Do future climate conditions change volatile organic compound emissions from Artemisia annua? Elevated CO 2 and temperature modulate actual VOC emission rate but not its emission capacity

Juliane Daussy, Michael Staudt

HAL Id: hal-03019778

https://hal.science/hal-03019778

Submitted on 23 Nov 2020

Contents lists available at ScienceDirect

Atmospheric Environment: X

Do future climate conditions change volatile organic compound emissions from *Artemisia annua*? Elevated CO_2 and temperature modulate actual VOC emission rate but not its emission capacity

Juliane Daussy, Michael Staudt [*]

Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, Univ Montpellier, Univ Paul Valéry Montpellier 3, EPHE, IRD, 1919 Route de Mende, 34293, Montpellier, France

A B S T R A C T

Increasing temperature and atmospheric $[CO_2]$ can change plant VOC emissions, thereby providing a possible feedback to climate change by altering particle and greenhouse gas formation. While evidence gathered on isoprene emission generally indicated an inhibitory effect of elevated CO_2 on emission at moderate temperature, it is unclear whether the emissions of other volatiles respond in a similar way. In a greenhouse study, we grew *Artemisia annua* plants under two $[CO_2]$ regimes (400/800 ppm) combined with two temperature regimes (25/15 and 30/20 °C) and determined emissions at two assay $[CO_2]$ (400/800 ppm) combined with two assay temperatures (30/37 °C). Foliar VOC concentrations were unaffected by growth conditions and resembled emissions consisting mainly of monoterpenes (>92%) and sesquiterpenes (>7%). Mean emission rates determined at 30 °C and 400 ppm $[CO_2]$ ranged between 1.2 and 2.5 $\mu g\ g^{-1}h^{-1}$ (11–26 ng $m^{-2}s^{-1}$) with lowest values observed for elevated-$[CO_2]$-grown plants. However, this apparent negative effect of growth $[CO_2]$ was compensated by increased plant growth. Irrespective of growth conditions, doubling assay $[CO_2]$ significantly reduced emissions at 37 °C by about 30% and had no effect at 30 °C. Emissions always increased on the increase in assay temperature, with monoterpenes responding more strongly than sesquiterpenes (mean %-increase of 17.5 and 12.5 °C^{-1}). Our results suggest that the future evolution of VOC release from *A. annua* depends mainly on the antagonistic emission responses to temperature and $[CO_2]$, while long-term acclimations play a minor role.

1. Introduction

Terrestrial vegetation releases into the atmosphere a large variety of organic molecules commonly called Biogenic Volatile Organic Compounds (BVOC). BVOCs differ in size and physicochemical properties but have all in common a relative high vapor pressure at ambient temperature and a low boiling temperature (Kesselmeier and Staudt, 1999). Hence all BVOCs potentially evaporate out of the plant tissues and spread in the surrounding environment, where they may encounter other organisms. Many BVOCs are known to mediate interactions between plants and other organisms: they are involved in plant reproduction by attracting pollinators (Wright and Schiestl, 2009), have defense functions against pathogens and herbivores directly through their antimicrobial and repellent properties (Dudareva et al., 2013) or indirectly by attracting enemies of herbivores (Heil, 2008). Furthermore, certain compounds may confer protection against abiotic stresses and/or serve as "airborne phytohormones" signaling stress between the organs of a same plant, and between plants of a same or different species (Kessler et al., 2006).

However, once in the atmosphere BVOCs become part of the chemical processes that regulate several radiative forcing components and hence the earth's climate. Notably, they alter greenhouse gas concentrations by influencing the formation of ozone and the lifetime of methane in the troposphere and modify the optical properties of the atmosphere by favoring the formation and growth of secondary organic aerosols and cloud condensation nuclei (Sartelet et al., 2012; Ziemann and Atkinson, 2012). In particular larger molecules such as monoterpenes (C10) and sesquiterpenes (C15) are efficient precursors for aerosol formation over rural, forested areas, while smaller BVOCs such as isoprene (C5) - which is globally the most emitted BVOC - has rather a modest productivity of particles (Jokinen et al., 2015; Zhang et al., 2018; McFiggans et al., 2019). The emissions of these terpenes are therefore potential actors to be considered in global climate change research, because climate change factors may affect their fluxes, which

in turn could feedback on climate evolution (e.g. Harper and Unger, 2018; Sporre et al., 2019).

Global climate change is characterized by an increase in mean temperature and the concentrations of greenhouse gases such as carbon dioxide, methane and ozone but also by the increase in the frequency and intensity of precipitation, drought and extreme weather events in general. Temperature and CO_2 concentration have been continuously rising since the end of the 19th century. Climate models predict that mean global surface temperature further rises up to 2–6 °C and mean global $[CO_2]$ to 800 ppm by 2100 with no or relative moderate regional differences compared to other climate factors such as precipitation (Pachauri et al., 2014). Therefore and because both factors are key drivers of the plant's metabolism, rising temperature and $[CO_2]$ may substantially change the BVOC fluxes from all vegetation cover over the world (Kulmala et al., 2004; Peñuelas and Staudt, 2010). However, estimates of BVOC fluxes at large scales and how they evolve in a warmer CO_2-enriched world are associated with large uncertainties (Jiang et al., 2019).

Studies that investigate and model the influence of environmental factors on isoprenoid emissions typically dissociate between short-term and long-term effects (Grote and Niinemets., 2008; Niinemets et al., 2010; Guenther et al., 2012; Monson et al., 2012). Short-term effects imply fast changes of emission in response to changes of environmental drivers that come rapidly to a new steady state, usually within tenths of minutes (e.g. Monson et al., 1991; Staudt et al., 2003). By contrast long-term acclimation effects describe slow changes in the steady state emission rate at standard environmental conditions (30 °C temperature, 1000 μmol m^{-2} s^{-1} incident photosynthetic photon flux density and 400 ppm atmospheric $[CO_2]$) also called basal emission rate or emission capacity. Long-term changes typically proceed over hours, days or months and may involve gene activation, metabolic adjustments, phenological changes and leaf anatomical acclimations (Harrison et al., 2013; Staudt et al., 2017a). So far, the effects of temperature are better known and described than CO_2 effects, and in both, the short-term influences better than their longer-term influences on emissions (for overview see Peñuelas and Staudt, 2010; Holopainen et al., 2018; Feng et al., 2019). Furthermore, the emissions of isoprene has been widely studied by the scientific community and its response to environmental factors is relatively well simulated, while there is more variability and uncertainty in the emission responses of higher isoprenoids. The foliar emission rate of isoprene is closely related to its biosynthesis rate, because in isoprene producing tissues the pool sizes of isoprene and its direct precursors are small and sustain emission only few minutes (Sharkey et al., 2007). Its emission is regulated on the short-term by both light and temperature (Grote and Niinemets, 2008; Harrison et al., 2013). Isoprene emission increases in response to temperature exponentially to an optimum around 40 °C, above which the emission decreases. In response to light, isoprene emission increases in a hyperbola shape similar to that of photosynthesis. As regards the short-term CO_2 effect, many studies revealed that isoprene emission decreases with increasing $[CO_2]$ (Sharkey and Monson, 2014; Monson et al., 2016; Rasulov et al., 2018; Lantz et al., 2019 and references therein). The reported degrees of isoprene inhibition at elevated $[CO_2]$ depended on the applied CO_2 levels (e.g. Monson and Fall, 1989), but also on the actual leaf temperature (e.g. Loreto and Sharkey, 1990). Generally, the short-term inhibitory effect of high CO_2 on isoprene emission becomes reduced with increasing temperature and is completely offset at temperatures around 35 °C or higher. The CO_2 responsiveness of isoprene emissions can also vary among plant species and be modulated by the plant's growth conditions (e.g. Sharkey et al., 1991; Wilkinson et al., 2009).

Monoterpenes and sesquiterpenes are typically emitted by plants that store these BVOCs inside specialized secretory organs like resin ducts, glandular trichomes or oil cavities, where they are synthesized and accumulated during the leaf development (Turner et al., 2000; Franceschi et al., 2005; Tissier et al., 2017). Buffered by large storage pools, the release of terpenes from these organs is uncoupled from biosynthesis. It is essentially driven by the vapor pressure and the resistances along the diffusion paths (Staudt et al., 2017b). The emissions are not light dependent and the temperature response has no apparent temperature optimum. Emissions increase exponentially with temperature, where the slope of the log-linear relationship (β-value) is used to describe and model the emission responsiveness to temperature (Guenther et al., 2012). However, numerous studies have been questioned this paradigm by showing that terpene storing species also emit de-novo-synthesized terpenes that can be the same or different isomers with respect to their storage-derived emissions (e.g. Ghirardo et al., 2010; Staudt et al., 2019; Huang et al., 2018). It is therefore possible that a fraction of their emissions are also inhibited by elevated CO_2 and show a response pattern similar to that of isoprene as indicated by studies conducted on non-storing monoterpene emitters (e.g., Loreto et al., 1996; Rapparini et al., 2004). To our knowledge only seven studies have investigated the effects of elevated CO_2 (alone or along with other factors) on constitutive terpene emissions from ten terpene storing plants, nine of which were diverse coniferous species and one the aromatic shrub rosemary. Of the ten investigated species, two showed decreased emissions (*Taxodium distichum* (Llorens et al., 2009), hybrid larch (Mochizuki et al., 2017)), two increased emissions (*Metasequoia glyptostroboides* (Llorens et al., 2009), *Cryptomeria japonica* (Mochizuki et al., 2018)), and five unchanged emissions of terpenes in response to elevated CO_2 (*Pinus radiata* (Juuti et al., 1990), Rosemary (Peñuelas and Llusià, 1997), *Pinus ponderosa* and *Pseudotsuga menziesii* (Constable et al., 1999), *Pinus sylvestris* (Räisänen et al., 2008; Llorens et al., 2009)). The experiments of Constable et al. (1999) and Räisänen et al. (2008) combined growth CO_2 treatments with two growth temperature treatments, but only in the latter study emissions were reported for all treatments. Based on temperature normalized emission rates, Räisänen et al. (2008) observed significantly increased terpene emissions from Scots pine grown under both elevated CO_2 and elevated temperature, whereas growth under elevated CO_2 alone had no significant effect, and growth under elevated temperature alone significantly decreased the emission rate. However, neither this work on Scots pine nor any other above cited studies on terpene emissions, dissociated experimentally long-term acclimation effects of growth under elevated CO_2 from the fast responses of emissions to CO_2, and its possible modulation by temperature as evidenced for isoprene emission. Thus according to our literature survey, much more experimental work on terpene storing plants is needed to understand and predict how their BVOC emissions respond to future climate conditions.

In order to contribute filling this gap, we designed an experiment, in which we grew Sweet wormwood plants (*Artemisia annua* L., Asteraceae) under two CO_2 regimes combined with two temperature regimes and determined emissions at two assay $[CO_2]$ (400 and 800 ppm) combined with two assay temperatures (30 and 37 °C). More specifically, we addressed the following questions:

- Which VOCs are released at what rates from *A. annua* foliage under environmentally controlled conditions and how do emissions compare with the foliar VOC concentrations?
- Does the actual emission rate of BVOCs respond to changes in assay CO_2 and if so does the CO_2 responsiveness depend on the actual assay temperature ?
- Does growth under warmer and/or CO_2 enriched atmospheres modulate the fast emission responses to CO_2?
- Do growth conditions affect the overall VOC emission capacity and/or foliar VOC concentrations?

