Universitat Autònoma de Barcelona

Volatile organic compounds (VOCs) in plants and soils of Mediterranean ecosystems

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PhD Thesis

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Abstract

Biogenic volatile organic compounds (BVOCs) play important roles in ecology and atmospheric chemistry. Their emissions from terrestrial ecosystems are driven mainly by plants, and are greatly influenced by the variations in environmental variables and therefore are altered by increasing global environmental change **(**GEC). However, these emissions and their influences are not well known in natural Mediterranean ecosystems.

While soil BVOCs apparently play a minor role in atmospheric chemistry, owing to their usually low fraction with respect to the total ecosystem emissions in Mediterranean-type ecosystems, they play important ecological roles in soil processes. Much previous studies on soil VOCs have looked at fluxes, while the actual VOC concentrations in soils from Mediterranean ecosystems have never been considered.

In this work, I carry out the monitoring of environmental manipulation experiments in typical Mediterranean ecosystems to study the exchanges of VOCs, including emissions from plants, and exchanges and concentrations of soils, and their possible drivers.

The shrub *Erica multiflora* L. abundant species in Mediterranean shrublands and the tree *Quercus ilex* L. dominant species in many Mediterranean forests were the species studied, the former in the Garraf Natural Park (shrubland) and the latter in Les Muntanyes de Prades (forest), two typically Mediterranean ecosystems. Both species emitted terpenes, but a significant emission of isoprene was only detected from *E. multiflora*. α-Pinene and limonene were the most abundant emitted terpenes.

Isoprenoid emissions increased with air temperature and generally decreased as the amount of soil moisture increased. Terpene emissions increased synergistically due to heat stress and drought in summer. The plants responded more in their isoprenoid emissions to the availability of soil water than that of N in soil. Nitrogenous fertilizer did not significantly affect the emission of isoprene, but it significantly increased the total emissions of terpenes and decreased their diversity. The results suggest that higher isoprenoid emissions can be expected as the Mediterranean region becomes warmer and drier over the next few decades and that N deposition could further stimulate these emissions.

For the soils of the same shrubland and holm oak forest ecosystems, those of shrubland showed higher total VOC concentrations than forest. There were the largest concentrations of monoterpenes and methanol in the shrubland and forest, respectively, and concentrations of methanol, acetic acid, formaldehyde, ethanol, and acetaldehyde were the dominant compounds in both ecosystems. Soil temperature and water content, CO_2 efflux, and enzyme activity were the best explanatory variables for variation in soil VOC concentrations in the two ecosystems: there was a stronger association between concentration of dominant compounds, except formaldehyde, with soil temperature and/or CO_2 efflux than with soil water content. Activity of C- and N-degrading enzymes was positively associated with the concentration of VOCs, depending on ecosystem, and consistently correlated with high soil water content. In the holm oak forest soils, net photosynthetic rate (*A*) was positively correlated with soil concentration of monoterpenes. These results show that soil VOC concentrations in these Mediterranean

ecosystems are driven by soil temperature and water content, and microbial activity, in combination with ecosystem plant activity.

I also analyzed a Mediterranean turf with *Pinus pinea* L. trees. I quantified the exchange of isoprenoids between soil (with litter) and atmosphere along a horizontal gradient from the trunks of *Pinus pinea*. Isoprenoid emissions were greatest and most diverse, and also can be roughly estimated by litter dry weight near the trunk, where the needle litter was denser. Irregular emission and adsorption of isoprenoids with low exchange rates were recorded, and exchange rates were correlated positively with soil temperature and negatively with soil moisture in open turf.

In conclusion, higher BVOC emissions can be expected owing to the increases in foliar emissions, and exchanges and concentrations of soils, and N deposition will also further stimulate these emission trends by increasing foliar isoprenoid emissions in the warmer and drier conditions predicted for the coming decades in the Mediterranean region.

General introduction

Biogenic volatile organic compounds (BVOCs) represent about 90% of total volatile organic compound (VOC) emitted into the atmosphere (Atkinson, 2002). They are very diverse and consist of various organic classes such as isoprenoids, alkanes, alkenes, carbonyls, alcohols, esters, ethers and acids (Kreuzwieser et al., 1999; Naik et al, 2004; Peñuelas and Llusià, 2001). Among them, isoprenoids (isoprene, monoterpenes and sesquiterpenes), which account for >50% of the total emission of BVOCs (Guenther et al., 1995), are the most dominant components of the biosphere-atmosphere exchange of BVOCs (Sharkey and Monson, 2017).

The study of these reactive hydrocarbons is mainly motivated by their key role in the physiology and ecological relationships of living organisms, especially plants and their significance for atmospheric processes that influence the atmospheric chemistry and climate change (Leff and Fierer, 2008; Peñuelas and Llusià, 2003; Peñuelas and Staudt, 2010). In addition, BVOCs play roles in the global carbon cycling and budget, as around 0.1–2% of the assimilated carbon is released in the form of BVOCs from vegetation (Portillo-Estrada et al., 2018; Sharkey and Yeh, 2001) and a non-negligible fraction of CO_2 derives from BVOCs oxidation in the atmosphere (Kesselmeier et al., 2002; Peñuelas and Staudt, 2010). Moreover, the capacity to emit volatile compounds could be used as a basis for studying taxonomic relationships in plants (Llusià et al., 2010; Loreto et al., 1998).

