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# Seasonal and diurnal variations of plant isoprenoid emissions from two dominant species in Mediterranean shrubland and forest submitted to experimental drought

Zhaobin Mu<sup>1,2</sup>, Joan Llusà<sup>1,2</sup>, Daijun Liu<sup>1,2</sup>, Romà Ogaya<sup>1,2</sup>, Dolores Asensio<sup>1,2</sup>, Chao Zhang<sup>1,2</sup>, Josep Peñuelas<sup>1,2</sup>

<sup>1</sup>CSIC, Global Ecology Unit CREAM-CSIC-UAB, E08193 Bellaterra, Catalonia, Spain

<sup>2</sup>CREAF, E08193 Cerdanyola, Catalonia, Spain

## Abstract

We tested the effect of increasing drought conditions in the Mediterranean Basin on isoprenoid emissions for the coming decades by analyzing their effect experimentally on the dominant Mediterranean species *Erica multiflora* in a Garraf shrubland and *Quercus ilex* in a Prades forest in Catalonia (Spain). Drought was simulated in Garraf using automatically sliding curtains to decrease the amount of soil moisture by 5% and in Prades by partial rainfall exclusion and runoff exclusion for a 25% decrease. We measured photosynthetic rates (A), stomatal conductance (g<sub>s</sub>) and rates of isoprenoid emission in the morning and at midday for four seasons and determined the relationship of emission rates with environmental conditions. Terpenes were emitted by both species, but only *E. multiflora* emitted isoprene.  $\alpha$ -Pinene and limonene were the most abundant terpenes. Isoprenoid emissions increased with air temperature and generally decreased as the amount of soil moisture increased. The results of this study suggest that higher isoprenoid emissions can be expected in the warmer and drier conditions predicted for the coming decades in the Mediterranean region.

**Keywords** Mediterranean ecosystems; Experimental drought; Seasonal variations; Diurnal variations; BVOC emissions; Isoprenoids; *Erica multiflora*; *Quercus ilex*.

## 1. Introduction

Mediterranean-type ecosystems provide important ecological services, such as the conservation of biodiversity and nutrient cycling (Peñuelas et al., 2013; Seddon et al., 2016). Precipitation is low for these ecosystems, especially in hot periods, and climate change has contributed to the increasing drought in recent decades (Llusà et al., 2011). Models of global circulation, climate and ecophysiology predict a further reduction in the availability of water in Mediterranean regions around the world (Piñol et al., 1998; IPCC, 2007; Sabaté, et al., 2002; Peñuelas and Boada, 2003), which are naturally water-limited (Sardans and Peñuelas, 2007) due to the high temperatures and the consequent high rates of evapotranspiration (Peñuelas and Llusà, 2001). Drought stress can affect numerous physiological and biochemical processes governing plant growth, leading to a reduction in stem elongation, leaf expansion and stomatal conductance (Daie and Patrick, 1988; Alexieva et al., 2001; Liu et al., 2016). Plants, however, can survive these hydropenic stress conditions after long periods of acclimation (Chaves et al., 2002; Bai et al., 2008; Rubio-Casal et al., 2010) by adjusting their metabolism (Hsiao, 1973) and reorganizing their energy resources (Dobrota, 2006), including changes in photosynthetic rate (A) and stomatal conductance (g<sub>s</sub>). Biogenic volatile organic

compounds (BVOCs) are also an important tool for resisting drought (Peñuelas and Llusà, 2003; Filella et al., 2007; Porcar-Castell et al., 2009).

Approximately half of all plant species growing in Mediterranean-type ecosystems, especially shrubland and forest ecosystems, produce and emit a large variety of BVOCs (Peñuelas and Llusà, 1999). These compounds are formed in various plant organs such as flowers, fruits, leaves, bark and roots (Niederbacher et al., 2015) during diverse physiological processes (Laothawornkitkul et al., 2009), which are then emitted directly or stored in specialized structures (Loreto and Schnitzler, 2010). BVOCs provide protection against high temperatures, high irradiation, oxidative stress and drought stress (Velikova et al., 2005; Holopainen and Gershenzon, 2010; Loreto and Schnitzler, 2010). They can also act as herbivore deterrents, attractants of pollinators and enemies of herbivores (Peñuelas and Munné-Bosch, 2005; Llusà and Peñuelas, 2000) and plant–plant communication signals (Peñuelas and Llusà, 2003). BVOCs are thus a vital and central component of plants due to their significance to the survival of individual plants but in addition they exert a strong influence on atmospheric chemistry (Dicke and Loreto, 2010; Seco et al., 2013; Niederbacher et al., 2015). BVOCs play a key role in atmospheric processes that influence the atmospheric burden of pollutants (Kroll and Seinfeld, 2008), which can also interact with climate change in several ways (Peñuelas and Llusà, 2003; Yuan et al., 2009; Riipinen et al., 2012). BVOCs are the main biogenic precursors of ozone and by consuming hydroxyl radical prolong the persistence of other compounds such as the greenhouse gas methane (Di Carlo et al., 2004). Furthermore, the photo-oxidation of BVOCs generates secondary organic aerosols, which have the potential for complex climatic feedbacks (Claeys et al., 2004; Carslaw et al., 2009).

BVOCs are very diverse and consist of various organic classes such as isoprenoids, fatty acid derivatives, alcohols, alkanes, alkenes, esters and acids (Kreuzwieser et al., 1999; Peñuelas and Llusà, 2001). Isoprenoids such as isoprene and monoterpenes, the most common and abundant BVOCs, confer protection against the high temperatures and drought stress under the current trend of climatic warming (Peñuelas and Llusà, 2001; Copolovici et al., 2005).

Several studies have demonstrated the importance of abiotic and biotic factors for the emissions of BVOCs (Peñuelas and Llusà, 2001 and 2003; Paris et al., 2010). Among abiotic factors, water availability has a strong effect on BVOC emission, especially under Mediterranean conditions characterized by long dry summers with high solar irradiation and temperatures (Tsakiris et al., 2007; Llusà et al., 2011 and 2013). Plant behavior is complex under these integrated environmental influences and may differ among biological species. Rates of isoprenoid emission increase and help plants to resist stress under moderate drought conditions but decrease under severe drought conditions (Gershenzon et al., 1978; Llusà et al., 2011 and 2013; Hansen and Seufert, 1999).

The dominant tree species in the Mediterranean Basin have two patterns of terpene emission depending on if they have the ability to store terpenes (Lerdau et al., 1997; Llusà and Peñuelas, 2000). Pool size in resin ducts and internal or external glands in terpene-storing species (Lerdau et al., 1997; Peñuelas and Llusà, 2001; Llusà et al., 2014) affect emission rates, and the short-term response of terpene-emission rates to photosynthetic rates may be stronger and faster in non-storing than storing species (Gershenzon et al., 1978; Staudt and Seufert, 1995). Terpene-emission rates in terpene-storing plants, though, are not necessarily determined by terpene concentration; their response to drought can be involved in the short-

term control of emissions, either increasing (Rennenberg et al., 2006) or decreasing (Bertin and Staudt, 1996; Llusà et al., 2006) the emission rates depending on the intensity of the water stress (Llusà and Peñuelas, 2000; Llusà et al., 2011).