We chose *A. annua* in the present study, because it is a widely distributed, strongly aromatic herb. Native to Asia, it was naturalized throughout the world and is cropped in many countries under warm temperate climates. In fact, *A. annua* is the only source of artemisinin, a non-volatile endoperoxide sesquiterpene lactone, identified as the

compound active against protozoan parasites of the genus *Plasmodium* responsible for malaria (Gershenzon and Dudareva, 2007; Xiao et al., 2016). *A. annua* culture involves environmental risks due to the toxicity of artemisinin leached in soil and groundwater (Herrmann et al., 2013). *A. annua* is also considered as an invasive weed species, whose distribution has continuously expanded in Europe, and is expected to further increase with global climate warming (Follak et al., 2013). Its essential oil, which is also used in perfumery and cosmetics, is produced and stored in peltate glandular trichomes and contains mainly monoterpenes and sesquiterpenes (Woerdenbag et al., 1993; Tissier et al., 2017; Desrosiers et al., 2019).

2. Material and methods

### 2.1. Plant material and long-term treatments

A. annua seeds were obtained from the supplier ChilternSeeds (UK). They were grown from end of April to beginning August 2018 in four identical glasshouse compartments at the Center for Functional and Evolutionary Ecology under two temperature regimes combined with two CO_2 regimes: normal temperature and ambient CO_2 (25/15 °C day/night temperature and 400 ppm CO_2 concentration, referred to hereafter as: "Ambient"), elevated temperature and ambient CO_2 (30/20 °C, 400 ppm [CO_2], "+T"), normal temperature and elevated CO_2 (25/15 °C, 800 ppm [CO_2], "+CO_2"), and both elevated temperature and CO_2 (30/20 °C, 800 ppm [CO_2], "+T + CO_2"). Plants were repeatedly sown during the period in order to have tests plants of similar size and development stage at any time of the experiment. For sowing, seeds were mixed with fine sand and then scattered on the surface of a mixture of compost and sand. Individual, well developed seedlings were transplanted into homemade cylindrical pots (2.8 L volume, 15 cm diameter, 16 cm height) containing the same substrate mixture used previously. The pots were inside lined with a Teflon sheet (FEP, 50 µm thickness), which at the upper open pot side was double folded over the border and

fixed with a silicon sealing ring taking care to let about 10 cm of Teflon sheet extending beyond the pot border.

2.2. Plant exposure system

Volatile emissions were determined by dynamic headspace technique using a system allowing to study VOC emissions from the whole foliage of small plants under environmentally controlled conditions. The system was composed of two double walled glass chambers settled side by side, closed on top by a removable glass lid and open underside to enable the installation of the potted plants (Fig. 1). The inner headspace volume of the chambers was about 5 L. Homogenous mixing of the air in the chambers was maintained by small Teflon fans fixed at the chamber lids (motor outside). Temperature control of the chambers was carried out by circulating water from a water-bath around the double walled chamber section. A programmable temperature controller regulated constantly the temperature of the water bath to achieve the target air temperature measured by a thermocouple inside the chamber headspace. Additional thermocouples (Chrom-Constantan, OMEGA) were inserted to survey the chamber air temperatures. Photosynthetic Photon Flux Density (PPFD) was monitored with two quantum sensors (LI-COR, PAR-SB 190, Lincoln, NE, USA) located outside the chambers at middle height of the plants. A programmable LED lamp (LX60 Heliospectra AB, Göteborg, Sweden) illuminated the chambers with a PPFD of approx. 600 µmol m^{-2} s^{-1}. Temperature and PPFD values were recorded by a data logger (21x; Campbell Scientific Ltd., Shepsherd, UK).

The chambers were continuously flushed with charcoal filtered ambient air via PFA tubing at constant rates regulated by mass flow controllers (Mass Stream, M + W Instruments GmbH, Leonhardsbuch, Germany). Pure CO_2 was injected from a 5 L tank via high precision mass flow regulators (El-Flow Select, Bronkhorst France S.A.S., Montigny-les-Cormeilles, France) to achieve the warranted CO_2 concentrations inside the chambers. CO_2 concentrations were manually adjusted before and during the acclimation phases of the plants to assay conditions and
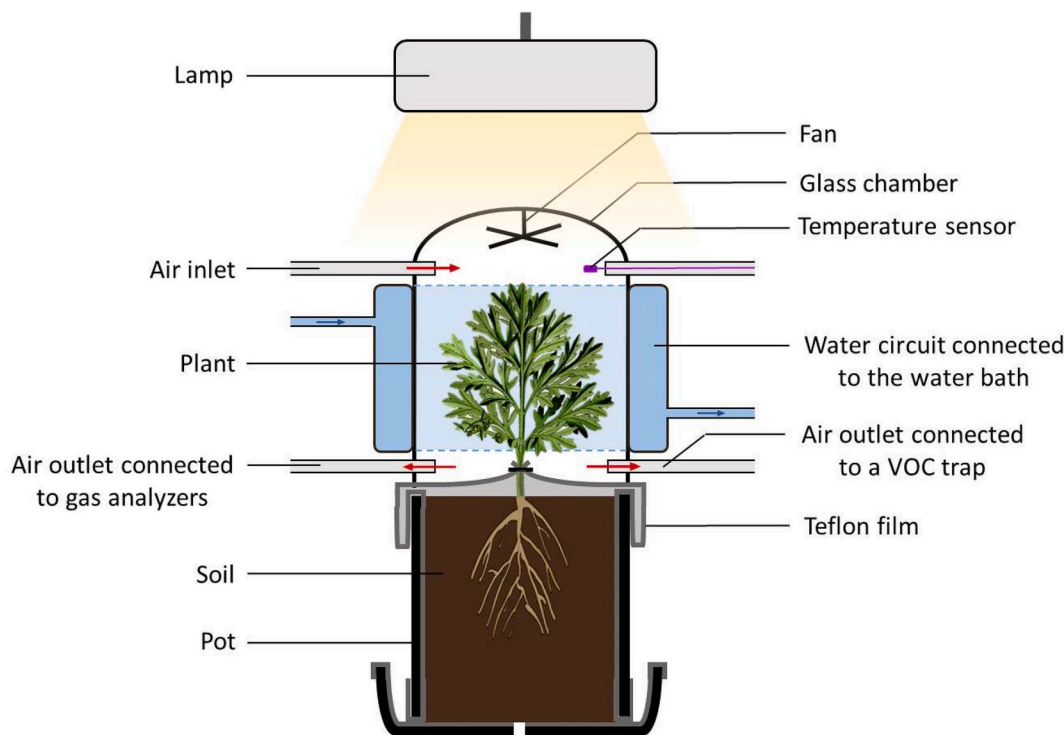
Fig. 1. Enclosure system used for the measurement of VOC emissions from the entire foliage of *Artemisia annua* plants. The red arrows indicate the direction of the airflow. The double walled middle section of the chamber with the temperature-regulated water circuit is shown in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

generally changed little once photosynthesis had reached steady state. The CO_2 concentrations of the air entering and leaving the chambers were continuously measured and recorded by infrared gas analyzers (LI-COR 840 combined with LI-COR 7000, LI-COR Inc., Lincoln, Nebraska, USA).

2.3. VOC sampling and measurement protocol

Prior installation of the plants inside the chambers, the Teflon sheet protruding the upper pot border was gently wrapped around the stem basis and loosely sealed with a Teflon tape to separate the soil with the root system from the above ground organs (Fig. 1). The glass chambers having the same diameter as the pots were then carefully placed on the pots with plants and sealed outside with a sealing ring to improve the air tightness of the system. The plants were mounted in the experimental device the night before the experiment started (on average 17 h) and flushed with charcoal filtered ambient air at a high rate ($\sim$3 L min^{-1}) to remove any residuals of VOC bursts that potentially occurred during plant installation (Niinemets et al., 2011). Indeed, preliminary tests showed that even tiny movements of the leaves caused for example by switching on the enclosure fan triggered temporary emission increases that were still detectable after 1 h.

The measurements were started in the next morning around 9 a.m. To measure VOC emissions, the chamber flow rate was reduced to 1 L min^{-1} (chamber flushing half-time ca. 3.4 min) and CO_2 concentration and air temperature were set to one of the following four assay conditions: Standard temperature and normal CO_2 (30 °C, 400 ppm [CO_2], referred to hereafter as: "30 × 400"), high temperature and normal CO_2 (37 °C, 400 ppm [CO_2], "37 × 400"), standard temperature and high CO_2 (30 °C, 800 ppm [CO_2], "30 × 800"), and both high temperature and high CO_2 (37 °C, 800 ppm [CO_2], "37 × 800"). These assay temperatures were chosen to detect the possible interaction of temperature on CO_2 responsiveness as shown for isoprene emissions. At 37 °C, which lies in the range of temperatures optimal for VOC emissions and supra-optimal for net-photosynthesis, the inhibitory effect of high CO_2 on emissions typically disappears (Sharkey and Monson, 2014; and other references cited above). Furthermore, 30 °C is the standard temperature commonly applied to determine the plant's emission capacity for emission modelling (Guenther et al., 2012). VOC emissions of each replicate plant were subsequently measured at the four assay conditions, whereby the order of the assay conditions was randomly reversed to avoid a potential bias associated with potential intrinsic diel variation in emissions. Before VOC sampling plants were maintained at target assay conditions for at least 30 min after photosynthesis had reached a new steady-state. After the last measurement around 3 p.m., plants were removed and the foliage was harvested for the determination of surface area and dry weight and the extraction of stored VOCs. On the whole, six replicate plants per growth treatment were measured at the 4 assay conditions, whereby the order of growth treatments was randomly changed throughout the duration of the experiment.

VOCs in the chamber air were trapped on adsorbent cartridges (stainless steel, Perkin Elmer, Villebon, France) packed with two absorption polymers (Tenax TA and Carbotrap). 2 L of air from the chambers with or without plants (i.e., background measurement) was drawn through the tubes at 0.2 L min^{-1} by mean of pumps and mass flow regulators. Background measurements were made approx. 50 min after plants were removed from the chambers while continuously flushed with clean air at the same rate. To close the open bottom of the chambers, two pots filled with substrate and lined with Teflon sheets (identical to the pots with plants) were fitted under the chambers. Additional empty chamber measurements were made at extra days without any plant measurements to check temporal variations of background concentrations and memory effects in the device.

2.4. Solvent extraction of stored VOCs

After VOC emission measurements, five leaflets of the plant were cut, rapidly weighed on a microbalance (on average 0,52 g) and plunged into 10 mL of dichloromethane. The extraction tube was placed for 10 min in an ultrasonic bath, then vortexed for 1 min and left for 1 h at room temperature. After maceration, the plant material was removed. An internal standard of 4 mL biphenyl (0.1 mg mL^{-1}) was added to the extract before concentration to a volume of 225 µL in a moderate nitrogen flow. The concentrated sample was stored at −20 °C until analysis.