BVOC emission profiles from terrestrial ecosystems tend to be driven mainly by plant BVOCs, which is linked to species composition (Kesselmeier and Staudt, 1999),

phenology and climate (Peñuelas and Staudt, 2010; Svendsen, et al, 2016). Plant BVOCs 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 and/or stored in specialized structures (Loreto and Schnitzler, 2010). BVOCs are a crucial group of plant compounds due to their significance to the survival of individual plants (Dicke and Loreto, 2010; Niederbacher et al., 2015), they play important roles in plant responses to both abiotic and biotic stresses (Holopainen and Gershenzon, 2010; Loreto and Schnitzler, 2010; Peñuelas and Llusià, 2003). BVOCs provide protection against high temperatures, high irradiation, oxidative stress and drought stress (Holopainen and Gershenzon, 2010; Loreto and Schnitzler, 2010; Velikova et al., 2005). They can also act as herbivore deterrents, attractants of pollinators and enemies of herbivores (Llusià and Peñuelas, 2000; Peñuelas and Munné-Bosch, 2005) and plant–plant communication signals (Peñuelas and Llusià, 2003).

Regarding atmospheric chemistry and climate change firstly, BVOCs contribute to the production of tropospheric ozone in the presence of NO_x compounds and sunlight (Dicke and Loreto, 2010; Tiiva et al., 2017). Secondly, BVOCs affect the oxidizing capacity of the atmosphere by consuming hydroxyl radical, which can 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 (Carslaw et al., 2009; Claeys et al., 2004; Scott et al., 2017).

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 Llusià, 1999). These ecosystems are undergoing intense ecological impacts of global environmental change **(**GEC). Models of global circulation, climate and ecophysiology predict a reduction in the availability of water in Mediterranean regions around the world (Peñuelas and Boada, 2003; Piñol et al., 1998; Sabaté, et al., 2002), which are naturally water limited (Sardans and Peñuelas, 2007) due to the high temperatures and the consequent high rates of evapotranspiration (Peñuelas and Llusià, 2001). Moreover, global atmospheric nitrogen (N) deposition has increased globally in recent decades (Galloway et al., 2004, 2008; Peñuelas et al., 2012), especially in temperate areas of Eurasia and North America (Peñuelas et al., 2012; Wang et al., 2017). Current models predict that soil N content, air temperature and soil moisture are expected to change concurrently and understanding their influences on BVOC emissions is essential for predicting future BVOC dynamics (Llusià et al., 2014; Tiiva et al., 2017; Zhang et al., 2017). The effect of N deposition, drought and warming, representing the most realistic future, on BVOC emissions remains uncertain.

Plants can acclimate to the new conditions generated by global changes (Bai et al., 2008; Chaves et al., 2002; Rubio-Casal et al., 2010) by adjusting their metabolism (Hsiao, 1973) and reorganizing their energy resources (Dobrota, 2006). BVOCs are an important tool for resisting stress and acclimate to the changing environmental conditions (Peñuelas and Llusià, 2003; Porcar-Castell et al., 2009; Loreto and Schnitzler, 2010; Pivovaroff et al., 2016). Rates of BVOC emission increase and help

plants to resist stress under moderate drought conditions but decrease under severe drought conditions (Gershenzon et al., 1978; Hansen and Seufert, 1999; Llusià et al., 2011 and 2013). The influence of N deposition on BVOC emissions is not uniform (Blanch et al., 2007; Peñuelas and Staudt, 2010; Yuan et al., 2017) due to the variations in response to N availability, acquisition and strategies of use (Pivovaroff et al., 2016; Vourlitis and Pasquini, 2009) by different plant species.

This key role of terrestrial plants in BVOCs has received much research attention (Albers et al., 2018; Leff and Fierer, 2008; Peñuelas et al., 2014; Peñuelas and Staudt, 2010). However, there is also emerging evidence that a wide range of BVOCs are also released from terrestrial ecosystem soils (Peñuelas et al., 2014; Leff and Fierer, 2008; Peñuelas et al., 2014; Potard et al., 2007). Soil emissions to the atmosphere are often 1–2 orders of magnitude lower than those from aboveground vegetation (Peñuelas et al., 2014), but may also represent up to 50% of net canopy BVOC flux, depending on the type of ecosystem, litter and soil (Tang et al., 2019), environmental condition (Peñuelas et al., 2014; Rossabi et al., 2018) and season of the year (Tang et al., 2019). However, these emissions usually constitute a very low fraction with respect to the total ecosystem emissions in Mediterranean type ecosystems (Hayward et al, 2001; Asensio et al, 2007b, c; Greenberg et al, 2012; Tang et al., 2019). As a result, the study of soil BVOCs in Mediterranean-type ecosystems is mainly motivated by their important role in root–soil microbe ecological processes (Asensio et al., 2007b; Leff and Fierer, 2008; Peñuelas et al., 2014). Soil BVOCs affect microbial process such as methane oxidation, nitrification, nitrogen mineralization, and aerobic respiration (Leff and Fierer, 2008;

Paavolainen et al., 1998; Smolander et al., 2006). They play roles in biological interactions, most importantly, they are key compounds in communication among soil microorganism and plant roots (Svendsen et al., 2018; Tang et al., 2019) that release carbon-rich root exudates and thus feed associated populations of bacteria, fungi, arthropod and nematode within the rhizosphere (Leff and Fierer, 2008), some bacterial volatiles promote growth in plants as well (Asensio et al., 2007b; Ryu et al., 2003; Tang et al., 2019).