Climatic experiments have been widely used on various time scales to predict the potential physiological and phenological changes in plants under simulated future climatic scenarios (Beier et al., 2012; Leuzinger et al., 2011; De Boeck et al., 2015; Ogaya et al., 2014). Numerous field experiments in various ecosystems have demonstrated the effectiveness of identifying the physiological adjustments of plants in response to climate change, despite the variety and complexity of the environmental conditions (Prieto et al., 2009; Limousin et al., 2010; Liu et al., 2016). The short-term diurnal (Peñuelas and Llusà, 1999) and long-term seasonal (Guenther, 1997; Llusà and Peñuelas, 2000) cycles under experimental drought also determine the status of isoprenoid emission (Llusà and Peñuelas, 2000; Llusà et al., 2006). Variations in emissions have been linked to corresponding changes in temperature, radiation, air humidity and water availability (Llusà et al., 2006) but also to leaf development and physiological activity (Llusà and Peñuelas, 2000). These factors are also involved in the variations among isoprenoids due to their diverse physicochemical traits (Llusà and Peñuelas, 2000).

We studied the net photosynthetic rates ( $A$ ), the stomatal conductance ( $g_s$ ) and the rates of isoprenoid emissions in the shrub *Erica multiflora* L. and the tree *Quercus ilex* L., which are widely distributed in the western and central Mediterranean Basin and are among the dominant species at our two study sites, Garraf (shrubland) and Prades (forest), respectively (Llusà et al., 2006 and 2013; Ogaya et al., 2014). Our aims were to determine the relationship between plant physiology and abiotic factors under Mediterranean field conditions, especially gas exchange and isoprenoid emissions, for predicting the effects of increasing drought stress expected in the coming decades and to improve the algorithms for isoprenoid emission used in models.

## 2. Material and methods

### 2.1. Study sites and species descriptions

The study was carried out in the Garraf and Prades Mountains in Catalonia, northeastern Spain. The climate and vegetation at the two sites are typically Mediterranean. Annual rainfalls were 510.2 mm in Garraf and 661.4 mm in Prades during the measurement year.

Garraf Natural Park is a dry shrubland (Rosmarino-Ericion) south of Barcelona (41°18'08"N, 1°49'05"E; 210 m a.s.l.). This site suffered large fires in the summers of 1982 and 1994, the regenerated vegetation has a coverage of 50–60% and a maximum height of 70 cm. The dominant species at the study site are *Erica multiflora* L., *Globularia alypum* L., *Pinus halepensis* L. and *Rosmarinus officinalis* L. (Llusà et al., 2006 and 2013). All are common evergreens of the coastal shrubland in the western Mediterranean Basin. We chose one dominant species *Erica multiflora* L. as research object in this site.

The Prades Mountains are in southwestern Catalonia (41°20'42"N, 1°02'04"E; 970 m a.s.l.) and about 30 km from the Mediterranean coast. The Prades sampling site is a holm oak forest with tree heights between 1.5 and 10 m, dominated by *Quercus ilex* (Boldòs and Vigo, 1990; Llusà et al., 2013; Ogaya et al., 2014). The site contains other important evergreen tree and shrub species (*Phillyrea latifolia* L., *Arbutus unedo* L., *Pinus sylvestris* L., *Erica arborea* L. and *Juniperus oxycedrus* L.) and deciduous species such as

*Sorbus torminalis* L. and *Acer monspessulanum* L. (Llusià et al., 2013). We chose the dominant species *Quercus ilex* as research object in this site.

## 2.2. Experimental design

The drought experiments were carried out from 1999 to 2014 (16 years) for both sites. In Garraf, six plots (5 × 4 m) were randomly organized in three blocks for replication, with each block having one drought and one control plot. Transparent and waterproof plastic curtains were activated in the drought treatments by rain sensors to cover the plants and soil during rain for four seasons. Control plots had the same scaffolding but without the curtains. All measurements were conducted in the central 12 m<sup>2</sup> to reduce margin effects. The outer 0.5 m of each plot served as an open buffer zone.

In Prades, four plots (15 × 10 m) were delimited at the same altitude along the slope, two as drought and two as control plots. The drought treatment consisted of partial rain exclusion using PVC strips suspended 0.5–0.8 m above the soil covering approximately 30% of the total plot surface. A ditch 0.8 m deep was excavated along the entire top edge of the drought plots to intercept runoff water.

Emissions were measured in winter 2014 (12, 13 and 14 February in Garraf and 23, 24 and 25 January in Prades), spring 2014 (1, 2 and 3 May in Garraf and 14, 15 and 16 May in Prades), summer 2014 (5, 7 and 9 August in Garraf and 29, 30 and 31 July in Prades) and autumn 2014 (29 and 30 October and 1 November in Garraf and 21, 22 and 23 October in Prades) in the morning (9:00–13:00 solar time) and at midday (13:00–17:00 solar time). Emissions from sunlit and healthy *E. multiflora* needle clusters and *Q. ilex* leaves were measured from three random plants in each plot. Air temperature was measured by an automatic meteorological station, and soil moisture was measured by time domain reflectometry (Tektronix 1502C, Beaverton, United States), both about every 30 min on the day of sampling.

## 2.3. Gas-exchange measurements and sampling of isoprenoid emissions

A and  $g_s$  were measured and isoprenoid emissions were collected simultaneously using a Licor-6400XT (4647 Superior Street P.O. Box 4425 Lincoln, Nebraska USA) gas-exchange system. A and  $g_s$  were measured at a quantum flux density of  $1080 \pm 19 \mu\text{mol m}^{-2} \text{s}^{-1}$  under a controlled CO<sub>2</sub> concentration of  $400 \pm 2$  ppm. One *E. multiflora* needle cluster or one *Q. ilex* leaf was enclosed in a clamp-on gas-exchange cuvette with a surface area of 2 cm<sup>2</sup>. The isoprenoids were collected by pumping air which was generated using a Qmax air-sampling pump (Supelco, Bellefonte, USA) from the cuvette through a glass cartridge (8 cm long and 0.3 cm internal diameter). Sampling time was 10 min, and the flow was about 400 mL min<sup>-1</sup>. The cartridges were manually filled with adsorbents Carbotrap B, Carboxen 1003 and Carbopack Y (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool. The hydrophobic properties of the activated adsorbents minimized any sample displacement by water, without chemical transformation in the tube. Isoprenoid concentrations were determined by reference to trapped standards ( $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, camphene, myrcene, limonene, sabinene, camphor and dodecane). The tubes were conditioned before terpene sampling with a stream of purified helium for 35 min at 350 °C. The trapping and desorption efficiencies of liquid and volatilized standards such as  $\alpha$ -pinene, 3-carene or limonene (the main terpenes accounting for about 65–85% of total emission) were practically 100%. Blank samples of air without leaves in the cuvette were collected for 10 min immediately before each measurement. Sampled leaves were cut

and stored in a portable cooler box at 4 °C and taken to the laboratory where they were oven-dried at 60 °C to constant weights. The metallic tubes (with trapped BVOCs) were stored at -22 °C until analysis.

