased α-pinene) would be expected to increase the yields of atmospheric particles, but that increase would be offset by the overall reduction in total emissions. The influence on ozone formation is more complicated since BVOCs can both deplete or lead to the formation of ozone, depending on environmental conditions. One way to evaluate the effect of shifting BVOC composition on ozone formation potential is to estimate the maximum incremental reactivity (MIR) - a parameter developed to rank the photochemical ozone formation reactivities of different VOCs (Carter, 1994). In general, sesquiterpenes have lower MIR values than monoterpenes (SAPRC Chemical Mechanism; https://intra.engr.ucr.edu/~carter/SAPRC/). This means a shift to more monoterpenes would increase the ozone formation potential of the emissions, but again, this would be offset by the overall reduction in emissions. Based on these results, we would not expect the shift in composition at 300 ppb ozone exposure to substantially influence secondary pollutant generation in urban environments. Follow-up studies should integrate these findings into a chemical transport model to evaluate the overall effects of reduced emissions and shifts in composition, but that is outside the scope of this study. We note that we do not have a measurement of active ozone uptake by the plants, and it is possible that plants responded to the acute ozone exposure by closing stomata in the short-term, thus limiting their ozone uptake. It is also possible that the degree to which plants limited their active ozone uptake differed depending on the dosage. However, even if this is the case, that would merely indicate a mechanism of resistance to the ozone stress. This does not prevent us from concluding that some BVOC emissions decreased after acute ozone exposure at the 200 ppb and 300 ppb levels.

4.2. Ozone stress response did not scale with ozone dose

There was no clear dose-dependent relationship between ozone mixing ratio and plant response (Fig. 2, 5-6). It is possible that ozone uptake by the plant did not scale directly with the ozone mixing ratio in the enclosure. At very high ozone concentrations, plants may close their stomata as a defense mechanism, so the actual ozone uptake might be lower at higher ozone mixing ratios in the enclosure (Emberson et al., 2000; Pleijel et al., 2004). BVOC emission rates from other plant types have generally exhibited a positive dose-dependent relationship when ozone uptake/deposition was used as the quantitative metric for the "treatment dose" (Beauchamp et al., 2005; Kanagendran et al., 2018). Furthermore, Kanagendren et al. (2018) saw the highest monoterpene and sesquiterpene emission rates immediately after exposure at the intermediate, rather than the maximum, ozone exposure concentrations. Future studies should measure the ozone uptake by the plant, in addition to ozone mixing ratio in the exposure chamber, to develop improved parameterizations of the ozone-BVOC stress response.

5. Conclusion

Plant stress caused by acute and chronic ozone exposure can alter BVOC emission rates and change the types of compounds emitted. This is concerning, particularly for urban forests, because BVOC chemistry in the presence of urban nitrogen oxides can lead to the formation of tropospheric ozone, potentially forming a positive feedback loop between BVOC emissions and ozone pollution. However, different plants have drastically different responses to plant stressors, so it is possible to target species for urban greening programs that will be less sensitive to the stressors typically found in urban environments. We studied the acute ozone stress response from Canary Island Pines, which are commonly used in urban landscaping in Southern California. We found that 24 h after acute ozone exposure, emission rates of monoterpenes and sesquiterpenes were either the same or lower than before exposure. Our findings suggest that *P. canariensis* is either resistant to, or quickly recovers from, acute ozone stress. As ozone concentrations and peak ozone levels are expected to rise, *P. canariensis* would be unlikely to exacerbate urban air quality issues due to acute ozone exposure, at least under the conditions studied here, potentially making it a good candidate for urban greening. We acknowledge there are other potential stressors and combinations of stressors that we have not considered in this study that could also influence emissions in an urban environment. We also note there are many factors to consider when selecting trees for urban greening programs, including considerations related to desirable ecosystem services such as reducing the urban heat island effect and supporting local insect and bird populations. We hope that future decisions will include consideration of plant emissions, particularly under the types of stress conditions typical of urban areas. With the rapid expansion of urban forests through greening programs, more studies need to be conducted to ensure the most appropriate plant species are being targeted for these programs and maximize the benefits associated with urban green spaces. The ideal plant species to target from an air quality perspective include low BVOC emitters that are resistant to pollution stress. Future work should target BVOC measurements from real urban plants located along a natural ozone gradient to complement the greenhouse study results reported here. Furthermore, regional chemical transport models should incorporate improved biogenic emission inventories in urban areas, including plant stress emissions, to better evaluate the impact of urban greening programs on urban air quality.

Author contributions

TV and CF designed the experiment and wrote and revised the manuscript. TV conducted the experiments and analyzed the data under the guidance of CF.

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Declaration of Competing Interest

The authors have no competing interests to declare.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2023.109416.

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