The role of soil VOC fluxes in atmospheric chemistry has led to a number of studies (Asensio et al., 2007b, 2008b; Leff and Fierer, 2008; Peñuelas et al., 2014; Potard et al., 2007). However, VOC concentrations in soils have rarely been examined (Wester-Larsen, et al., 2020), despite their important ecological role in soils. Soils may function as either sources or and sinks of gaseous VOCs (Asensio et al., 2007b; Leff and Fierer, 2008; Peñuelas et al., 2014; Ramirez et al., 2010), owing to the biotic production (Leff and Fierer, 2008; Mu et al., 2020; Peñuelas et al., 2014; Tang et al., 2019) and consumption (Albers et al., 2018; Asensio et al., 2007b; Cho et al., 2005; Owen et al., 2007) processes from roots and microorganisms, and abiotic processes including desorption/sorption, dissolution/solution, exchanges with atmospheres and reactions with soil chemicals (Peñuelas et al., 2014; Tang et al., 2019). Thus, soil VOC concentrations are dependent on temperature, water availability, and soil physical traits, which affect plant and soil microorganism activities, and atmospheric concentration of VOC compounds near the soil surface (Asensio et al., 2007b; Greenberg et al., 2012; Peñuelas et al., 2014; Tang et al., 2019). The flux studies suggest that Mediterranean

soil often behave more as a sink of VOCs than as a source, owing to overall VOC uptake under the most standard soil moisture and temperature conditions and in most seasons of the year (Asensio et al., 2007a, b, c, 2008b). Terpenoids and oxygenated VOCs are the most exchanged soil VOCs (Aaltonen et al., 2013; Asensio et al., 2007b; Mäki et al., 2017) in these ecosystems with low and randomly fluctuating exchange rates (Asensio et al., 2007b; Asensio et al., 2008). However, there is still no concentration study published in Mediterranean ecosystems, so research into soil VOC concentrations is warranted.

Objectives of the thesis

The main objective of this thesis was to study the emissions of BVOC in Mediterranean ecosystems. To accomplish this main objective, we divided the thesis into three blocks. The first block is focused on foliar BVOC emissions from dominant species in a Mediterranean shrubland and a Mediterranean forest and their responses to changing experimental conditions simulating two main global environmental changes, drought and N deposition (chapter 1 and 2). The second block is focused on soil VOC concentrations in the same Mediterranean shrubland and forest (chapter 3). The third block is focused on ground level BVOC exchanges associated with distances to the trunks of the trees (chapter 4).

In chapter 1, we aim to study the effects of experimental drought on seasonal and diurnal foliar isoprenoid emissions from a dominant species in a Mediterranean shrubland (*Erica multiflora* L.) and a dominant species in a Mediterranean forest (*Quercus ilex* L.), and to determine the relationship of emission rates with environmental conditions.

In chapter 2, we aim to study the effects of experimental nitrogen deposition on the foliar isoprenoid emissions from the same Mediterranean dominant species, *E. multiflora* and *Q. ilex*.

In chapter 3, we aim to study annual and seasonal soil VOC concentrations in the same Mediterranean shrubland and holm oak forest, and to determine their relationships with environmental variables. Here, we also aim to study the similarity and difference between the dominant Mediterranean-type ecosystems.

In chapter 4, we aim to study isoprenoid exchanges between soil, litter and atmosphere, and their potential drivers in a Mediterranean turf along a horizontal gradient from dense *P. pinea* litter to open herbaceous turf.

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Block 1. Foliar isoprenoid emissions from Mediterranean dominant shrub and tree species under global environmental change

Chapter 1. Seasonal and diurnal variations of plant isoprenoid emissions from two dominant species in Mediterranean shrubland and forest submitted to experimental drought

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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_s) 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. α-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 (Llusià 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 Llusià, 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_s). Biogenic volatile organic compounds (BVOCs) are also an important tool for resisting drought (Peñuelas and Llusià, 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 Llusià, 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; Llusià and Peñuelas, 2000) and plant–plant communication signals (Peñuelas and Llusià, 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 Llusià, 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 Llusià, 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 Llusià, 2001; Copolovici et al., 2005).

Several studies have demonstrated the importance of abiotic and biotic factors for the emissions of BVOCs (Peñuelas and Llusià, 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; Llusià 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; Llusià 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; Llusià and Peñuelas, 2000). Pool size in resin ducts and internal or external glands in terpene-storing species (Lerdau et al., 1997; Peñuelas and Llusià, 2001; Llusià 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; Llusià et

al., 2006) the emission rates depending on the intensity of the water stress (Llusià and Peñuelas, 2000; Llusià 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 Llusià, 1999) and long-term seasonal (Guenther, 1997; Llusià and Peñuelas, 2000) cycles under experimental drought also determine the status of isoprenoid emission (Llusià and Peñuelas, 2000; Llusià et al., 2006). Variations in emissions have been linked to corresponding changes in temperature, radiation, air humidity and water availability (Llusià et al., 2006) but also to leaf development and physiological activity (Llusià and Peñuelas, 2000). These factors are also involved in the variations among isoprenoids due to their diverse physicochemical traits (Llusià 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 (Llusià 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. (Llusià 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*

(Bolòs and Vigo, 1990; Llusià 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^2 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 ± 19 μmol m^{-2} s^{-1} under a controlled CO_2 concentration of 400 ± 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^2. 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^{-1}. 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 (α-pinene, β-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 α-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^{-1} 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^{-1}, 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^{-1} from 40 to 60 °C and then at 10 °C min^{-1} to 150 °C, maintained for 3 min, increased at 70 °C min^{-1} 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 α-pinene, 3-carene, β-pinene, myrcene, limonene, sabinene and α-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.