#### 2.4. Isoprenoid analyses

The isoprenoids were analyzed using a GC-MS system (HP59822B, Hewlett Packard, Palo Alto, USA) with an automatic sample processor (Combi PAL, FOCUS-ATAS GL International BV 5500 AA Veldhoven, The Netherlands). The desorber was an OPTIC3 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands), and the temperature was increased at 16 °C s<sup>-1</sup> from 60 °C to 300 °C. The desorbed isoprenoids were cryofocused at -20 °C for 2 min after which the cryotrap was heated rapidly to 250 °C, and conducted into a 30 m × 0.25 mm × 0.25 µm film capillary column (HP-5, Crosslinked 5% pH Me Silicone; Supelco, Bellefonte, USA). The flow of helium was 1 mL min<sup>-1</sup>, and the total run time was 29 min including the solvent delay for about 4 min. The initial oven temperature was increased at 30 °C min<sup>-1</sup> from 40 to 60 °C and then at 10 °C min<sup>-1</sup> to 150 °C, maintained for 3 min, increased at 70 °C min<sup>-1</sup> to 250 °C and maintained for another 5 min.

The monoterpenes were identified by comparing their retention times with those of standards from Fluka (Buchs, Switzerland), published spectra, GCD ChemStation G1074A HP and the Wiley7n mass-spectra library. Terpene concentrations were determined from calibration curves for common terpenes such as  $\alpha$ -pinene, 3-carene,  $\beta$ -pinene, myrcene, limonene, sabinene and  $\alpha$ -humulene, every five analyses using three terpene concentrations (always  $r^2 > 0.99$  for the relationships between the signal and terpene concentrations). The most abundant terpenes had very similar sensitivities, with differences <5% among the calibration factors.

#### 2.5. Statistical analyses

The ANOVAs were conducted using STATISTICA v.8.0 for Windows (StatSoft, Inc. Tulsa, USA). Statistical differences between treatments were analyzed with a Student's *t*-test. Differences were considered significant at  $P < 0.05$ . The analysis of the effects of season, treatment and sampling time were conducted by repeated measurements ANOVA. Regression analyses were conducted using Sigma Plot v. 11.0 for Windows (Systat Software, Chicago, USA).

### 3. Results

#### 3.1. Seasonal and diurnal variation of air temperature and soil moisture

Mean air temperature on the sampling dates in Garraf ranged between 14.7 ± 1.17 °C in winter mornings and 35.3 ± 0.51 °C at summer middays. Soil moisture ranged between 7.2 ± 0.33% (v/v) at summer middays and 24.7 ± 1.40% (v/v) in winter mornings (Fig. 1). Compared to control treatment, the drought treatment decreased soil moisture an average of 3.7% in mornings and 4.7% at midday throughout the year, with decreases ranging between 1.3% in winter mornings and 14.7% in spring afternoons.

Mean air temperature on the sampling dates in Prades ranged between 9.4 ± 0.84 °C in winter mornings and 31.8 ± 0.97 °C at summer middays. Soil moisture ranged between 2.4 ± 0.28% (v/v) at summer middays and 38.2 ± 2.69% (v/v) in winter mornings (Fig. 1). Compared to control treatment, the drought treatment

significantly decreased soil moisture an average of 26.2% in mornings and 25.7% at midday throughout the year, with decreases ranging between 21.0% in winter mornings and 48.8% at summer middays.

### 3.2. Seasonal and diurnal variation of A and $g_s$

A and  $g_s$  for *E. multiflora* seasonally varied similarly (Fig. 2A). A and  $g_s$  were highest in autumn mornings, at 6.7–7.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 0.079–0.090  $\text{mol m}^{-2} \text{s}^{-1}$ , and were lowest at winter middays, at 1.2–2.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 0.020–0.027  $\text{mol m}^{-2} \text{s}^{-1}$ , respectively. A and  $g_s$  tended to be lower in the drought than the control treatments in most seasons but were significantly lower only at autumn midday for A ( $P < 0.05$ ) and summer midday for  $g_s$  ( $P < 0.05$ ).

A and  $g_s$  for *Q. ilex* were highest in spring and winter mornings (Fig. 2B), at 9.2–10.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 0.208–0.215  $\text{mol m}^{-2} \text{s}^{-1}$ , and lowest at autumn middays at 2.8–4.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 0.028–0.064  $\text{mol m}^{-2} \text{s}^{-1}$ , respectively. A in the drought treatments was significantly highest in winter mornings ( $P < 0.05$ ), and A and  $g_s$  were significantly lowest in spring mornings ( $P < 0.05$ ) and at autumn middays ( $P < 0.05$ ), respectively.

### 3.3. Seasonal and diurnal variation of isoprenoid emissions

Isoprene was the main compound emitted by *E. multiflora*. The emission rates ranged between  $0.40 \pm 0.15 \mu\text{g g}^{-1} \text{dw h}^{-1}$  at winter middays and  $4.77 \pm 1.51 \mu\text{g g}^{-1} \text{dw h}^{-1}$  at summer middays (Fig. 3). The drought treatments did not affect isoprene-emission rates except for decreasing them when they were already low at winter midday ( $P < 0.05$ ). Isoprene emission was not detected for *Q. ilex*. Both species, however, emitted volatile terpenes.  $\alpha$ -Pinene and limonene were the two most abundant terpenes for both species and were detected in all periods both sampling times, with trends similar to those for total terpene emission. Total terpene emissions for *E. multiflora* were very low, ranging between  $0.10 \pm 0.05 \mu\text{g g}^{-1} \text{dw h}^{-1}$  in winter mornings and  $1.05 \pm 0.32 \mu\text{g g}^{-1} \text{dw h}^{-1}$  at summer middays (Fig. 4A). Emissions did not globally differ significantly between the treatments but were lower in the drought plots at summer midday.  $\alpha$ -Pinene and limonene were emitted mostly at summer midday, at about 0.7 and 0.3  $\mu\text{g g}^{-1} \text{dw h}^{-1}$ , respectively. *Q. ilex* emitted terpenes at much higher rates than *E. multiflora*, ranging between  $1.8 \pm 0.3 \mu\text{g g}^{-1} \text{dw h}^{-1}$  at winter middays and  $44.1 \pm 3.2 \mu\text{g g}^{-1} \text{dw h}^{-1}$  at summer middays (Fig. 4B). Overall terpene emissions from *Q. ilex* were 19.3% higher in the morning and 35.5% higher at midday in the drought than the control treatments. Total terpene emissions from *Q. ilex* were significantly higher in the drought treatments in summer mornings ( $P < 0.05$ ) and at summer middays ( $P < 0.01$ ) by 39.7% and 68.0%, coinciding with significantly higher  $\alpha$ -pinene ( $P < 0.05$ ) and limonene ( $P < 0.05$ ) emissions, respectively.  $\alpha$ -Pinene and limonene were emitted mostly at summer middays, at rates of about  $13 \mu\text{g g}^{-1} \text{dw h}^{-1}$  for both.