2.5. VOC analysis

All VOC analysis were carried out at the Ecological Chemical Analysis Platform of the CEFE institute. Volatiles trapped on adsorption cartridges were analyzed by GC-MS using an automatic thermal desorption system (TD-20, Shimadzu France, Marne-la-Vallée, France) coupled to a Shimadzu GC-MS QP2010 Plus equipped with a DB5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Trapped VOCs were thermally desorbed and injected in splitless mode into the column after pre-concentration in a cold trap with the following temperature program: desorption 15 min at 230 °C, preconcentration at −30 °C on a Tenax TA cold trap, injection 5 min at 230 °C, transfer-line 200 °C. The GC oven program was: 2 min at 40 °C, 5 °C min^{-1} to 200 °C, 10 °C min^{-1} to 270 °C held for 6 min. Leaf extracts were analyzed by a second Shimadzu GC-MS QP2010 Plus instrument equipped with the same DB5 column. An aliquot of 1 µL of the concentrated leaf extracts was injected with a split ratio of 4. The GC oven temperature program was: 40 °C for 1 min, 3.2 °C min^{-1} to 100 °C, 2.90 °C min^{-1} to 170 °C, 10 °C min^{-1} to 250 °C held for 6 min. Both GC-MS used helium as carrier gas at a constant flow of 1 mL min-1. The MS ion sources were operated at 70 eV and 200 °C.

GC-MS data were processed using the GC-MS Solution package, Version 4.11 (Shimadzu Corporation 2013). Identification of the compounds was realized by comparing mass spectra and retention indices with those of authentic VOC standards, databases (Wiley 9, Nist 11, Adams) and alkane standards. For quantification, we used standard solutions of (Z)-3-hexenylacetate, linalool, methyl salicylate and β-caryophyllene in three different concentrations (approximately 100, 10 and 1 ng µl^{-1} each compound), which were prepared from authentic pure standards (Fluka, Sigma) using methanol as solvent. Each substance represents a class of VOCs typically found in A. annua. Multipoint calibrations for all standards revealed a good linear dependency of peak area to the respective compound concentration. For each standard VOC, the slope of the linear relationship was used as calibration factor (peak area ng^{-1}) for all molecules of the same VOC class (i.e. LOX, monoterpenes, phenolic compounds, sesquiterpenes). On average, the calibration factors of the four selected standard VOCs diverged by 30% with the lowest sensibility observed for methyl salicylate and the highest for β-caryophyllene.

2.6. Calculations and statistics

The emission rate of a given VOC was calculated as the difference between the VOC concentration in the chamber with plant and the concentration in the empty chamber (zero in many cases), multiplied by the chamber air flow rate and divided by either the dry weight or the projected leaf area of the enclosed foliage. VOC amounts in the leaf extracts were calculated from the amount of internal standard weighed for differences in the calibration factors of the VOC classes. Foliar VOC concentrations were expressed per leaf dry weight, which was estimated from the fresh weight of the measured leaflets and the ratio of fresh weight to dry weight determined on adjacent leaflets. Fresh and dry weights (24 h at 60 °C) were measured on a microbalance (Mettler, model AE 100) and leaf area from scans using the Image J software. It should be noted that losses of stored VOCs occurring during foliage

harvest, leaf area determination and oven drying have likely lead to a slight underestimation of the foliage dry weight (<1%, cf. Table 1, see also Portillo-Estrada et al. (2015) on this issue).

The photosynthesis rate A was calculated according to Von Caemmerer and Farquhar, 1981 (equation (1)):

$$A = \frac{u_e}{s} \times \left[c_e - c_o \times \left(\frac{1 - w_e \times 10^{-3}}{1 - w_o \times 10^{-3}} \right) \right], \quad (\mu \text{ mol } \text{m}^{-2}\text{s}^{-1}) \qquad (1)$$

where u_e, the molar flows of air entering the glass chambers (mol s^{-1}), s is the leaf area (m^2), c_e/c_o the mole fractions of CO_2 entering/leaving the glass chambers (μmol mol^{-1}), and w_e/w_o the mole fraction of water vapor entering/leaving the glass chambers (mmol mol^{-1}). It should be noted that water condensation occasionally occurred at high assay temperature in the chamber outlet and tubing. Therefore, transpiration and stomatal conductance data were not considered in this study. The underestimation of w_o due to water condensation causes however only small errors in the calculation of the photosynthesis rates (relative error <5%).

Statistical analyses were performed using R Software (version 3.5.2.). Variables were log transformed after testing the normality and homoscedasticity of residuals. Differences were considered as significant at a probability level of $P<0.05$ and as marginally significant at a probability level of $P<0.1$. For VOC emission and photosynthesis data, no interactions between the effects of assay conditions and growth conditions were found (Linear mixed model fit by REML with log-transformed values: $F = 0.842$; degrees of freedom (df) = 9; $P = 0.58$ and $F = 0.916$; df = 9; $P = 0.52$). The effects of assay and growth treatments were therefore treated independently in our study. The effect of assay treatments on VOC emission (total and single compound classes) and photosynthesis rate were tested with a linear mixed model with repetition as a random effect and growth condition as a fixed effect. Differences between assay treatments were tested with post-hoc t-tests using Satterthwaite's method and the rates of increase and decrease in emissions were compared using paired Wilcox test. The effect of growth conditions on VOC emission (total and single compound classes) and photosynthesis rate measured at standard conditions was tested with a one-way Anova followed by a Tukey post-hoc test, and further on the pooled emission or photosynthesis data measured at the four different assay conditions with a linear mixed model with repetition as a random effect and assay conditions as a fixed effect. The effect of growth conditions on foliar VOC concentration, leaf dry mass per projected leaf area (LMA), leaf water content and plant biomass was tested with a one-way Anova, followed by a Tukey post-hoc test.

3. Results

3.1. Volatiles stored and emitted by A. annua

Overall, 29 compounds were found in *A. annua* leaf extracts and 23 in the emissions, of which 21 were common (Fig. 2, Table 1, see also supplementary data Table S1). The great majority of VOCs were isoprenoids, mostly non-oxygenated and oxygenated monoterpenes (MTs) and non-oxygenated sesquiterpenes (SQs). In addition, one alkene and several non-identified compounds could be detected. Artemisia ketone was by far the most emitted compound accounting for about the half of the total VOC release (42.5–52.3%). The second major emitted VOCs were α-pinene (4.0–14.9%) and 1.8-cineole (8.3–12.1%) followed by camphor (5.0–8.2%), β-pinene (2.6–5.2%), artemisia alcohol (2.3–4.8%) and the SQ (E)-β-caryophyllene (3.6–5.2%).

The other compounds found in the emissions represented less than

Table 1

Mean ± SEM (n = 6) VOC emission rates and foliar VOC concentrations of *Artemisia annua* plants grown under four temperature and CO_2 regimes: Ambient: 25 °C and 400 ppm, +T: 30 °C and 400 ppm, +CO_2: 25 °C and 800 ppm, +T + CO_2: 30 °C and 800 ppm. All emissions were determined at standard assay conditions (30 °C and 400 ppm [CO2]). Emissions and contents are expressed per g foliage dry weight. Tr: Trace amounts (<0.01 μg g^{-1} h^{-1}); -: not detected in the samples; NI: Non-Identified peak with retention time; oxMT: oxygenated MT; MTs, SQs, Other semi-volatiles: Sum of monoterpenes, sesquiterpenes and other non-emitted, non-identified compounds; *: tentative identification (for more details see Supplementary Table S1).

| VOC	Ambient		+T		+CO$_2$		+T + CO$_2$	
	Emission	Content	Emission	Content	Emission	Content	Emission	Content
	[μg g^{-1} h^{-1}]	[μg g^{-1}]	[μg g^{-1} h^{-1}]	[μg g^{-1}]	[μg g^{-1} h^{-1}]	[μg g^{-1}]	[μg g^{-1} h^{-1}]	[μg g^{-1}]
α-Pinene	0.13 ± 0.07	17.82 ± 10.2	0.20 ± 0.07	26.95 ± 6.14	0.16 ± 0.02	29.42 ± 3.75	0.41 ± 0.13	31.24 ± 9.2
Camphene	0.03 ± 0.01	5.83 ± 1.74	0.02 ± 0.01	3.91 ± 1.29	0.02 ± 0.01	4.26 ± 0.89	0.06 ± 0.02	4.15 ± 1.16
β-Pinene	0.06 ± 0.02	10.87 ± 3.68	0.03 ± 0.01	4.05 ± 0.62	0.06 ± 0.02	6.84 ± 1.77	0.10 ± 0.04	4.41 ± 1.15
1,8-Cineole	0.26 ± 0.06	17.68 ± 5.06	0.17 ± 0.04	13.36 ± 5.06	0.11 ± 0.04	11.78 ± 5.32	0.20 ± 0.09	5.94 ± 2.73
Artemisia ketone	1.32 ± 0.39	100.03 ± 22.04	0.58 ± 0.12	55.71 ± 19.88	0.62 ± 0.07	50.36 ± 8.95	0.99 ± 0.25	59.64 ± 7.07
Sabinene hydrate	0.02 ± 0.01	1.39 ± 0.57	tr	1.93 ± 0.70	tr	1.79 ± 0.47	0.01 ± 0.01	1.96 ± 0.43
Artemisia alcohol	0.08 ± 0.02	11.1 ± 3.23	0.03 ± 0.01	8.00 ± 2.64	0.03 ± 0.01	6.84 ± 1.51	0.10 ± 0.03	9.05 ± 1.95
(E)-Pinocarveol	0.02 ± 0.01	2.26 ± 1.49	0.01 ± 0.01	4.39 ± 1.6	0.01 ± 0.004	3.82 ± 0.43	0.02 ± 0.01	4.93 ± 1.32
Camphor	0.17 ± 0.05	26.06 ± 9.35	0.08 ± 0.04	20.18 ± 6.54	0.06 ± 0.02	16.9 ± 4.1	0.27 ± 0.17	20.94 ± 7.18
(Z)-Verbenol*	–	1.04 ± 0.34	–	1.24 ± 0.29	–	0.94 ± 0.16	–	0.93 ± 0.14
Pinocarvone	0.01 ± 0.01	3.72 ± 2.35	0.02 ± 0.01	6.26 ± 2.36	0.01 ± 0.003	5.39 ± 0.83	0.04 ± 0.01	7.53 ± 1.61
Myrtenal	tr	1.86 ± 0.5	tr	0.89 ± 0.28	tr	1.09 ± 0.07	0.01 ± 0.003	1.10 ± 0.28
Myrtenol	–	1.35 ± 0.33	tr	0.81 ± 0.22	tr	0.85 ± 0.09	tr	1.09 ± 0.29
Tridec-1-ene*	0.04 ± 0.01	0.66 ± 0.19	0.03 ± 0.01	0.59 ± 0.19	0.03 ± 0.01	0.33 ± 0.09	0.04 ± 0.01	0.64 ± 0.14
OxMT*	–	0.28 ± 0.24	–	0.52 ± 0.25	tr	0.48 ± 0.21	tr	0.19 ± 0.1
NI:22.89	tr	–	0.01 ± 0.01	–	tr	–	–	–
α-Copaene	tr	0.98 ± 0.43	tr	0.44 ± 0.08	tr	0.84 ± 0.1	tr	1.34 ± 0.51
(E)-β-Caryophyllene	0.09 ± 0.03	4.48 ± 2.35	0.06 ± 0.02	2.53 ± 0.45	0.04 ± 0.01	3.99 ± 0.7	0.11 ± 0.05	7.31 ± 2.13
Geranyl acetone	tr	–	0.01 ± 0.01	–	tr	–	tr	–
α-Humulene	tr	0.3 ± 0.2	tr	0.14 ± 0.04	tr	0.24 ± 0.05	0.01 ± 0.004	0.46 ± 0.14
(E)-β-Farnesene	0.03 ± 0.02	3.32 ± 1.05	tr	0.37 ± 0.13	tr	3.13 ± 0.85	tr	2.52 ± 1.02
β-Chamigrene*	tr	0.09 ± 0.09	tr	0.04 ± 0.04	tr	0.01 ± 0.01	tr	0.03 ± 0.03
Germacrene-D	0.02 ± 0.01	6.96 ± 3.75	tr	2.52 ± 0.45	tr	5.22 ± 0.79	0.01 ± 0.002	7.62 ± 1.97
β-Selinene	0.04 ± 0.01	5.76 ± 1.74	0.04 ± 0.03	8.11 ± 5.21	tr	0.14 ± 0.03	0.03 ± 0.02	1.84 ± 1.47
Arteannuin b*	–	33.28 ± 8.91	–	31.22 ± 8.82	–	15.28 ± 2.4	–	24.95 ± 6.77
Other semi-volatiles	–	18.66 ± 4.90	–	18.00 ± 5.14	–	10.31 ± 1.56	–	13.55 ± 4.03
MTs	**2.12 ± 0.54**	**201.27 ± 45.50**	**1.15 ± 0.27**	**148.20 ± 42.37**	**1.11 ± 0.14**	**140.76 ± 25.18**	**2.23 ± 0.62**	**153.08 ± 20.55**
SQs	**0.19 ± 0.07**	**55.16 ± 13.19**	**0.13 ± 0.04**	**45.37 ± 13.47**	**0.06 ± 0.02**	**28.85 ± 2.75**	**0.18 ± 0.07**	**46.07 ± 11.07**
Total	**2.35 ± 0.52**	**275.75 ± 60.41**	**1.34 ± 0.29**	**212.16 ± 60.91**	**1.20 ± 0.15**	**180.25 ± 26.71**	**2.45 ± 0.68**	**213.35 ± 29.31**
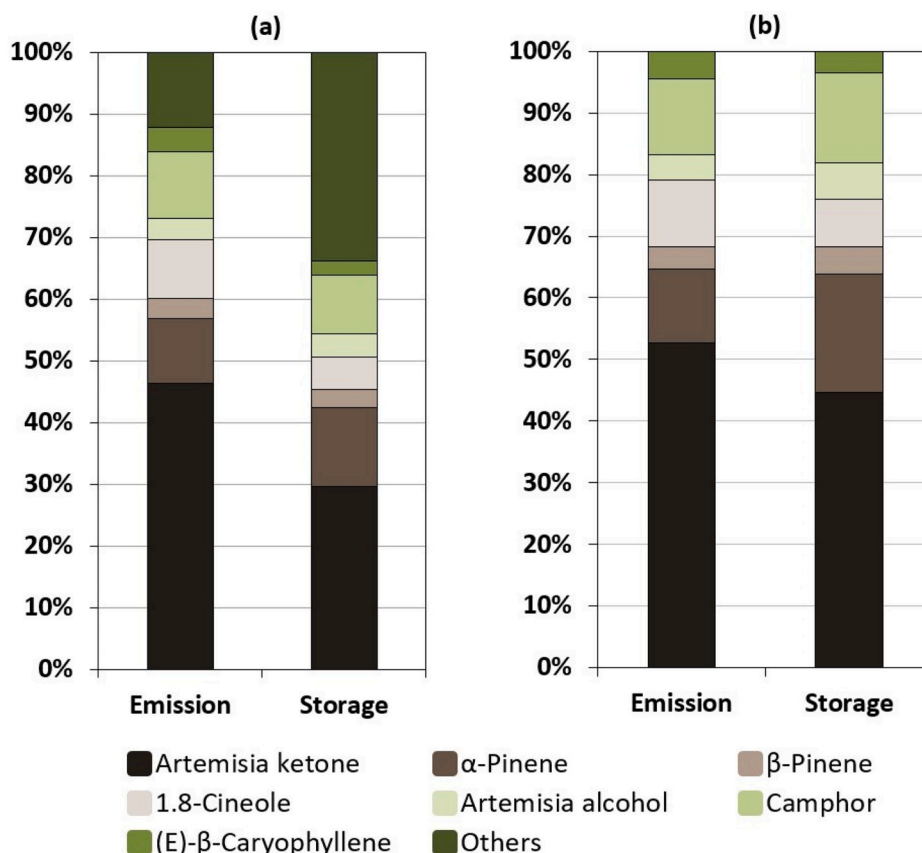
Fig. 2. Comparison of the composition of emitted and stored VOCs of *Artemisia annua* foliage. (a) Percentage contributions of the seven major compounds plus the sum of all other detected VOCs and (b) Percentage contributions of the seven main only. Values are means of n = 24.