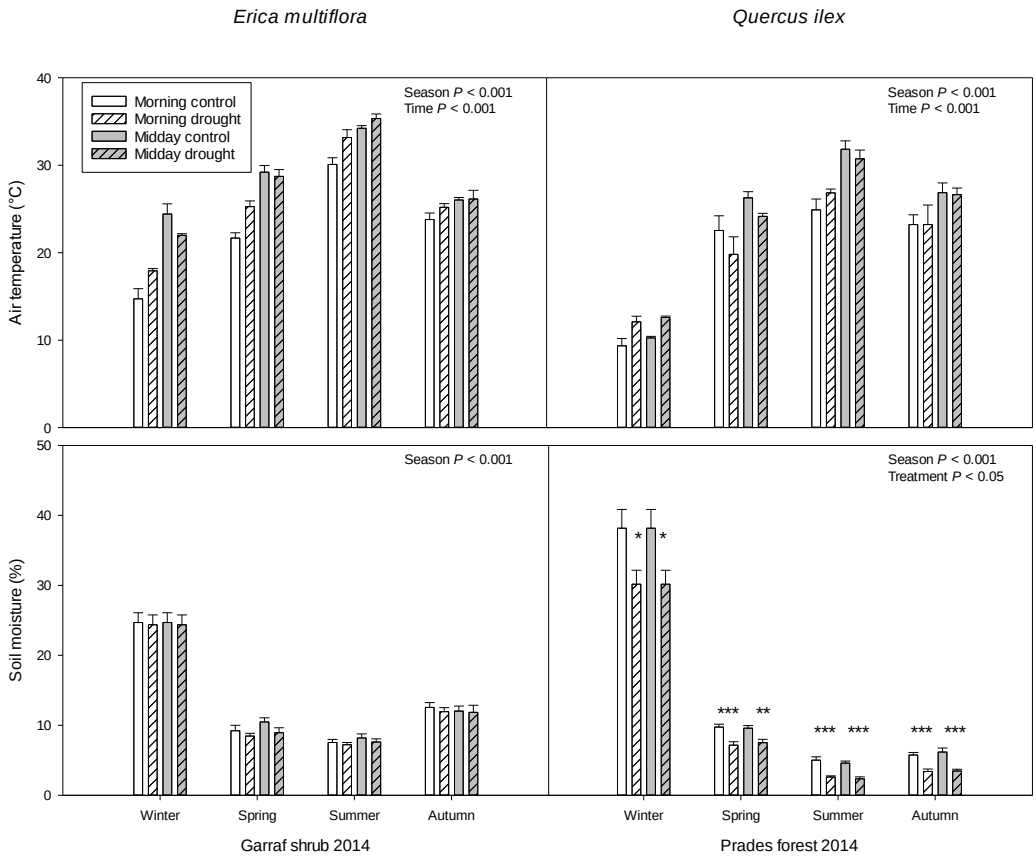


Fig. 1. Seasonal morning and midday time-courses of mean air temperature and soil moisture in Garraf and Prades. Error bars indicate standard errors of the means; $n = 6$

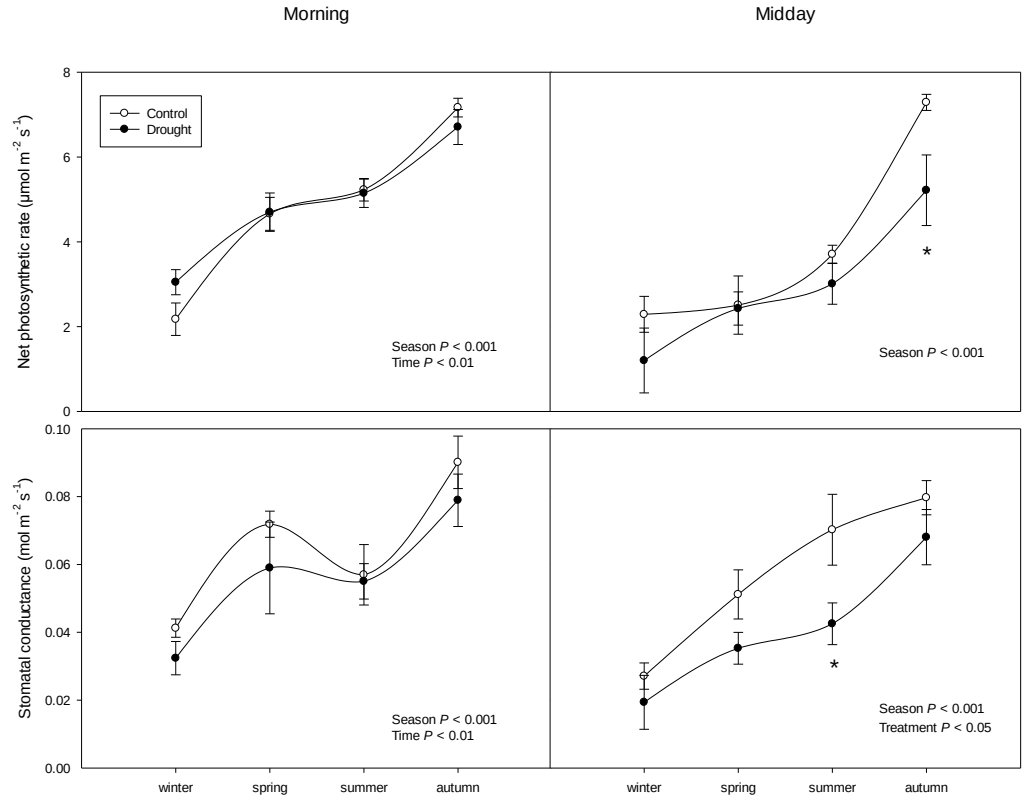
(* P < 0.05, ** P < 0.01 and *** P < 0.001 indicate significant differences between treatments identified by Student's t-tests). The significances of the effects of season, treatment and sampling time (repeated measurements ANOVA) are depicted in each panel.

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 μmol m^{-2} s^{-1} and 0.079–0.090 mol m^{-2} s^{-1}, and were lowest at winter middays, at 1.2–2.3 μmol m^{-2} s^{-1} and 0.020–0.027 mol m^{-2} 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 μmol m^{-2} s^{-1} and 0.208–0.215 mol m^{-2} s^{-1}, and lowest at autumn middays at 2.8–4.7 μmol m^{-2} s^{-1} and 0.028–0.064 mol m^{-2} 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.