## 4. Discussion

Plants in Mediterranean-type climates have similar physiological trends, with A and  $g_s$  highest in spring or autumn when environmental conditions are favorable (Llusià et al., 2013; Liu et al., 2016). A and  $g_s$  for *Q. ilex* in our study were highest in winter, probably due to the extremely uneven distribution of water availability in 2013–2014 (Fig. 1). A and  $g_s$  tended to decrease from winter to summer with increasing heat and drought (Fig. 2B). Plants generally accumulate carbon under moderate drought stress due to growth

restriction by water deficiency but may temporarily decrease photosynthetic activity when water stress is severe because of the increased resistance to CO<sub>2</sub> in both the stomata and mesophyll (Centritto et al., 2003; Ogaya and Peñuelas, 2003; Llusà et al., 2006). These low rates of photosynthesis and stomatal conductance indicate that plants can successfully adapt to severe stress caused by extreme climatic environments by slowing growth and reproduction (Llusà et al., 2013; Matesanz and Valladares 2014; Bussotti et al., 2015).

The emission of isoprenoids differed between the species (Llusà and Peñuelas, 2000; Peñuelas and Llusà, 2001) but followed a similar seasonal pattern (Figs. 3, 4A and B). The seasonal pattern agreed with previous results of isoprenoid emissions in most Mediterranean species, with a maximum in summer and a minimum in cold seasons (Llusà and Peñuelas, 2000; Llusà et al., 2011). The seasonality is due to the dependence of metabolic regulation on many abiotic factors, but temperature is likely the dominant driver of emission (Llusà and Peñuelas, 2000).

Isoprenoid-emission rates for *E. multiflora* were inside the range of 0.5–20 µg g<sup>-1</sup> dw h<sup>-1</sup> reported by Owen et al. (1997) and also inside the ranges for isoprene emissions of 0.15–4.4 µg g<sup>-1</sup> dw h<sup>-1</sup> and monoterpene emissions of 0.08–0.4 µg g<sup>-1</sup> dw h<sup>-1</sup> reported by Llusà et al. (2009). 2014 was a relatively wet and warm year for Garraf (Fig. 1). The drought treatment decreased soil moisture by only about 5%, and emissions were similar in all seasons except summer (Figs. 3 and 4A). The differences of emission rates between treatments may have been due to a combination of factors dominated by temperature variation. The small differences in soil moisture caused little change in plant physiology and did not substantially influence emission rates on a yearly scale.

*Q. ilex* is a non-stored species with strong emission capacity of terpenes, especially in hot seasons, and is highly sensitive to drought (Llusà and Peñuelas, 2000; Loreto et al., 2001; Llusà et al., 2011; Llusà et al., 2013). Heat and water distribution were extremely unbalanced in Prades (Fig. 1). Terpene-emission rates for *Q. ilex* were higher than previously reported (Llusà et al., 2011), especially for the temperate seasons due to the warmer and drier environmental conditions, but the drought treatment had no significant effects, probably because the treatment was not severe enough for *Q. ilex* to adjust emissions for adapting to drought. The plants were able to maintain a stable status after a long acclimation to a moderate drought in these seasons (Fig. 4B). Terpene emissions from *Q. ilex*, however, were significantly higher only in summer in the drought treatment. An increase in emission in response to moderate drought has also been reported in other studies (Llusà et al., 2006; Llusà et al., 2011) and supports the existence of an interaction between drought and high temperature (Blanch et al., 2009). The increase in terpene emissions under drought conditions may be also attributed to a combination of other factors such as high radiation and temperature (Osmond et al., 1997; Llusà et al., 2006) for avoiding damage to cellular membranes caused by oxygenated products generated under summer stressful environmental conditions (Gershenzon et al., 1978; Peñuelas and Llusà, 2001; Loreto et al., 2001).

The monoterpenes α-pinene and limonene were the main terpenes emitted by the two species. Their emission trends were similar to those for total terpenes, with a maximum in summer and a minimum in winter (Fig. 4A and B). α-Pinene and limonene emissions from *E. multiflora* were low and lower in the drought treatments than in control treatments at summer midday (Fig. 4A). α-Pinene emission from *Q. ilex* was highly sensitive to temperature, increasing sharply from winter to spring (Fig. 4B), and the emission rates even increased ( $P < 0.05$ ) with air temperature in the control treatment in spring mornings (Figs. 1



and 4B), indicating that temperature was a more powerful driving force than moderate drought on  $\alpha$ -pinene emissions. Limonene emission responded strongly to water deficiency, most obviously at the driest summer midday ( $P < 0.001$ ), coinciding with a significantly higher emission ( $P < 0.01$ ) (Fig. 4B). The increased emissions of the two main terpenes in response to temperature and moderate drought has also been found in other studies (Bertin and Staudt, 1996; Llusà et al., 2011). Not all terpene emissions, however, were higher in the drought treatment in summer. The various responses may have been due to the activities of synthases and to the potential protective roles of the various terpenes under drought conditions (Blanch et al., 2009).

Photosynthetic rates and stomatal conductance were higher in both species in the morning than at midday for most seasons, but isoprenoid emissions had the opposite trends (Fig. 2A and B). A and g<sub>s</sub> ( $P < 0.01$ ) and isoprene emissions ( $P < 0.05$ ) for *E. multiflora* differed significantly between the two diurnal periods, and drought treatments in spring to autumn days decreased photosynthetic rates and stomatal conductance ( $P < 0.05$ ) more at midday (Fig. 2A). These two opposite trends identified an important aspect of photosynthetic carbon fixation at midday that is still used for having a larger terpene production than in the morning (Peñuelas and Llusà 1999; Vallat et al., 2005; Blanch et al., 2009), and emissions in the drought treatment were much higher at summer midday (Figs. 3, 4A and B). However, isoprenoid emissions from plants generally do not only depend on the current environmental drivers, but also the preceding environmental and physiological status (Llusà et al., 2013; Tiiva et al., 2017) which may also suggest a high emission potential at more severe midday conditions if there is high A in morning (Fig. 2, 3 and 4). The higher percentages of fixed carbon devoted to terpene emission at midday also indicate a successful adaptation of plants by adjusting metabolism under environmental stress (Litvak et al., 1996; Loreto et al., 2001).

Environmental conditions such as air temperature and soil moisture are the main factors that determine plant physiological responses, including BVOC emissions (Llusà et al., 2006, 2009 and 2011; Peñuelas and Llusà, 2001; Filella, et al., 2007; Blanch et al., 2009). We analyzed the corresponding correlations and generalized them with linear or exponential algorithms (Table 1). The emission rates of the main isoprenoids were correlated positively with air temperature and negatively with soil moisture for both species (Fig. 5). The relationships with environmental conditions were stronger for *Q. ilex* than *E. multiflora*. The production of isoprenoids has been linked to an increased tolerance to water stress in some species (Peñuelas and Llusà, 2001; Blanch et al., 2009), and plants under severe drought conditions may even decrease their emissions (Llusà and Peñuelas, 2000). These results indicate that higher isoprenoid emissions can be expected in the warmer and drier conditions projected by climatic and ecophysiological models for the coming decades in the Mediterranean region (Peñuelas and Llusà, 2001; IPCC, 2014). The most widely used Model of Emissions of Gasses and Aerosols from Nature (MEGAN) model (Guenther et al., 2012) initially estimated the emissions depending on species-specific capacities of foliar emissions, light and temperature (Bertin and Staudt, 1996; Guenther et al., 2012; Llusà et al., 2013). In its latest version, MEGAN2.1 (Guenther et al., 2012) has included a simple drought algorithm for isoprene emissions derived from the observations of Pegoraro et al. (2004). This improvement of the model MEGAN could still not describe the actual emission rates in response to drought since that algorithm only predicts reduction in emissions (Guenther et al., 2012) and not possible increases at mild drought conditions. It cannot,