4% of the total VOC release.

Among the emitted VOCs, geranyl acetone and one non-identified compound (RT 22.89) were not detected in the leaf extracts but almost regularly in the emissions albeit at trace level. All other emitted VOCs were also observed in the leaf extracts with relative abundances similar to those of the emissions. However, the second major stored compound, a highly oxygenated SQ tentatively identified as arteannuin B, as well as a MT alcohol tentatively identified as verbenol and six non-identified compounds were exclusively found in the leaf extracts. Except verbenol, these stored but apparently non-emitted VOCs eluted all very late on our analytical column, hence were compounds having a very low volatility.

3.2. Emission responses to changes in assay temperature and CO_2

Changing assay [CO_2] and/or temperature significantly changed total VOC emission rates ([CO_2]: $F = 6.30$; df = 1; $P = 0.014$; temperature: $F = 239.95$; df = 1; $P<0.001$) (Fig. 3a) regardless of the climate conditions where plants were grown ($F = 0.84$; df = 9; $P = 0.58$). The effect of assay [CO_2] on emissions depended on assay temperature (CO_2-temperature interaction: $F = 2.79$; df = 1; $P = 0.09$). At 30 °C, switching assay [CO_2] from 400 to 800 ppm had no significant effect on VOC emission rates, whereas at 37 °C it significantly decreased volatile emissions by about a third. The inhibitory CO_2 effect on emissions at 37 °C was significant for both VOC classes (MTs, SQs) and all individual major compounds with the exception of β-pinene and artemisia alcohol. Increasing assay temperature from 30 to 37 °C always significantly increased emission rates of the individual compounds and the sum of VOCs. On average, the total VOC release of *A. annua* was about 3 times higher at 37 °C than at 30 °C (17% increase per °C). Assuming a log-linear relationship between temperature and emission as applied in

emission models for storage-derived VOCs (Guenther et al., 2012), the resulting global slope β is 0.16 °C^{-1} (see supplementary data Table S2 and Fig. S1a). Due to the negative CO_2 effect on emission at 37 °C, the apparent rate of emission increase with temperature increase was marginally significantly greater at 400 ppm (19% °C^{-1}, $\beta = 0.17$) than at 800 ppm (15% °C^{-1}, $\beta = 0.14$) ($V = 204$, $P = 0.07$). Among compound classes, MT emissions responded more strongly to temperature than SQ emissions ($F = 8.14$, df = 1, $P = 0.005$). However, this compound specific difference in the temperature responses was very similar at both assay [CO_2] ($F = 0.22$, df = 1, $P = 0.64$; MTs: 20%- and 15%- increase °C^{-1}; SQs: 15%- and 10%-increase °C^{-1} at 400 and 800 ppm assay [CO_2], respectively; see also supplementary data Table S2 for β-values and Figs. S1a and b).

Switching assay temperature from 30 °C to 37 °C consistently decreased photosynthesis rates (Fig. 3b). The rate of decrease was significantly greater at 400 ppm than at 800 ppm (respectively 15.6% and 9.4%. $V = 38$, $P = 0.0007$). On the contrary, the photosynthesis rates of *A. annua* plants significantly increased when assay CO_2 concentration was doubled from 400 to 800 ppm. The rate of increase was significantly greater at 37 °C than at 30 °C (respectively 29.8% and 20.9%. $V = 32$, $P = 0.0003$).

3.3. Acclimation effects of growth conditions on VOC production

Growth conditions had only minor effects on the VOC production of *A. annua* foliage. The mean emission rates measured at the standard assay conditions of 30 °C and 400 ppm CO_2 ranged between 1.2 and 2.5 μg g^{-1} h^{-1} (11–26 ng m^{-2} s^{-1}) of the four populations with the lowest and highest value observed for the plants grown under elevated CO_2 and normal temperature and under elevated CO_2 and elevated temperature respectively (Table 1).
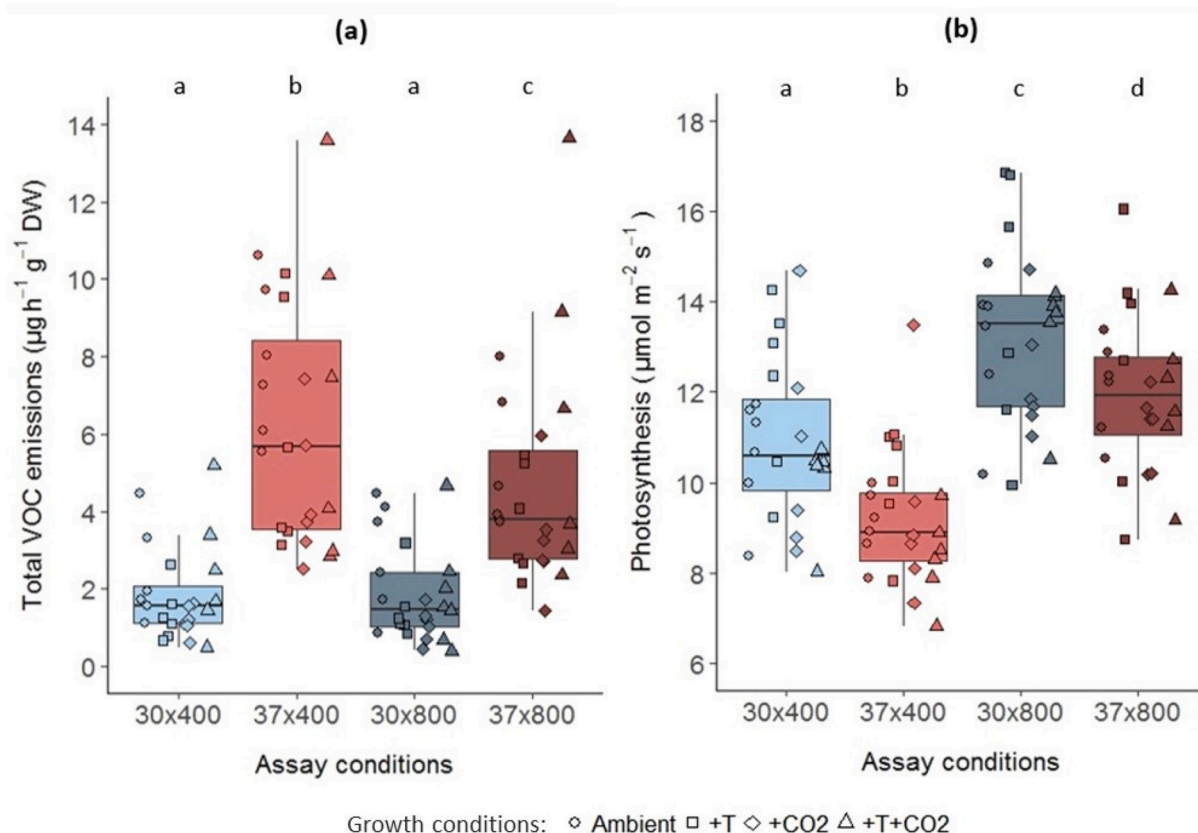
Fig. 3. VOC emissions (**a**) and photosynthesis (**b**) of whole *Artemisia annua* foliage measured at two assay $[CO_2]$ (400, 800 ppm) combined with two assay temperatures (30, 37 °C). Boxes display the medians (central lines), the first and third quartiles (bottom and top borders of the box) respectively. The whiskers extend to the most extreme data points. Symbols indicate the different CO_2 and temperature conditions, in which the plant were grown. Superscript letters denote significant differences between the means of the pooled data (n = 24, VOC emissions: $F = 85.69$; df = 3; $P<0.0001$; photosynthesis: $F = 51.99$; df = 3; $P<0.0001$).