A) *Erica multiflora*



B) *Quercus ilex*

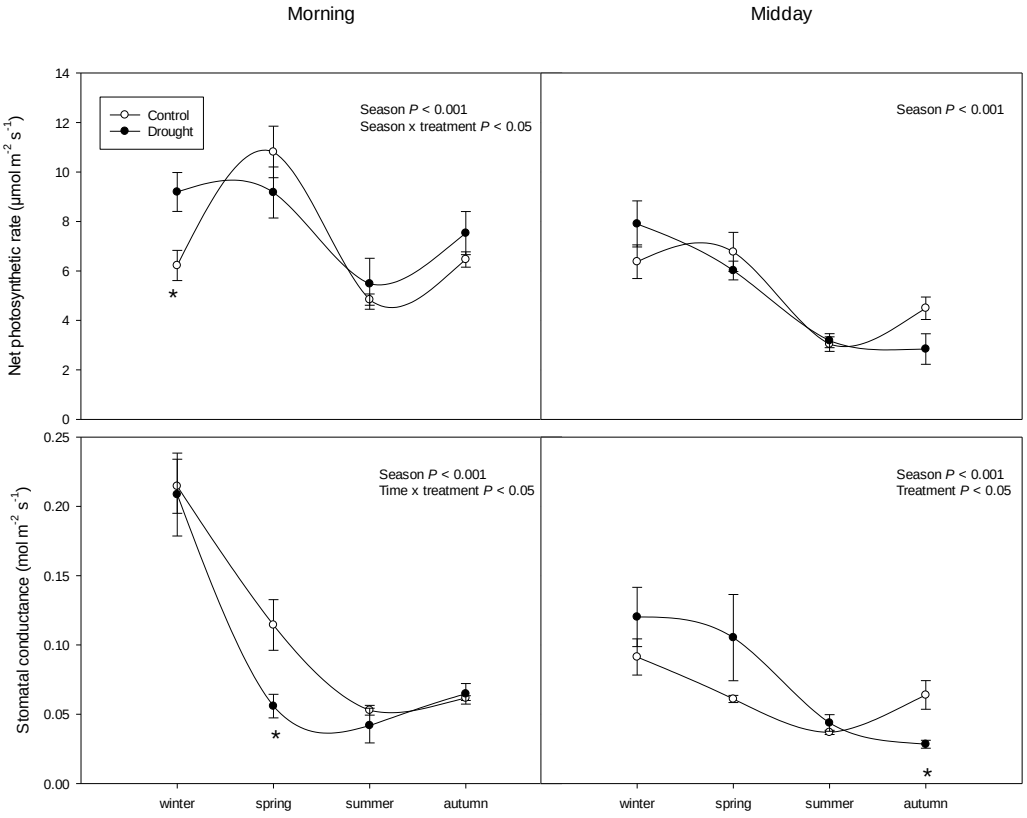


Fig. 2. Seasonal net photosynthetic rates and stomatal conductances for *E. multiflora* (A) and *Q. ilex* (B) in the morning and at midday. Error bars indicate standard errors of the means; $n = 9$ (* $P < 0.05$ indicates significant differences between treatments identified by Student's *t*-tests).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

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 ± 0.15 μg g^{-1} dw h^{-1} at winter middays and 4.77 ± 1.51 μg g^{-1} 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. α-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 ± 0.05 μg g^{-1} dw h^{-1} in winter mornings and 1.05 ± 0.32 μg g^{-1} 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. α-Pinene and limonene were emitted mostly at summer midday, at about 0.7 and 0.3 μg g^{-1} dw h^{-1}, respectively. *Q. ilex* emitted terpenes at much higher rates than *E. multiflora*, ranging between 1.8 ± 0.3 μg g^{-1} dw h^{-1} at winter middays and 44.1 ± 3.2 μg g^{-1} 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 α-pinene ($P < 0.05$) and limonene ($P < 0.05$) emissions, respectively. α-Pinene and limonene were emitted mostly at summer middays, at rates of about 13 μg g^{-1} dw h^{-1} for both.