moreover, account properly for the seasonal variation of the enzymatic activity regulating the basal emission factor (BEF) employed in the model (Schnitzler et al. 1996; Brillì et al., 2016). Neglecting seasonal drought stress could lead to large misestimates of drought influences on isoprenoid emissions. Failing to fully take into consideration the capacity of plants to acclimate, which varies widely among seasons and even within a season if the environment changes, may also lead to misestimates. Although previous studies have shown that terpenes, especially for monoterpenes and sesquiterpenes, are to a large extent emitted in a manner similar to that of isoprene depending on both temperature and light (Sindelarova et al. 2014), an improvement of current models is also required to better predict the dynamics of both basal and total isoprenoid emissions, especially under the increasing intensity of drought stress (Filella et al., 2018; IPCC, 2014). The trends of isoprenoid emissions are very important due to their potential roles in plant flammability (Alessio et al., 2008; Llusà et al., 2011) and atmospheric chemical processes contributing to ozone formation and even affecting climate (Thompson, 1992; Peñuelas and Llusà, 2003; Dicke and Loreto, 2010; Seco et al., 2013).

## Acknowledgements

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## Figure captions

**Fig. 1.** Seasonal morning and midday time-courses of mean air temperature and soil moisture in Garraf and Prades. Error bars indicate standard errors of the means;  $n = 6$  (\*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$  indicate significant differences between treatments identified by Student's  $t$ -tests). The significances of the effects of season, treatment and sampling time (repeated measurements ANOVA) are depicted in each panel.

**Fig. 2.** Seasonal net photosynthetic rates and stomatal conductances for *E. multiflora* (A) and *Q. ilex* (B) in the morning and at midday. Error bars indicate standard errors of the means;  $n = 9$  (\*  $P < 0.05$  indicates significant differences between treatments identified by Student's  $t$ -tests). The significances of the effects of season, treatment and sampling time (repeated measurements ANOVA) are depicted in each panel.

**Fig. 3.** Seasonal time courses of the rate of isoprene emission for *E. multiflora*. Error bars indicate standard errors of the means;  $n = 6$  (\*  $P < 0.05$  indicates significant differences between treatments identified by Student's  $t$ -tests). The effects of season, treatment and sampling time are depicted in the panels when significant.

**Fig. 4.** Seasonal time courses for the rates of emission of total terpenes,  $\alpha$ -pinene and limonene for *E. multiflora* (A) and *Q. ilex* (B). Error bars indicate standard errors of the means;  $n = 6$  (\*  $P < 0.05$  and \*\*  $P < 0.01$  indicate significant differences between treatments identified by Student's  $t$ -tests). The effects of season, treatment and sampling time are depicted in the panels when significant.

**Fig. 5.** Relationships for the emissions of the main isoprenoids with air temperature and soil moisture for *E. multiflora* and *Q. ilex*.

## Table caption

**Table 1.** Relationships for the emissions of the main isoprenoids (isoprene for *E. multiflora* and total terpenes for *Q. ilex*) with air temperature and soil moisture (SE, standard error).

## References

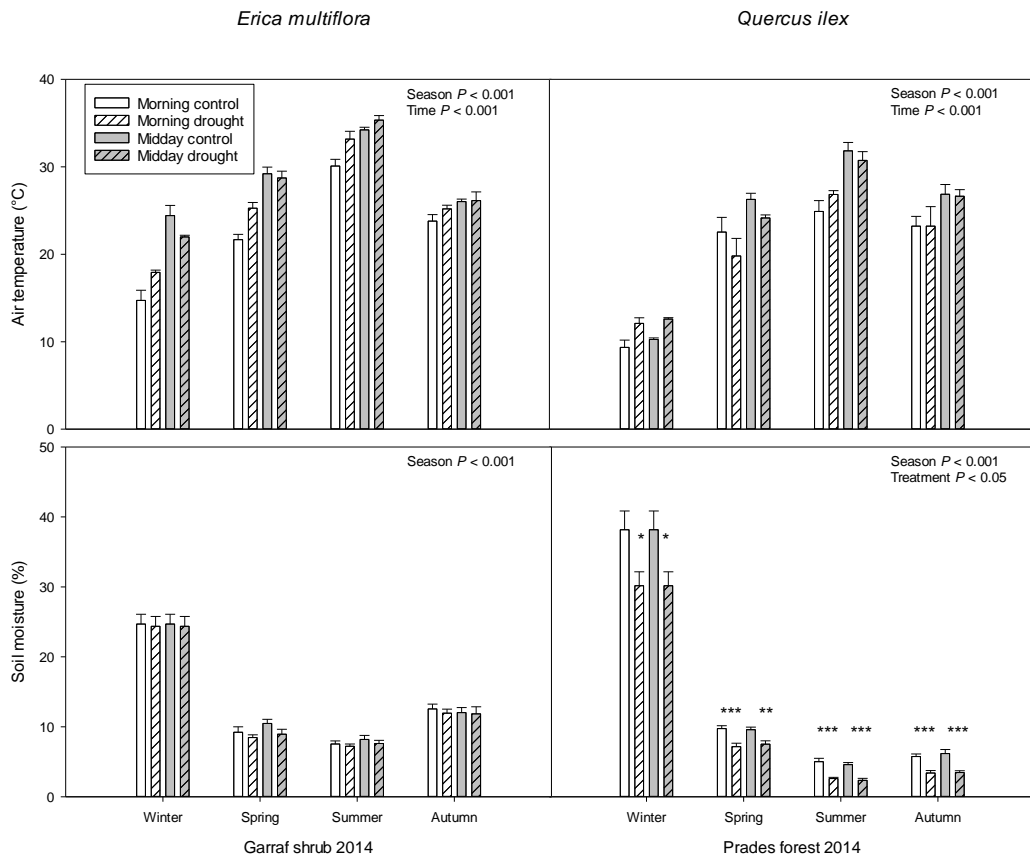
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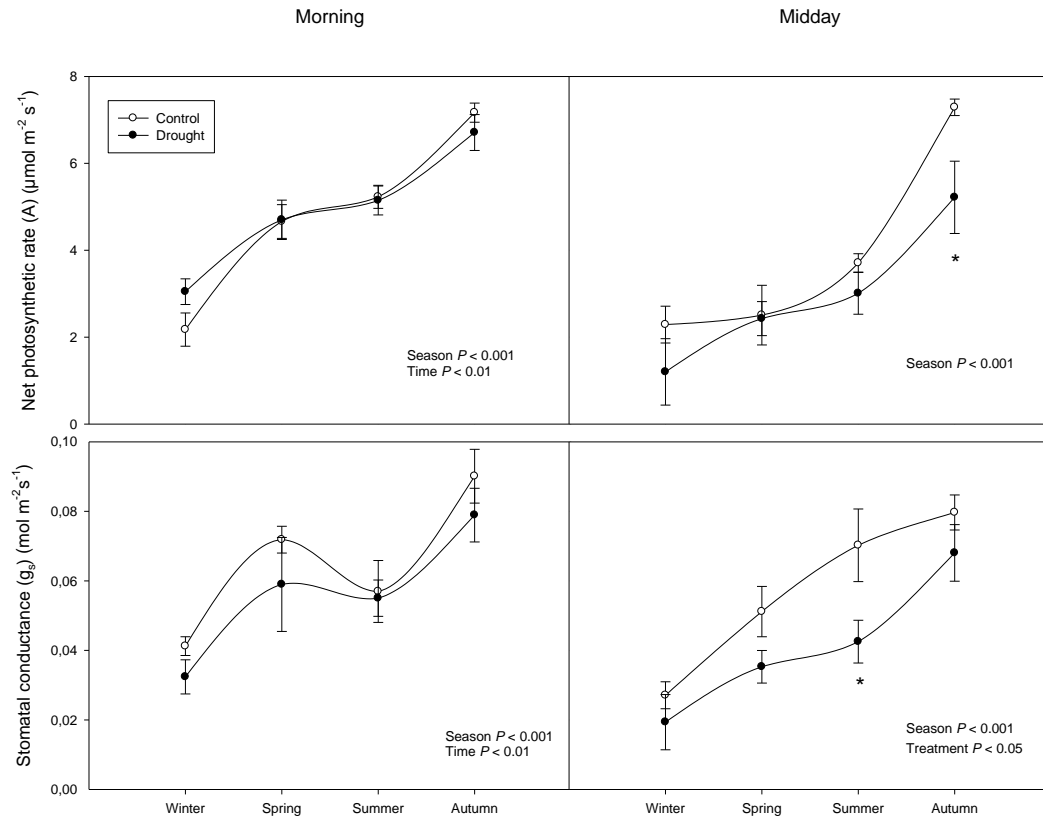