This apparent acclimation effect on the foliage emission capacities was however not significant for the sum of VOC emissions, for the sum of MTs and SQs emissions and for the major VOCs except for artemisia alcohol that was marginally higher in emissions of plants from the +T + CO_2 treatment than emissions of plants from "+T" and "+CO_2" treatments ($P = 0.05$ and $P = 0.09$ respectively) (see supplementary data Fig. S2a). Using the pooled emission data from the measurements made at the four different assay condition, our statistical analysis indicated reduced VOC emissions of the plants grown at elevated CO_2 and normal temperature with respect to plants grown at normal CO_2 and temperature (Fig. 4a).

This negative acclimation effect of elevated growth CO_2 on total mean VOC emission rates was also marginally significant for the emissions of the main compound artemisia ketone ($F = 1.21$, df = 3, $P = 0.055$), but not significant for the emissions of the other major VOCs or the sum of MTs and SQs. However, there was no more significant effect of growth CO_2 when Anova analyses were run with emission rates expressed per leaf area. Likewise, average VOC emissions per plant were not different among growth treatments suggesting that at whole plant scale any negative effect of elevated CO_2 on the foliar emission capacity was compensated by the CO_2 fertilization effect on plant growth. Total or individual VOC emission rates per dry weight or leaf area from plants grown under elevated temperature with either normal or elevated CO_2 were not significantly different from all others. Growth conditions had also no significant effect on the foliar concentration of individual VOCs or VOC classes (Fig. 4b). In fact, foliar VOC concentrations were highly variable among plants, especially within the population grown under ambient conditions.

3.4. Acclimation effects of growth conditions on primary metabolism

Growth conditions did not significantly affect the mean photosynthesis rate ($F = 1.44$, df = 3, $P = 0.26$, measured at same assay conditions), plant height ($F = 0.80$, df = 3, $P = 0.51$) and total leaf area ($F = 0.69$, df = 3, $P = 0.57$) of *A. annua* plants (Table 2). However, growth under elevated CO_2 had a global positive effect on the mean dry and fresh biomass of the plants (dry biomass: $F = 6.91$, df = 1, $P = 0.02$; fresh biomass: $F = 4.25$, df = 1, $P = 0.05$). Consequently, the leaf mass per area (LMA) and the relative water content were respectively higher and lower in the +T + CO_2 treatment than in the ambient treatment (LMA: $F = 5.05$, df = 3, $P = 0.01$; water content: $F = 2.83$, df = 3, $P = 0.06$).

4. Discussion

One purpose of our study was to identify the VOCs emitted by *A. annua* plants and to quantify the emission rates under undisturbed, environmentally controlled conditions. The relative composition we observed in the foliar VOC emissions closely resembled that of the foliar VOC contents, especially when considering that some asymmetry between the compositions is expected due to differences in the volatility of the individual VOCs and due to limitations in the VOC extraction method, notably unaccounted losses of light VOCs during the concentration step of leaf extracts (Ormeno et al., 2011). The compositional similarity of foliar VOC emissions and extract suggests that the great majority of the emitted VOCs came from the essential oil stored at the tip of the glandular trichomes covering foliage and stems. However, two minor emitted compounds were not detected in the leaf extracts, perhaps because our extraction method was not sensitive enough. Alternatively, these emissions are not part of the essential oil but derive
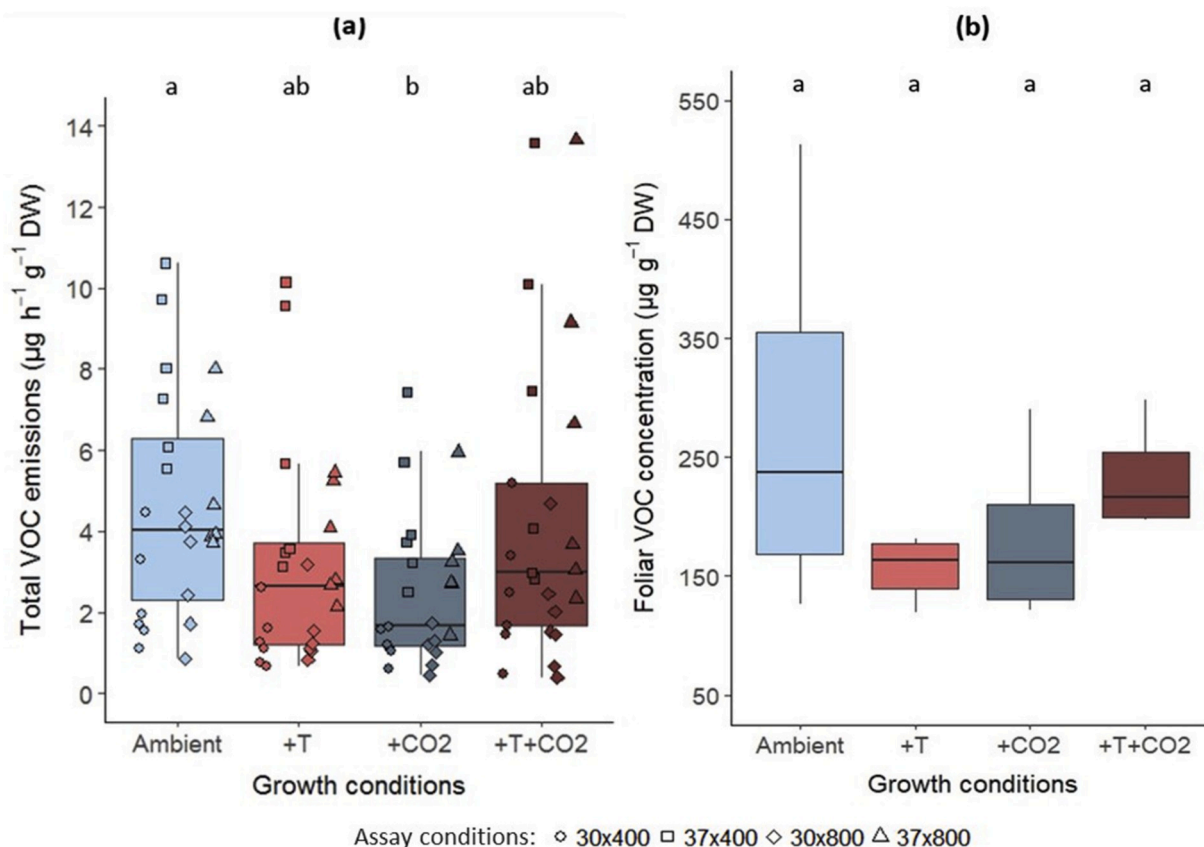
Fig. 4. Effect of growth under different temperature and CO_2 regimes on **(a)**, total amount of VOCs emitted under the four assay conditions (symbols) and **(b)**, total foliar VOC concentration (μg g^{-1} DW) of *Artemisia annua* plants. Boxes display the medians (central lines), the first and third quartiles (bottom and top borders of the box) respectively. The whiskers extend to the most extreme data points. Superscript letters denote significant differences between the means of n = 6 replicate plants per growth treatment (VOC emissions: $F = 2.60$; df = 3; $P = 0.08$; foliar VOC concentration: $F = 0.66$; df = 3; $P = 0.59$).

Table 2

Effect of growth under elevated temperature (T) and/or [CO_2] on photosynthesis rate, plant height, foliage dry, fresh weight and leaf area, LMA and water content of *Artemisia annua*. Means ± SEM (n = 24) annotated with different letters are significantly different and * indicate an effect of elevated CO_2 as a single factor. Mean photosynthesis rates were calculated from the measurements made at standard assay conditions (30 °C and 400 ppm [CO_2]).

Growth conditions	Photosynthesis (μmol m^{-2} s^{-1})	Height (cm)	Dry weight (g)	Fresh weight (g)
Ambient	10.62 ± 0.52	12.83 ± 0.79	0.82 ± 0.08	5.47 ± 0.61
+CO_2	10.75 ± 0.97	11.83 ± 0.85	1.05 ± 0.12 *	6.58 ± 0.70 *
+T	12.16 ± 0.79	11.50 ± 0.48	0.82 ± 0.13	5.08 ± 0.83
+T + CO_2	10.07 ± 0.41	12.33 ± 0.49	1.15 ± 0.12 *	6.33 ± 0.34 *

Growth conditions	Leaf area (cm^2)	LMA (g m^2)	Water content (%)
Ambient	293.59 ± 24.04	27.96 ± 0.66 **a**	84.80 ± 0.36 **a**
+CO_2	326.64 ± 44.70	31.88 ± 1.16 **ab**	84.05 ± 0.32 **ab**
+T	281.79 ± 34.61	29.36 ± 1.52 **ab**	84.01 ± 0.52 **ab**
+T + CO_2	320.66 ± 15.83	35.49 ± 2.22 **b**	81.93 ± 1.29 **b**

from other small VOC pools. Geranyl acetone for example might be secondary formed from the breakdown of non-volatile metabolites such as carotenoids (Fruekilde et al., 1998; Kask et al., 2016). On the whole, the VOC composition we observed in the emissions as well as in the leaf extract agrees with the results of previous studies on *A. annua*. Artemisia ketone was always identified as the main compound (Woerdenbag et al.,

1993; Rapparini et al., 2008; Nibret and Wink, 2010), though its proportion may vary with the chemotype (Woerdenbag et al., 1993), the season and the method of VOC extraction (Ormeno et al., 2011; Nekoei et al., 2012).

As a highly aromatic plant that stores large amounts of VOCs in its glandular trichomes, *A. annua* emissions may essentially contribute to the atmospheric VOC load in rural landscapes where it is cultivated or commonly appears as ruderal or weed species. However, VOC emission rates of aromatic plants determined by enclosure techniques might be easily overestimated, since any mechanical stress leads to the rupture of trichomes, which causes large emission bursts (Niinemets et al., 2011). To avoid such artifacts we used large enclosures avoiding any contact between foliage and chamber wall and flushed chambers overnight after installation of the plants. Proceeding in this way, we observed that the whole foliage of individual *A. annua* plants released VOCs at rates of 1–5 μg g^{-1} h^{-1} under standard assay temperature. This is in the range of those reported by other terpene storing plants (Kesselmeier and Staudt, 1999; Biogenic VOC emission database http://www.es.lancs.ac.uk/cnh group/download.html; Šimpraga et al., 2019) but 1–2 orders of magnitude lower than those reported by Rapparini et al. (2008) for the same species. The aforementioned emission enhancement by mechanical stress might be the main reason for this discrepancy in addition to other methodological and biological sources of variation such as the choice of cultivar, measurement scale and conditions.