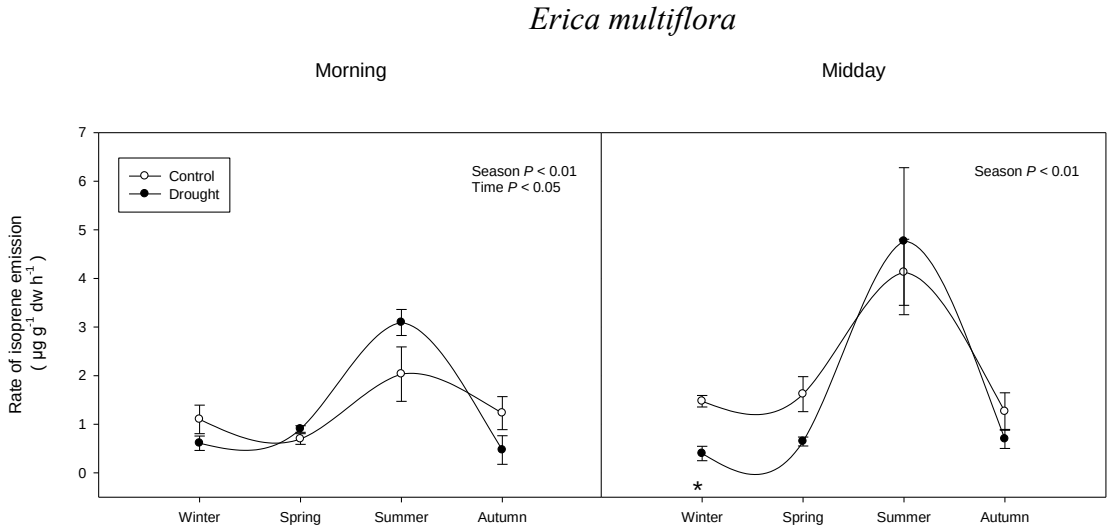
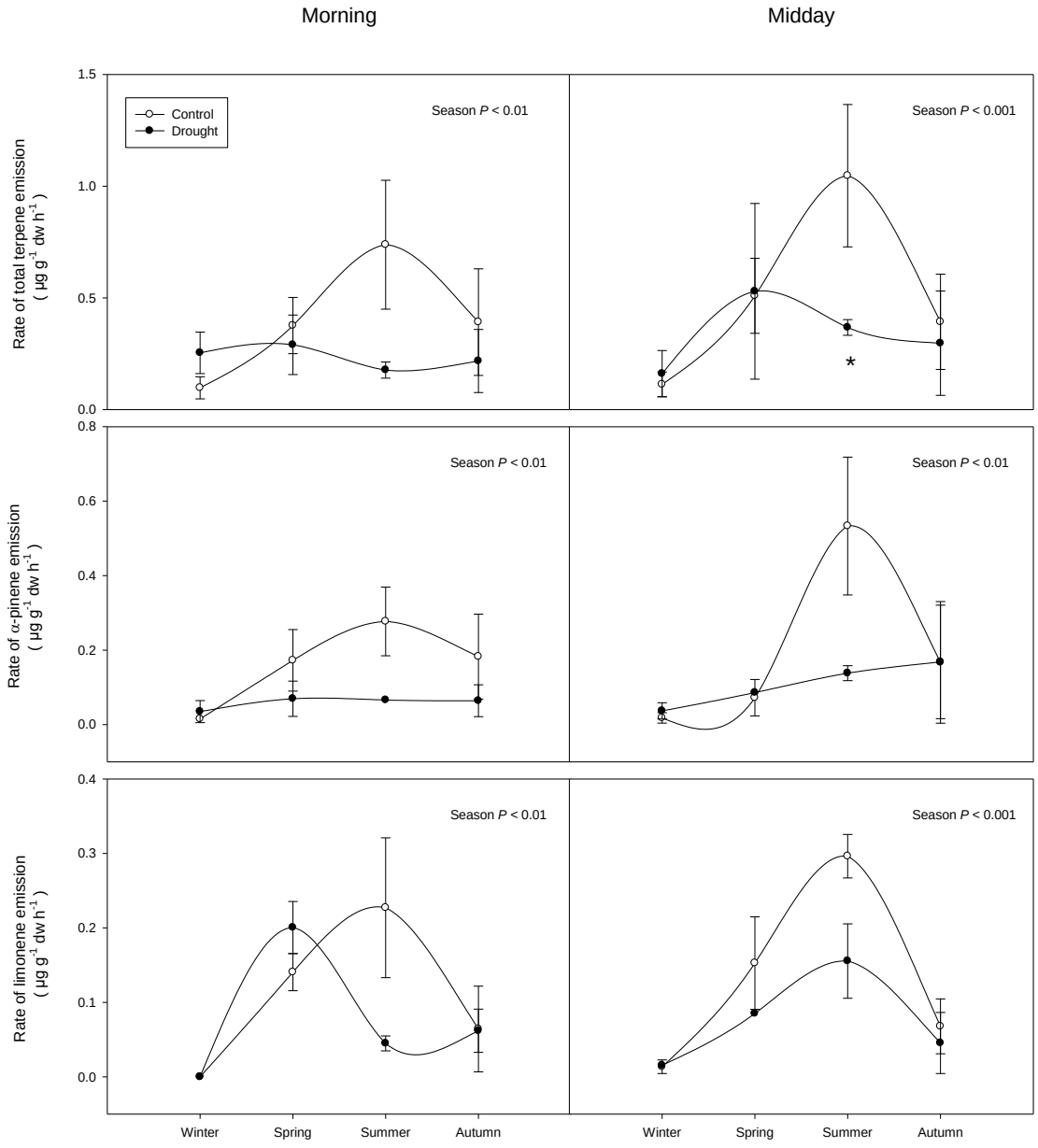


Fig. 3. Seasonal time courses of the rate of isoprene emission for *E. multiflora*. Error bars indicate standard errors of the means; $n = 6$ (* $P < 0.05$ indicates significant differences between treatments identified by Student's *t*-tests). The effects of season, treatment and sampling time are depicted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