**Fig. 1.** Seasonal morning and midday time-courses of mean air temperature and soil moisture in Garraf and Prades. Error bars indicate standard errors of the means;  $n = 6$  (\*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$  indicate significant differences between treatments identified by Student's t-tests). The significances of the effects of season, treatment and sampling time (repeated measurements ANOVA) are depicted in each panel.



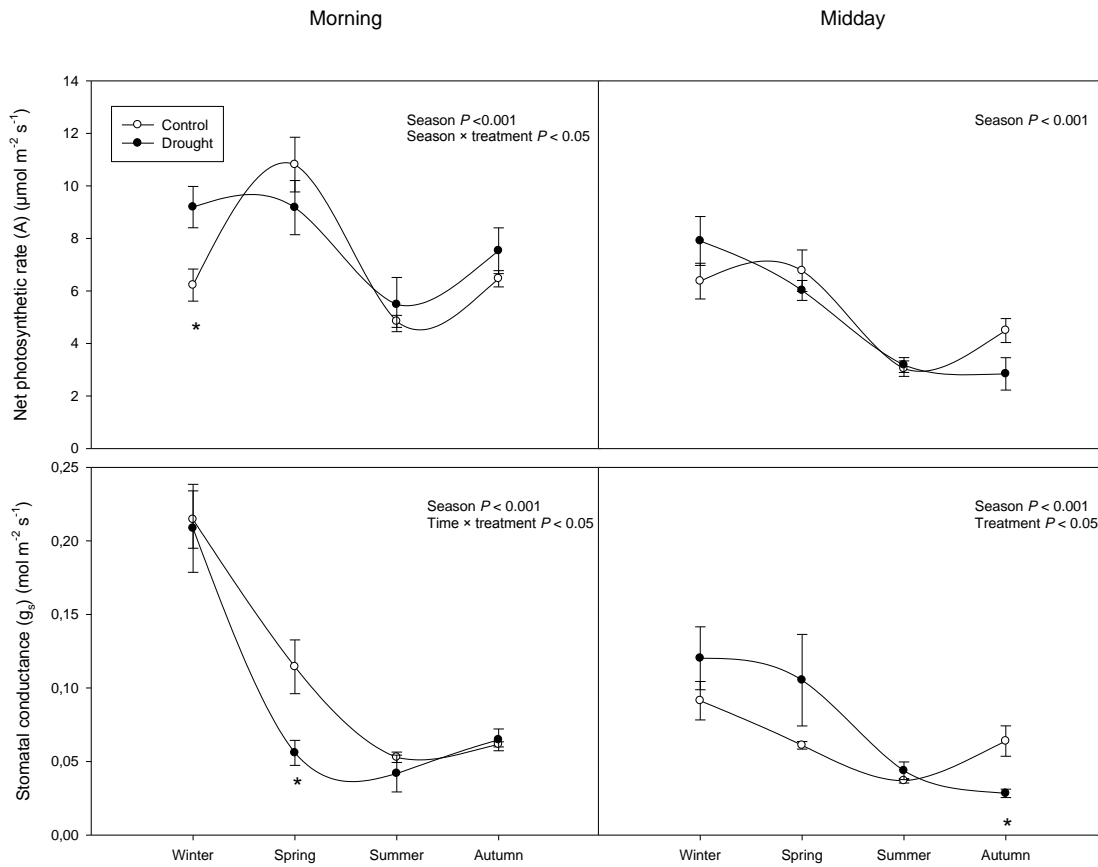
A)

*Erica multiflora*



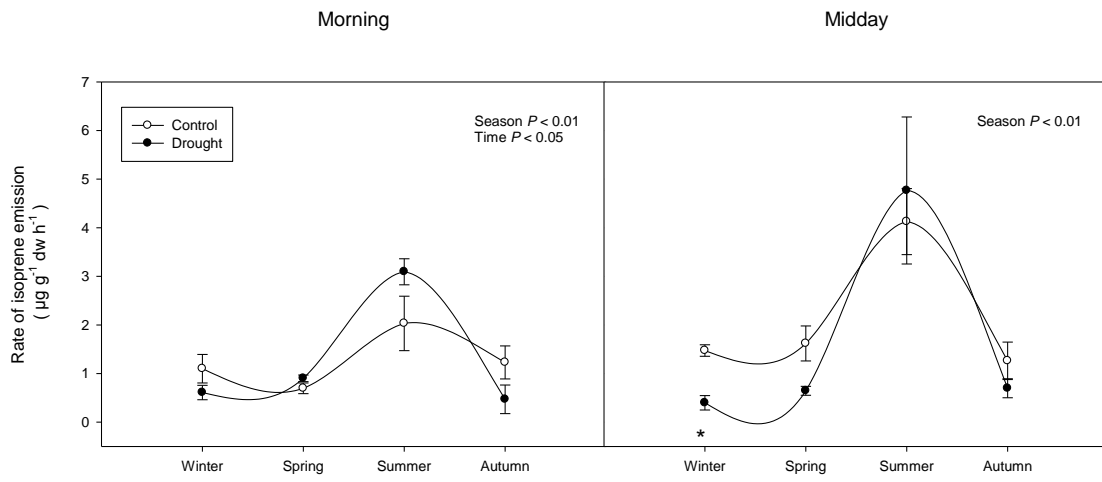
B)

*Quercus ilex*



**Fig. 2.** Seasonal net photosynthetic rates and stomatal conductances for *E. multiflora* (A) and *Q. ilex* (B) in the morning and at midday. Error bars indicate standard errors of the means;  $n = 9$  (\*  $P < 0.05$  indicates significant differences between treatments identified by Student's *t*-tests). The significances of the effects of season, treatment and sampling time (repeated measurements ANOVA) are depicted in each panel.