Growing *A. annua* under warmer and/or CO_2-enriched atmospheres hardly affected their overall BVOC emission capacities and had no significant effect on the short-term responses of emissions to assay CO_2 and temperature. Nevertheless, elevated-[CO_2]-grown plants tended to have a lower BVOC emission capacity per foliage dry weight, although this

effect was compensated by higher growth rates. Furthermore, the lower emission capacity was attributed to an increase in LMA (Table 2) rather than to a true change of the emission source strength. The increase in LMA was mostly due to an increase in the leaf's dry matter density (for example due to increased starch accumulation under elevated [CO_2]) as indicated by the significant decrease in leaf water content. Changes in leaf structure and composition are commonly observed in plants grown under elevated CO_2 (Zvereva and Kozlov, 2006; Poorter et al., 2009; Sun et al., 2012).

However, growth conditions had no significant effect on VOC contents of *A. annua* foliage that largely varied among individual plant replicates. Intraspecific chemical diversity in VOC production commonly exists in VOC storing plants (see e.g. Woerdenbag et al., 1993; Wu et al., 2011), which could have also increased the data variability in our study of both, VOC contents and emissions. In addition, sporadic losses of VOCs during the extraction might have been contributed to data scattering, most likely some inevitable rupture of trichomes during the leaf sample preparation (i.e. the dissection, weighing and transfer of leaflets to the solvent). Finally as for emissions, variation in foliar VOC contents based on concentrations may not necessarily reflect changes in VOC production but can also result from dilution or concentration effects (Koricheva, 1999) thus further increasing the inconsistency of results within and among studies. Indeed, previous studies examining changes in the yield and composition of essential oils of aromatic herbs or oleoresin of conifers grown under elevated CO_2 and/or elevated temperature reported contrasting effects, of which the majority found no or negative effects of elevated CO_2 (Table 3).

Regarding the short-term influences of [CO_2] and temperature, doubling [CO_2] decreased emissions at high assay temperature but not at standard temperature, whereas switching temperature from 30 to 37 °C always strongly increased emissions, though the degree of increase was apparently higher at low assay [CO_2] than at high assay [CO_2]. Thus, our results revealed a possible interaction in the temperature and CO_2 responses of emissions, suggesting that either the positive effect of temperature is attenuated by high [CO_2] or the inhibitory effect of [CO_2] is enhanced by high temperature. This observation discords with the aforementioned conclusion of storage derived emissions. If *A. annua* VOC emissions are sustained by the large preformed reservoirs in the tips of its glandular trichomes, they should be insensitive to changes of [CO_2] and any temporary variation in the plant's metabolism. Even changes in stomatal aperture should not affect the emission rate, because it is unlikely that the VOCs stored in the extracellular subcuticular cavity of the apical trichome cells diffuse back inside and along the subapical, stalk and basal trichome cells into the leaf parenchyma to leave the leaf via the stomata. Hence, emissions should depend solely on temperature driving the volatilization and diffusion through the cuticle of the trichome tips. A possible explanation for this apparent discrepancy is that a second, CO_2-responsive de-novo synthesis VOC pool exists in the photosynthetic leaf parenchyma, which fuels a fraction of the total *A. annua* VOC release. Indeed, Tellez et al. (1999) found small amounts of SQs in the foliage extracts of glandless *A. annua* phenotypes and traces of ocimene indicating that isoprenoids are also produced outside trichomes. However, we found no ocimene emission in our study and there was no difference between the apparent CO_2 responses of compound classes. Furthermore, high-CO_2 inhibition of de-novo synthesized isoprenoid emissions in photosynthesizing tissues occurs typically at low temperatures and not at high temperatures (Sharkey and Monson, 2014), which is the opposite what we have observed in the present study on *A. annua* emissions. An alternative explanation is that the CO_2 sensitive part of the emissions stemmed from the young growing leaves and apex of the enclosed foliage. Räisänen et al. (2008) observed that elevated CO_2 and temperature affects terpene emissions from Scots pine saplings only during the period of shoot growth indicating that the responsiveness of emissions could be associated with oleoresin synthesis in the growing tissues. In *A. annua* as for many other VOC storing plants

Table 3

Overview of the effects of growth under elevated CO_2 concentrations or elevated temperature on foliar VOC concentrations of aromatic herbs or conifers reported in the present and previous studies.

CO_2 effect	Plant species	References
↗	*Rosmarinus officinalis*	Peñuelas and Llusià, (1997)
	Thymus vulgaris	Vurro et al. (2009)
	Valeriana jatamansi	Kaundal et al. (2018)
↘	*Salvia officinalis*	Nowak et al. (2010)
	Melissa officinalis	Shoor et al. (2012)
	Pseudotsuga menziesii	Litvak et al., 2002; Snow et al., 2003
	Pinus sylvestris	Sallas et al. (2003)
	Picea abies	Sallas et al. (2003)
	Eucalyptus camaldulensis	Bustos-Segura et al. (2017)
=	*Artemisia annua*	Present study
	Artemisia tridentata	Johnson and Lincoln, (1990)
	Pinus sylvestris	Kainulainen et al., 1998, Heyworth et al., 1998 [a]; Sallas et al., 2001
	Pinus ponderosa	Constable et al. (1999)
	Pseudotsuga menziesii	Constable et al. (1999)
	Eucalyptus globulus	McKiernan et al. (2012)
	Eucalyptus pauviflora	McKiernan et al. (2012)
Temperature effect	**Plant species**	**References**
↗	*Picea abies*	Sallas et al. (2003)
	Pinus sylvestris	Sallas et al. (2003)
	Pseudotsuga menziesii	Litvak et al. (2002)
↘	*Pseudotsuga menziesii*	Snow et al., 2003; Duan et al., 2019 [b]
	Valeriana jatamansi	Kaundal et al. (2018)
=	*Artemisia annua*	Present study
	Pseudotsuga menziesii	Duan et al., 2019 [b]

[a] No effect except an increase in α-pinene.
[b] Depending on the tree's provenance.

(e.g. Turner et al., 2000; Vendemiatti et al., 2017), the production of essential oil takes place in developing trichomes and ceases when the reservoirs are filled and foliage becomes mature (Towler and Weathers, 2015; Tan et al., 2015). There, large amounts of isoprenoids are de-novo synthesized in the non-green apical trichome cells and subsequently actively excreted into the subcuticular space (Olsson et al., 2009; Tissier et al., 2017), during which a fraction could leak out in the air. Indeed, Lu et al. (2002) observed that that the transcript level of a terpene synthase is much higher in juvenile than in mature leaves of *A. annua* and that the temporal variations in transcript levels correlates with those of foliar emissions and contents. This suggests that some terpenes produced in juvenile foliage are not entirely sequestered and stored in glandular trichomes, but are released directly after synthesis (Lu et al., 2002). Given that the isoprenoid production there relies at least partly on photosynthate imports via phloem transport (Schuurink and Tissier, 2019), is influenced by stress hormones (Hao et al., 2017) and cross talks with other secondary metabolite pathways (Ma et al., 2018), it is plausible that it respond differently to CO_2 than the constitutive synthesis of isoprene in photosynthetic source tissues.

To conclude, we observed no relevant long-term, acclimation effect of elevated temperature and CO_2 on the overall VOC production capacity of *A. annua* plants, apart from a general CO_2 fertilization effect on plant growth and leaf structure. Thus our results suggest that considering short-term responses of emissions is sufficient to predict their future evolution. Contrary to isoprene emissions, *A. annua* emissions became CO_2-inhibited at high temperature and not at moderate

temperature thus reducing the apparent temperature response of 19% emission increase per °C to 15% °C^{-1}. More in deep studies over larger CO_2 and temperature ranges are needed to corroborate and precise the antagonistic interaction between these two factors and the underlying mechanisms. Furthermore, we need to know whether this phenomena is common in BVOC storing plant species and whether it is constrained to the growing vegetative phase. If so, the release of VOCs from *A. annua* and possibly other aromatic plants would increase less rapidly in a warmer and more CO_2-enriched world due to the more frequent negative feedback of CO_2 on their emissions.

Credit author statement

Juliane Daussy: Investigation, Formal analysis, Resources, Validation; Visualization, Writing - Original Draft - Review & Editing

Michael Staudt: Conceptualization, Methodology, Validation, Writing - Original Draft - Review & Editing, Supervision, Project administration, Funding acquisition

Declaration of competing interest

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

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aeaoa.2020.100082.

References

Bustos-Segura, C., Dillon, S., Keszei, A., Foley, W.J., Külheim, C., 2017. Intraspecific diversity of terpenes of Eucalyptus camaldulensis (Myrtaceae) at a continental scale. Aust. J. Bot. 65, 257–269. https://doi.org/10.1071/BT16183.

Constable, J.V., Litvak, M.E., Greenberg, J.P., Monson, R.K., 1999. Monoterpene emission from coniferous trees in response to elevated CO2 concentration and climate warming. Global Change Biol. 5, 252–267. https://doi.org/10.1046/j.1365-2486.1999.00212.x.

Desrosiers, M.R., Towler, M.J., Weathers, P.J., 2019. Artemisia annua and artemisia afra essential oils and their therapeutic potential. In: Essential Oil Research. Springer, Cham, pp. 197–209. https://doi.org/10.1007/978-3-030-16546-8_6.

Duan, Q., Kleiber, A., Jansen, K., Junker-Frohn, L.V., Kammerer, B., Han, G., Zimmer, I., Rennenberg, H., Schnitzler, J.-P., Ensminger, I., Gessler, A., Kreuzwieser, J., 2019. Effects of elevated growth temperature and enhanced atmospheric vapour pressure deficit on needle and root terpenoid contents of two Douglas fir provenances. Environ. Exp. Bot. 166, 103819. https://doi.org/10.1016/j.envexpbot.2019.103819.

Dudareva, N., Klempien, A., Muhlemann, J.K., Kaplan, I., 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol. 198, 16–32. https://doi.org/10.1111/nph.12145.

Feng, Z., Yuan, X., Fares, S., Loreto, F., Li, P., Hoshika, Y., Paoletti, E., 2019. Isoprene is more affected by climate drivers than monoterpenes: a meta-analytic review on plant isoprenoid emissions. Plant Cell Environ. 42, 1939–1949. https://doi.org/10.1111/pce.13535.

Follak, S., Dullinger, S., Kleinbauer, I., Moser, D., Essl, F., 2013. Invasion dynamics of three allergenic invasive Asteraceae (Ambrosia trifida, Artemisia annua, Iva xanthiifolia) in central and eastern Europe. Preslia 85, 41–61.

Franceschi, V.R., Krokene, P., Christiansen, E., Krekling, T., 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytol. 167, 353–376. https://doi.org/10.1111/j.1469-8137.2005.01436.x.

Fruekilde, P., Hjorth, J., Jensen, N.R., Kotzias, D., Larsen, B., 1998. Ozonolysis at vegetation surfaces: a source of acetone, 4-oxopentanal, 6-methyl-5-hepten-2-one,

and geranyl acetone in the troposphere. Atmos. Environ. 32, 1893–1902. https://doi.org/10.1016/S1352-2310(97)00485-8.

Gershenzon, J., Dudareva, N., 2007. The function of terpene natural products in the natural world. Nat. Chem. Biol. 3, 408. https://doi.org/10.1038/nchembio.2007.5.

Ghirardo, A., Koch, K., Taipale, R., Zimmer, I.N.A., Schnitzler, J.P., Rinne, J., 2010. Determination of de novo and pool emissions of terpenes from four common boreal/alpine trees by 13CO2 labelling and PTR-MS analysis. Plant Cell Environ. 33, 781–792. https://doi.org/10.1111/j.1365-3040.2009.02104.x.

Grote, R., Niinemets, Ü., 2008. Modeling volatile isoprenoid emissions–a story with split ends. Plant Biol. 10, 8–25. https://doi.org/10.1055/s-2007-964975.