A) *Erica multiflora*



B) *Quercus ilex*

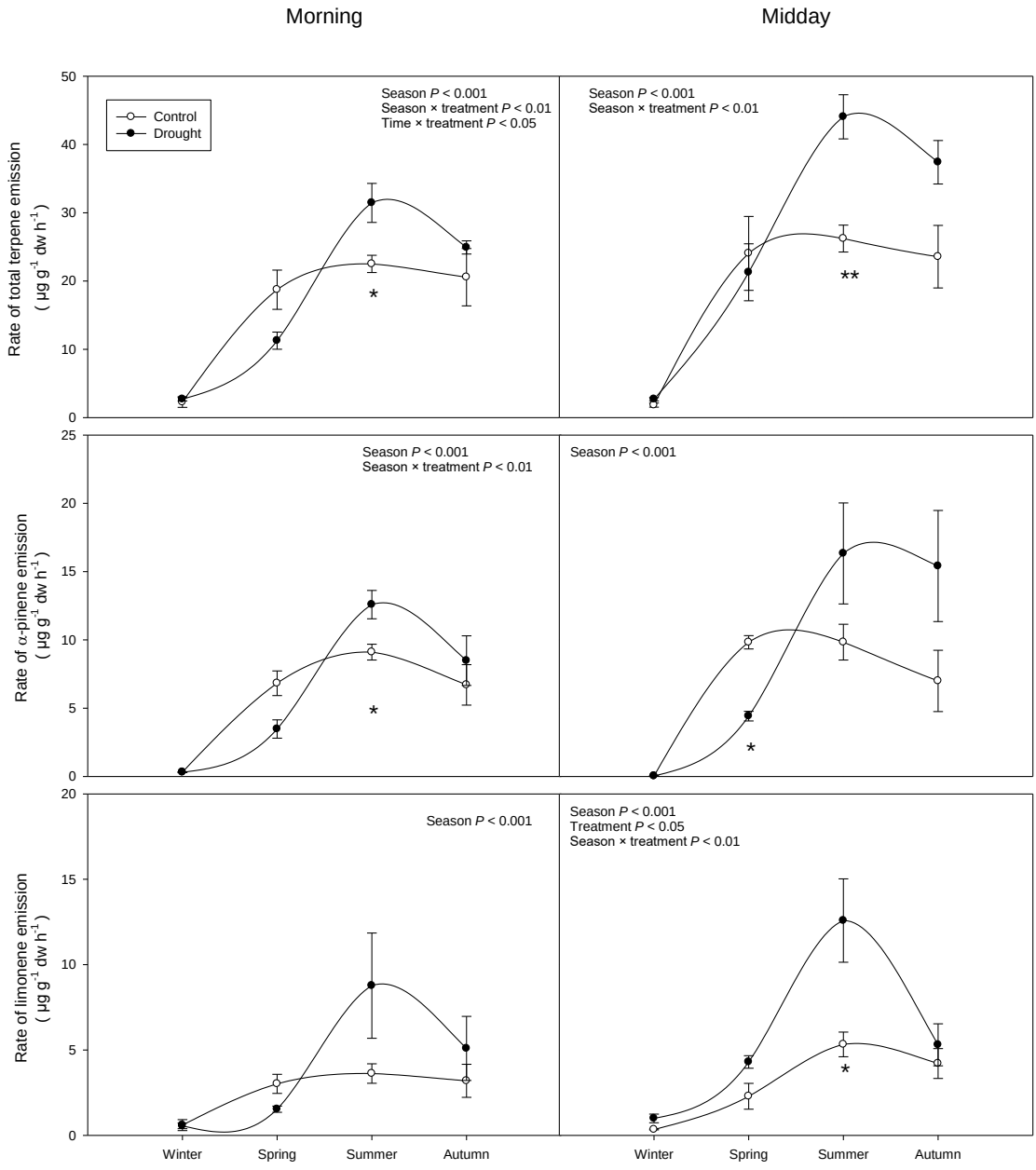


Fig. 4. Seasonal time courses for the rates of emission of total terpenes, α-pinene and limonene for *E. multiflora* (A) and *Q. ilex* (B). Error bars indicate standard errors of the means; $n = 6$ (* $P < 0.05$ and ** $P < 0.01$ indicate significant differences between treatments identified by Student's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

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_2 in both the stomata and mesophyll (Centritto et al., 2003; Ogaya and Peñuelas, 2003; Llusià 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 (Llusià et al., 2013; Matesanz and Valladares 2014; Bussotti et al., 2015).

The emission of isoprenoids differed between the species (Llusià and Peñuelas, 2000; Peñuelas and Llusià, 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 (Llusià and Peñuelas, 2000; Llusià 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 (Llusià and Peñuelas, 2000).

Isoprenoid-emission rates for *E. multiflora* were inside the range of 0.5–20 $\mu g\ g^{-1}$ dw h^{-1} reported by Owen et al. (1997) and also inside the ranges for isoprene emissions

of 0.15–4.4 μg g^{-1} dw h^{-1} and monoterpene emissions of 0.08–0.4 μg g^{-1} dw h^{-1} reported by Llusià 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 (Llusià and Peñuelas, 2000; Loreto et al., 2001; Llusià et al., 2011; Llusià 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 (Llusià 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 (Llusià et al., 2006; Llusià 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; Llusià 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 Llusià, 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 α-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; Llusià 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_s ($P < 0.01$) 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 Llusià 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 (Llusià 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).