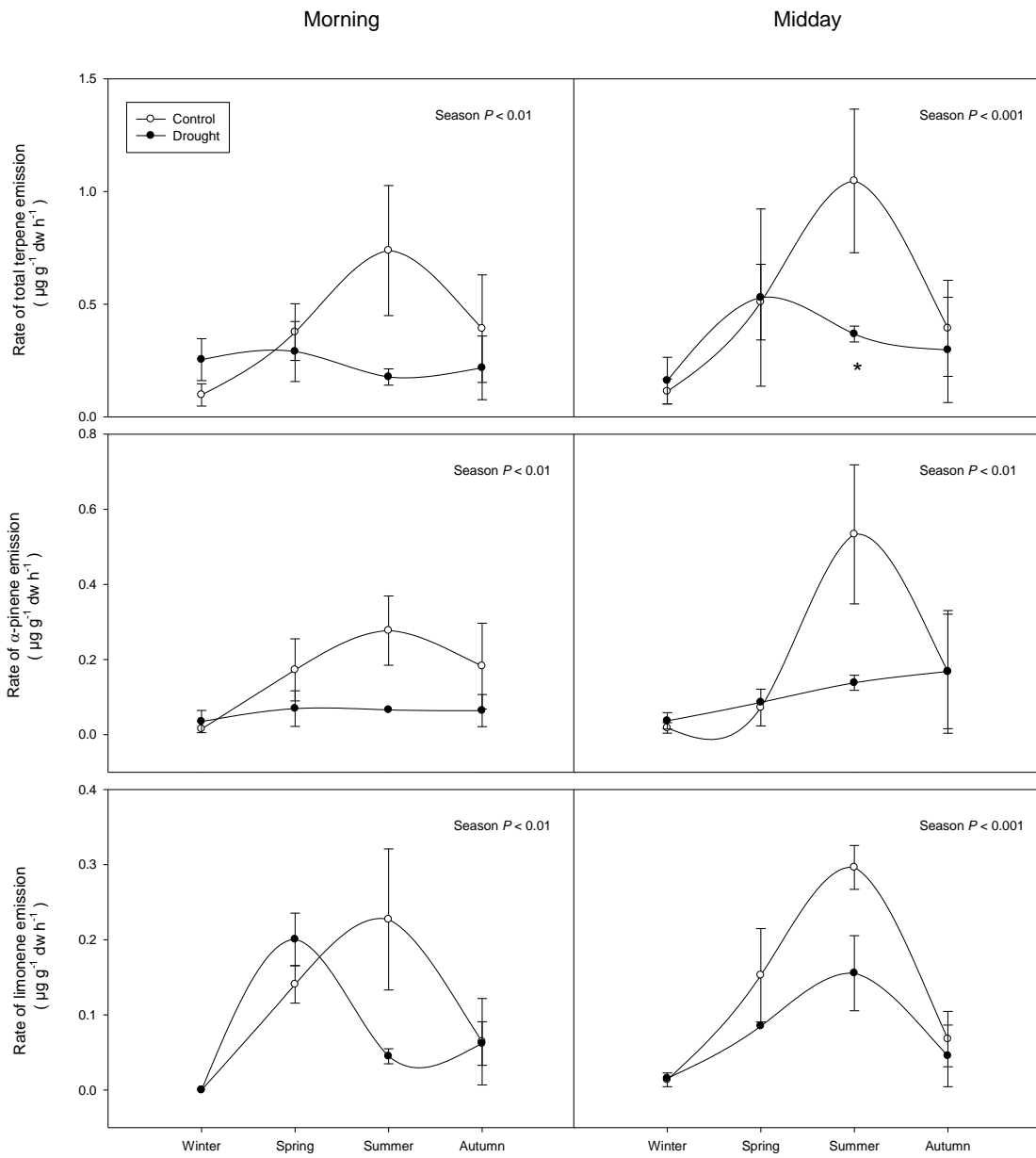
*Erica multiflora*



**Fig. 3.** Seasonal time courses of the rate of isoprene emission for *E. multiflora*. Error bars indicate standard errors of the means;  $n = 6$  (\*  $P < 0.05$  indicates significant differences between treatments identified by Student's *t*-tests). The effects of season, treatment and sampling time are depicted in the panels when significant.

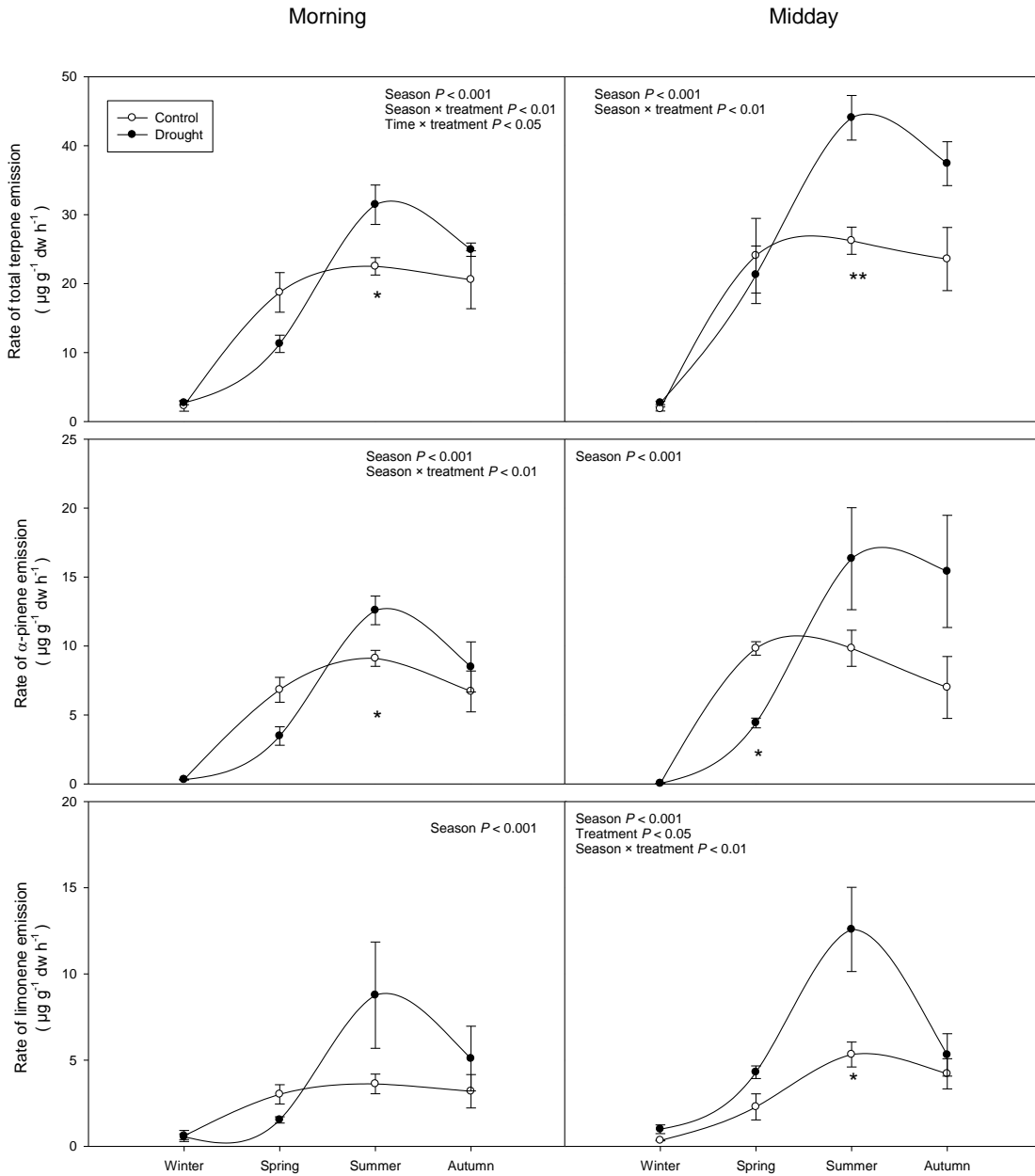
A)