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

Hao, X., Zhong, Y., Fu, X., Lv, Z., Shen, Q., Yan, T., Shi, P., Ma, Y., Chen, M., Lv, X., Wu, Z., Zhao, J., Sun, X., Li, L., Tang, K., 2017. Transcriptome analysis of genes associated with the artemisinin biosynthesis by jasmonic acid treatment under the light in artemisia annua. Front. Plant Sci. 8. https://www.frontiersin.org/article/10.3389/fpls.2017.00971.

Harper, K.L., Unger, N., 2018. Global climate forcing driven by altered BVOC fluxes from 1990 to 2010 land cover change in maritime Southeast Asia. Atmos. Chem. Phys. 18, 16931–16952. https://doi.org/10.5194/acp-18-16931-2018.

Harrison, S.P., Morfopoulos, C., Dani, K.G.S., Prentice, I.C., Arneth, A., Atwell, B.J., Barkley, M.P., Leishman, M.R., Loreto, F., Medlyn, B.E., Niinemets, Ü., Possell, M., Peñuelas, J., Wright, I.J., 2013. Volatile isoprenoid emissions from plastid to planet. New Phytol. 197, 49–57. https://doi.org/10.1111/nph.12021.

Heil, M., 2008. Indirect defence via tritrophic interactions. New Phytol. 178, 41–61. https://doi.org/10.1111/j.1469-8137.2007.02330.x.

Herrmann, S., Jessing, K.K., Jørgensen, N.O., Cedergreen, N., Kandeler, E., Strobel, B.W., 2013. Distribution and ecological impact of artemisinin derived from Artemisia annua L. in an agricultural ecosystem. Soil Biol. Biochem. 57, 164–172. https://doi.org/10.1016/j.soilbio.2012.08.011.

Heyworth, C.J., Iason, G.R., Temperton, V., Jarvis, P.G., Duncan, A.J., 1998. The effect of elevated CO 2 concentration and nutrient supply on carbon-based plant secondary metabolites in Pinus sylvestris L. Oecologia 115, 344–350.

Holopainen, J.K., Virjamo, V., Ghimire, R.P., Blande, J.D., Julkunen-Tiitto, R., Kivimäenpää, M., 2018. Climate change effects on secondary compounds of forest trees in the Northern Hemisphere. Front. Plant Sci. 9 https://doi.org/10.3389/fpls.2018.01445.

Huang, J., Hartmann, H., Hellen, H., Wisthaler, A., Perreca, E., Weinhold, A., Racker, A., van Dam, N.M., Gershenzon, J., Trumbore, S., Behrendt, T., 2018. New perspectives on CO2, temperature, and light effects on BVOC emissions using online measurements by PTR-MS and cavity ring-down spectroscopy. Environ. Sci. Technol. 52, 13811–13823. https://doi.org/10.1021/acs.est.8b01435.

Jiang, J., Aksoyoglu, S., Ciarelli, G., Oikonomakis, E., El-Haddad, I., Canonaco, F., O'Dowd, C., Ovadnevaite, J., Minguillon, M.C., Baltensperger, U., Prévôt, A.S.H., 2019. Effects of two different biogenic emission models on modelled ozone and aerosol concentrations in Europe. Atmos. Chem. Phys. 19, 3747–3768. https://doi.org/10.5194/acp-19-3747-2019.

Johnson, R.H., Lincoln, D.E., 1990. Sagebrush and grasshopper responses to atmospheric carbon dioxide concentration. Oecologia 84, 103–110. https://doi.org/10.1007/BF00665602.

Jokinen, T., Berndt, T., Makkonen, R., Kerminen, V.M., Junninen, H., Paasonen, P., et al., 2015. Production of extremely low volatile organic compounds from biogenic emissions: measured yields and atmospheric implications. Proc. Natl. Acad. Sci. Unit. States Am. 112, 7123–7128. https://doi.org/10.1073/pnas.1423977112.

Juuti, S., Arey, J., Atkinson, R., 1990. Monoterpene emission rate measurements from a Monterey pine. J. Geophys. Res.: Atmospheres 95 (D6), 7515–7519. https://doi.org/10.1029/JD095iD06p07515.

Kainulainen, P., Holopainen, J.K., Holopainen, T., 1998. The influence of elevated CO2 and O3 concentrations on Scots pine needles: changes in starch and secondary metabolites over three exposure years. Oecologia 114, 455–460. https://doi.org/10.1007/s004420050469.

Kask, K., Kännaste, A., Talts, E., Copolovici, L., Niinemets, Ü., 2016. How specialized volatiles respond to chronic and short-term physiological and shock heat stress in Brassica nigra. Plant Cell Environ. 39, 2027–2042. https://doi.org/10.1111/pce.12775.

Kaundal, M., Bhatt, V., Kumar, R., 2018. Elevated CO2 and temperature effect on essential oil content and composition of valeriana jatamansi jones. With organic manure application in a Western Himalayan region. J. Essential Oil Bearing Plants 21, 1041–1050. https://doi.org/10.1080/0972060X.2018.1497547.

Kessler, A., Halitschke, R., Diezel, C., Baldwin, I.T., 2006. Priming of plant defense responses in nature by airborne signaling between Artemisia tridentata and Nicotiana attenuata. Oecologia 148, 280–292. https://doi.org/10.1007/s00442-006-0365-8.

Kesselmeier, J., Staudt, M., 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. J. Atmos. Chem. 33, 23–88. https://doi.org/10.1023/A:1006127516791.

Koricheva, J., 1999. Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. Oecologia 119, 467–473. https://doi.org/10.1007/s004420050809.

Kulmala, M., Suni, T., Lehtinen, K.E.J., Maso, M.D., Boy, M., Reissell, A., et al., 2004. A new feedback mechanism linking forests, aerosols, and climate. Atmos. Chem. Phys. 4, 557–562. https://doi.org/10.5194/acp-4-557-2004.

Lantz, A.T., Allman, J., Weraduwage, S.M., Sharkey, T.D., 2019. Isoprene: new insights into the control of emission and mediation of stress tolerance by gene expression. Plant Cell Environ. 42, 2808–2826. https://doi.org/10.1111/pce.13629.

Litvak, M.E., Constable, J.V., Monson, R.K., 2002. Supply and demand processes as controls over needle monoterpene synthesis and concentration in Douglas fir [Pseudotsuga menziesii (Mirb.) Franco]. Oecologia 132, 382–391. https://doi.org/10.1007/s00442-002-0964-y.

Llorens, L., Llusià, J., Murchie, E.H., Peñuelas, J., Beerling, D.J., 2009. Monoterpene emissions and photoinhibition of "living fossil" trees grown under CO2 enrichment in a simulated Cretaceous polar environment. J. Geophys. Res.: Biogeosciences 114. https://doi.org/10.1029/2008JG000802.

Loreto, F., Sharkey, T.D., 1990. A gas-exchange study of photosynthesis and isoprene emission in Quercus rubra L. Planta 182, 523–531. https://doi.org/10.1007/BF02341027.

Loreto, F., Ciccioli, P., Cecinato, A., Brancaleoni, E., Frattoni, M., Fabozzi, C., Tricoli, D., 1996. Evidence of the photosynthetic origin of monoterpene emitted by Quercus ilex L. leaves by 13C labelling. Plant Physiol. 110, 1317–1322. https://doi.org/10.1104/pp.110.4.1317.

Lu, S., Xu, H., Jia, J.-W., Pang, J., Matsuda, S.P.T., Chen, X.-Y., 2002. Cloning and functional characterization of a beta-Pinene synthase from Artemisia annua that shows a circadian pattern of expression. Plant Physiol. 130, 477–486. http://www.plantphysiol.org/cgi/content/abstract/130/1/477.

Ma, D., Xu, C., Alejos-Gonzalez, F., Wang, H., Jiang, J., Judd, R., Xie, D.-Y., 2018. Overexpression of Artemisia annua cinnamyl alcohol dehydrogenase increases lignin and coumarin and reduces artemisinin and other sesquiterpenes. Front. Plant Sci. 9 article 828. https://www.frontiersin.org/article/10.3389/fpls.2018.00828.

McFiggans, G., Mentel, T.F., Wildt, J., Pullinen, I., Kang, S., Kleist, E., et al., 2019. Secondary organic aerosol reduced by mixture of atmospheric vapours. Nature 565, 587–593. https://doi.org/10.1038/s41586-018-0871-y.

McKiernan, A.B., O'Reilly-Wapstra, J.M., Price, C., Davies, N.W., Potts, B.M., Hovenden, M.J., 2012. Stability of plant defensive traits among populations in two Eucalyptus species under elevated carbon dioxide. J. Chem. Ecol. 38, 204–212. https://doi.org/10.1007/s10886-012-0071-4.

Mochizuki, T., Watanabe, M., Koike, T., Tani, A., 2017. Monoterpene emissions from needles of hybrid larch F1 (Larix gmelinii var. japonica× Larix kaempferi) grown under elevated carbon dioxide and ozone. Atmos. Environ. 148, 197–202. https://doi.org/10.1016/j.atmosenv.2016.10.041.

Mochizuki, T., Amagai, T., Tani, A., 2018. Effects of soil water content and elevated CO2 concentration on the monoterpene emission rate of Cryptomeria japonica. Sci. Total Environ. 634, 900–908. https://doi.org/10.1016/j.scitotenv.2018.04.025.

Monson, R.K., Fall, R., 1989. Isoprene emission from aspen leaves: influence of environment and relation to photosynthesis and photorespiration. Plant Physiol. 90, 267–274. https://doi.org/10.1104/pp.90.1.267.

Monson, R.K., Hills, A.J., Zimmerman, P.R., Fall, R.R., 1991. Studies of the relationship between isoprene emission rate and CO2 or photon-flux density using a real-time isoprene analyser. Plant Cell Environ. 14, 517–523. https://doi.org/10.1111/j.1365-3040.1991.tb01522.x.

Monson, R.K., Grote, R., Niinemets, Ü., Schnitzler, J.-P., 2012. Modeling the isoprene emission rate from leaves. New Phytol. 195, 541–559. https://doi.org/10.1111/j.1469-8137.2012.04204.x.

Monson, R.K., Neice, A.A., Trahan, N.A., Shiach, I., McCorkel, J.T., Moore, D.J., 2016. Interactions between temperature and intercellular CO2 concentration in controlling leaf isoprene emission rates. Plant Cell Environ. 39, 2404–2413. https://doi.org/10.1111/pce.12787.

Nekoei, M., Mohammadhosseini, M., Akhlaghi, H., 2012. Chemical composition of the volatile oils from the aerial parts of Artemisia annua L.(Asteraceae) by using head space solid phase microextraction and hydrodistillation methods prior to gas chromatographic-mass spectrometric determination: a comparative investigation. J. Essential Oil Bearing Plants 15, 926–933. https://doi.org/10.1080/0972060X.2012.10662595.

Nibret, E., Wink, M., 2010. Volatile components of four Ethiopian Artemisia species extracts and their in vitro antitrypanosomal and cytotoxic activities. Phytomedicine 17, 369–374. https://doi.org/10.1016/j.phymed.2009.07.016.

Niinemets, Ü., Arneth, A., Kuhn, U., Monson, R.K., Peñuelas, J., Staudt, M., 2010. The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses. Biogeosciences 7, 2203–2223. https://doi.org/10.5194/bg-7-2203-2010.