*Erica multiflora*

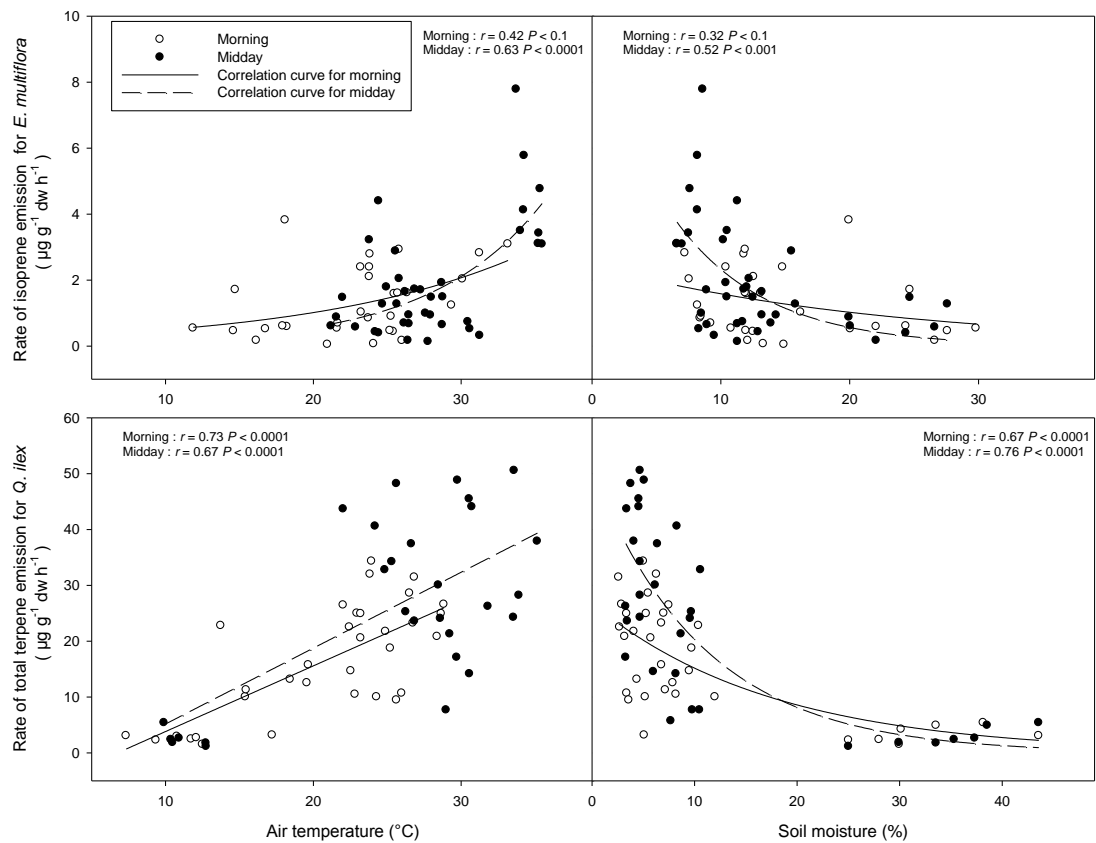


B)

*Quercus ilex*



**Fig. 4.** Seasonal time courses for the rates of emission of total terpenes,  $\alpha$ -pinene and limonene for *E. multiflora* (A) and *Q. ilex* (B). Error bars indicate standard errors of the means;  $n = 6$  (\*  $P < 0.05$  and \*\*  $P < 0.01$  indicate significant differences between treatments identified by Student's *t*-tests). The effects of season, treatment and sampling time are depicted in the panels when significant.



**Fig. 5.** Relationships for the emissions of the main isoprenoids with air temperature and soil moisture for *E. multiflora* and *Q. ilex*.

**Table 1.** Relationships of main isoprenoid emissions (isoprene for *E. multiflora* and total terpenes for *Q. ilex*) with air temperature and soil moisture (SE, Standard Error).

		Morning		Midday	
		Linear	Exponential	Linear	Exponential
<i>E. multiflora</i>	<i>R</i>	0.3832	<b>0.4187</b>	0.5680	<b>0.6280</b>
Air temperature	<i>P</i>	0.0366	<b>0.0213</b>	0.0002	<b>&lt;0.0001</b>
	SE	0.9875	<b>0.9710</b>	1.4307	<b>1.3528</b>
Correspondent equation		<b><math>y = 0.246 * 1.074 ^x</math></b>		<b><math>y = 0.042 * 1.139 ^x</math></b>	
	<i>R</i>	0.3125	<b>0.3165</b>	0.4409	<b>0.5182</b>
Soil moisture	<i>P</i>	0.0927	<b>0.0884</b>	0.0056	<b>0.0009</b>
	SE	1.1056	<b>1.0142</b>	1.5603	<b>1.4867</b>
Correspondent equation		<b><math>y = 2.449 * 0.958 ^x</math></b>		<b><math>y = 9.562 * 0.868 ^x</math></b>	
<i>Q. ilex</i>	<i>R</i>	<b>0.7266</b>	0.6995	<b>0.6701</b>	0.6124
Air temperature	<i>P</i>	<b>&lt;0.0001</b>	<0.0001	<b>&lt;0.0001</b>	0.0005
	SE	<b>6.9235</b>	7.2012	<b>12.3180</b>	13.1199
Correspondent equation		<b><math>y = -7.936 + 1.176x</math></b>		<b><math>y = -8.404 + 1.358x</math></b>	
	<i>R</i>	0.6664	<b>0.6814</b>	0.7067	<b>0.7587</b>
Soil moisture	<i>P</i>	<0.0001	<b>&lt;0.0001</b>	<0.0001	<b>&lt;0.0001</b>
	SE	7.4338	<b>7.2968</b>	11.7636	<b>10.8312</b>
Correspondent equation		<b><math>y = 26.83 * 0.945 ^x</math></b>		<b><math>y = 50.66 * 0.913 ^x</math></b>	