Niinemets, Ü., Kuhn, U., Harley, P.C., Staudt, M., Arneth, A., Cescatti, A., Ciccioli, P., Copolovici, L., Geron, C., Guenther, A., Kesselmeier, J., Lerdau, M.T., Monson, R.K., Peñuelas, J., 2011. Estimations of isoprenoid emission capacity from enclosure studies: measurements, data processing, quality and standardized measurement protocols. Biogeosciences 8, 2209–2246. https://doi.org/10.5194/bg-8-2209-2011.

Nowak, M., Kleinwächter, M., Manderscheid, R., Weigel, H.J., Selmar, D., 2010. Drought stress increases the accumulation of monoterpenes in sage (Salvia officinalis), an effect that is compensated by elevated carbon dioxide concentration. J. Appl. Bot. Food Qual. 83, 133–136.

Olsson, M.E., Olofsson, L.M., Lindahl, A.L., Lundgren, A., Brodelius, M., Brodelius, P.E., 2009. Localization of enzymes of artemisinin biosynthesis to the apical cells of glandular secretory trichomes of Artemisia annua L. Phytochemistry 70, 1123–1128. https://doi.org/10.1016/j.phytochem.2009.07.009.

Ormeno, E., Goldstein, A., Niinemets, Ü., 2011. Extracting and trapping biogenic volatile organic compounds stored in plant species. Trends Anal. Chem. 30, 978–989. https://doi.org/10.1016/j.trac.2011.04.006.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., et al., 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, p. 151, 10013/epic.45156.d001.

Peñuelas, J., Llusià, J., 1997. Effects of carbon dioxide, water supply, and seasonality on terpene content and emission by Rosmarinus officinalis. J. Chem. Ecol. 23, 979–993. https://doi.org/10.1023/B:JOEC.0000006383.29650.d7.

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

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., Villar, R., 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182, 565–588. https://doi.org/10.1111/j.1469-8137.2009.02830.x.

Portillo-Estrada, M., Copolovici, L., Niinemets, Ü., 2015. Bias in leaf dry mass estimation after oven-drying isoprenoid-storing leaves. Trees 29, 1805–1816. https://doi.org/10.1007/s00468-015-1262-8.

Räisänen, T., Ryyppö, A., Kellomäki, S., 2008. Effects of elevated CO2 and temperature on monoterpene emission of Scots pine (Pinus sylvestris L.). Atmos. Environ. 42, 4160–4171. https://doi.org/10.1016/j.atmosenv.2008.01.023.

Rapparini, F., Miglietta, R., Loreto, F., 2004. Isoprenoid emission in trees of Quercus pubescens and Quercus ilex with lifetime exposure to naturally high CO2 environment. Plant Cell Environ. 27, 381–391.

Rapparini, F., Llusià, J., Peñuelas, J., 2008. Effect of arbuscular mycorrhizal (AM) colonization on terpene emission and content of Artemisia annua L. Plant Biol. 10, 108–122. https://doi.org/10.1055/s-2007-964963.

Rasulov, B., Talts, E., Bichele, I., Niinemets, Ü., 2018. Evidence that isoprene emission is not limited by cytosolic metabolites. Exogenous malate does not invert the reverse sensitivity of isoprene emission to high [CO2]. Plant Physiol. 176, 1573–1586. https://doi.org/10.1104/pp.17.01463.

Sallas, L., Kainulainen, P., Utriainen, J., Holopainen, T., Holopainen, J.K., 2001. The influence of elevated O3 and CO2 concentrations on secondary metabolites of Scots pine (Pinus sylvestris L.) seedlings. Global Change Biol. 7, 303–311. https://doi.org/10.1046/j.1365-2486.2001.00408.x.

Sallas, L., Luomala, E.M., Utriainen, J., Kainulainen, P., Holopainen, J.K., 2003. Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. Tree Physiol. 23, 97–108. https://doi.org/10.1093/treephys/23.2.97.

Sartelet, K.N., Couvidat, F., Seigneur, C., Roustan, Y., 2012. Impact of biogenic emissions on air quality over Europe and North America. Atmos. Environ. 53, 131–141. https://doi.org/10.1016/j.atmosenv.2011.10.046.

Schuurink, R., Tissier, A., 2019. Glandular trichomes: micro-organs with model status? New Phytol. 225, 2251–2266. https://doi.org/10.1111/nph.16283.

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

Sharkey, T.D., Wiberley, A.E., Donohue, A.R., 2007. Isoprene emission from plants: why and how. Ann. Bot. 101, 5–18. https://doi.org/10.1093/aob/mcm240.

Sharkey, T.D., Monson, R.K., 2014. The future of isoprene emission from leaves, canopies and landscapes. Plant Cell Environ. 37, 1727–1740. https://doi.org/10.1111/pce.12289.

Shoor, M., Mondani, F., Aliverdi, A., Golzardi, F., 2012. Interaction effect of CO2 enrichment and nutritional conditions on physiological characteristics, essential oil and yield of lemon balm (Melissa officinalis L.). Not. Sci. Biol. 4. https://www.notulaebiologicae.ro/index.php/nsb/article/view/6682.

Šimpraga, M., Ghimire, R.P., Van Der Straeten, D., Blande, J.D., Kasurinen, A., Sorvari, J., et al., 2019. Unravelling the functions of biogenic volatiles in boreal and temperate forest ecosystems. Eur. J. For. Res. 138, 763–787. https://doi.org/10.1007/s10342-019-01213-2.

Snow, M.D., Bard, R.R., Olszyk, D.M., Minster, L.M., Hager, A.N., Tingey, D.T., 2003. Monoterpene levels in needles of Douglas fir exposed to elevated CO2 and temperature. Physiol. Plantarum 117, 352–358. https://doi.org/10.1034/j.1399-3054.2003.00035.x.

Sporre, M.K., Blichner, S.M., Karset, I.H.H., Makkonen, R., Berntsen, T.K., 2019. BVOC-aerosol-climate feedbacks investigated using NorESM. Atmos. Chem. Phys. 19, 4763–4782. https://doi.org/10.5194/acp-19-4763-2019.

Staudt, M., Joffre, R., Rambal, S., 2003. How growth conditions affect the capacity of Quercus ilex leaves to emit monoterpenes. New Phytol. 158, 61–73. https://doi.org/10.1046/j.1469-8137.2003.00722.x.

Staudt, M., Morin, X., Chuine, I., 2017a. Contrasting direct and indirect effects of warming and drought on isoprenoid emissions from Mediterranean oaks. Reg. Environ. Change 17, 2121–2133. https://doi.org/10.1007/s10113-016-1056-6.

Staudt, M., Bourgeois, I., Al Halabi, R., Song, W., Williams, J., 2017b. New insights into the parametrization of temperature and light responses of mono-and sesquiterpene emissions from Aleppo pine and rosemary. Atmos. Environ. 152, 212–221. https://doi.org/10.1016/j.atmosenv.2016.12.033.

Staudt, M., Byron, J., Piquemal, K., Williams, J., 2019. Compartment specific chiral pinene emissions identified in a Maritime pine forest. Sci. Total Environ. 654, 1158–1166. https://doi.org/10.1016/j.scitotenv.2018.11.146.

Sun, Z., Copolovici, L., Niinemets, Ü., 2012. Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species Populus tremula? J. Plant Res. 125, 263–274. https://doi.org/10.1007/s10265-011-0429-7.

Tan, H., Xiao, L., Gao, S., Li, Q., Chen, J., Xiao, Y., et al., 2015. Trichome and Artemisinin Regulator 1 is required for trichome development and artemisinin biosynthesis in Artemisia annua. Mol. Plant 8, 1396–1411. https://doi.org/10.1016/j.molp.2015.04.002.

Tellez, M.R., Canel, C., Rimando, A.M., Duke, S.O., 1999. Differential accumulation of isoprenoids in glanded and glandless Artemisia annua L. Phytochemistry 52, 1035–1040. https://doi.org/10.1016/S0031-9422(99)00308-8.

Tissier, A., Morgan, J.A., Dudareva, N., 2017. Plant volatiles: going 'in' but not 'out' of trichome cavities. Trends Plant Sci. 22, 930–938. https://doi.org/10.1016/j.tplants.2017.09.001.

Towler, M.J., Weathers, P.J., 2015. Variations in key artemisinic and other metabolites throughout plant development in Artemisia annua L. for potential therapeutic use. Ind. Crop. Prod. 67, 185–191. https://doi.org/10.1016/j.indcrop.2015.01.007.

Turner, G.W., Gershenzon, J., Croteau, R.B., 2000. Development of peltate glandular trichomes of peppermint. Plant Physiol. 124, 665–680. https://doi.org/10.1104/pp.124.2.665.

Vendemiatti, E., Zsögön, A., e Silva, G.F.F., de Jesus, F.A., Cutri, L., Figueiredo, C.R.F., et al., 2017. Loss of type-IV glandular trichomes is a heterochronic trait in tomato and can be reverted by promoting juvenility. Plant Sci. 259, 35–47. https://doi.org/10.1016/j.plantsci.2017.03.006.

Von Caemmerer, S.V., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387. https://doi.org/10.1007/BF00384257.

Vurro, E., Bruni, R., Bianchi, A., di Toppi, L.S., 2009. Elevated atmospheric CO_2 decreases oxidative stress and increases essential oil yield in leaves of Thymus vulgaris grown in a mini-FACE system. Environ. Exp. Bot. 65, 99–106. https://doi.org/10.1016/j.envexpbot.2008.09.001.

Wilkinson, M.J., Monson, R.K., Trahan, N., Stanfield, L., Brown, E., Jackson, R.B., Polley, H.W., Fay, P.A., Fall, R., 2009. Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. Global Change Biol. 15, 1189–1200. https://doi.org/10.1111/j.1365-2486.2008.01803.x.

Woerdenbag, H.J., Bos, R., Salomons, M.C., Hendriks, H., Pras, N., Malingré, T.M., 1993. Volatile constituents of artemisia annua L.(Asteraceae). Flavour Fragrance J. 8, 131–137. https://doi.org/10.1002/ffj.2730080303.

Wright, G.A., Schiestl, F.P., 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. Funct. Ecol. 23, 841–851. https://doi.org/10.1111/j.1365-2435.2009.01627.x.

Wu, W., Yuan, M., Zhang, Q., Zhu, Y., Yong, L., Wang, W., Qi, Y., Guo, D., 2011. Chemotype-dependent metabolic response to methyl jasmonate elicitation in Artemisia annua. Planta Med. 77, 1048–1053. https://doi.org/10.1055/s-0030-1250744.

Xiao, L., Tan, H., Zhang, L., 2016. Artemisia annua glandular secretory trichomes: the biofactory of antimalarial agent artemisinin. Sci. Bull. 61, 26–36. https://doi.org/10.1007/s11434-015-0980-z.

Zhang, H., Yee, L.D., Lee, B.H., Curtis, M.P., Worton, D.R., Isaacman-VanWertz, G., et al., 2018. Monoterpenes are the largest source of summertime organic aerosol in the southeastern United States. Proc. Natl. Acad. Sci. Unit. States Am. 115, 2038–2043. https://doi.org/10.1073/pnas.1717513115.

Ziemann, P.J., Atkinson, R., 2012. Kinetics, products, and mechanisms of secondary organic aerosol formation. Chem. Soc. Rev. 41, 6582–6605. https://doi.org/10.1039/C2CS35122F.

Zvereva, E.L., Kozlov, M.V., 2006. Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis. Global Change Biol. 12, 27–41. https://doi.org/10.1111/j.1365-2486.2005.01086.x.