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

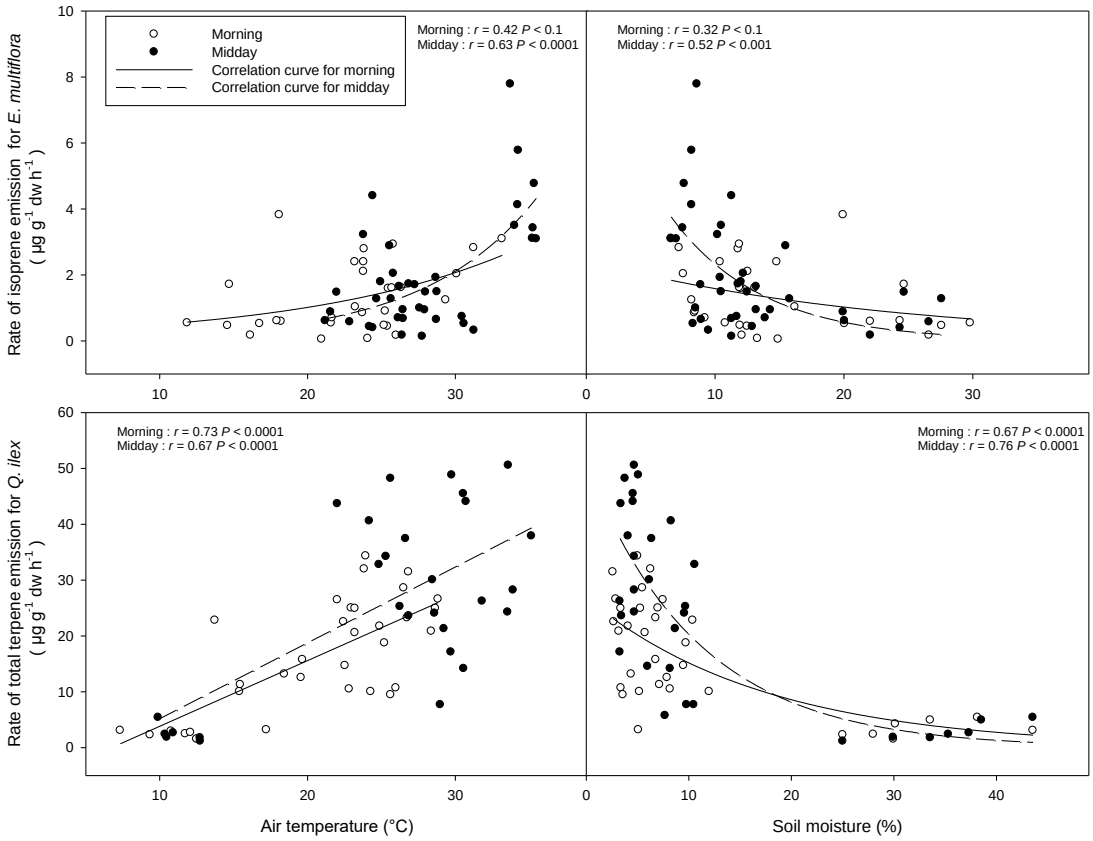


Fig. 5. Relationships for the emissions of the main isoprenoids with air temperature and soil moisture for *E. multiflora* and *Q. ilex*.

Environmental conditions such as air temperature and soil moisture are the main factors that determine plant physiological responses, including BVOC emissions (Llusià et al., 2006, 2009 and 2011; Peñuelas and Llusià, 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 Llusià,

2001; Blanch et al., 2009), and plants under severe drought conditions may even decrease their emissions (Llusià 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 Llusià, 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; Llusià 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; Brilli 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; Llusià et al., 2011) and atmospheric chemical processes contributing to ozone formation and even affecting climate (Thompson, 1992; Peñuelas and Llusià, 2003; Dicke and Loreto, 2010; Seco et al., 2013).

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Chapter 2. Profile of foliar isoprenoid emissions from Mediterranean dominant shrub and tree species under experimental nitrogen deposition

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Abstract

Biogenic volatile organic compounds play important roles in atmospheric chemistry, and their emissions can be greatly influenced by the variations in environmental conditions and physiological activities caused by continuously increasing global nitrogen (N) deposition. However, this influence is still poorly understood, especially in a natural ecosystem. We conducted a one-year (2015-2016) experiment adding N deposition (60 kg N ha^{-1}) with fertilization to a Mediterranean shrubland dominated by *Erica multiflora* and a Mediterranean forest dominated by *Quercus ilex* and compared the seasonal and daytime photosynthetic rates (A), stomatal conductances (g_s) and rates of isoprenoid emission with control (2015-2016) and pre-treatment (2014-2015) plots. N fertilization increased A in warm weather as soil moisture increased, and assimilation became saturated when the environment was sufficiently favorable, and excess soil N significantly restrained A in cold weather. The plants were much more sensitive to soil water availability than N content and terpene emissions increased synergistically due to heat and drought stress in hot weather. N fertilization did not significantly affect isoprene emission but significantly increased total terpene emissions and decreased the diversity of terpenes. Our results suggest a successful acclimation of plants by emitting more isoprenoids under environmental stress and that N deposition will further stimulate emissions as the Mediterranean region becomes warmer and drier. The results highlight the necessity for predicting the most realistic future of ecosystems under global environmental change and for assessing the impacts of multiple factors acting in concert on plant physiological and

ecosystem functioning including biogenic VOC emissions.

Keywords: Nitrogen deposition; Climate change; BVOC emissions; Isoprenoids; *Erica multiflora*; *Quercus ilex*.

Introduction

Global environmental change (GEC) is accelerating and becoming more intense around the world. The ecological impacts of the main drivers of GEC, such as climate change, nitrogen (N) deposition, land-use changes and species invasions, are all expected to become more conspicuous as human exploitation and pollution of the environment continue to increase (Sala et al., 2000). Climate change has been the most widely and thoroughly studied due to its influence. Other factors, however, are also receiving increasing attention, especially N deposition. Global N deposition has increased in recent decades and will likely double the existing levels to as much as 230 Mt N y^{-1} by 2050 (Galloway et al., 2004; Llusià et al., 2014; Yuan et al., 2017), especially in India, China and Europe, which are also the major global manufacturers and emitters of reactive N (Liu et al., 2011; Yuan et al., 2017). Higher N deposition from anthropogenic sources such as fertilizers, combustion of fossil fuels and cattle residuals (Blanch et al., 2009; Galloway et al., 2008; Peñuelas et al., 2013) has an important long-term impact on ecosystem structure and function (Phoenix et al., 2012; Meunier et al., 2016), including some potential threats to soil acidification that decreases the defensive capacity and biodiversity of plants (Phoenix et al., 2012; Valliere and Allen, 2016; Zhang et al., 2017). GEC research should therefore not ignore N deposition (Janssens et al., 2010).

More than 100 000 chemical products have been identified in plants (Dicke and Loreto, 2010), including many biogenic volatile organic compounds (BVOCs) (Loreto and Schnitzler, 2010) that contribute about 90% of the global emission of volatile

organic compounds (VOCs) into the atmosphere (Guenther et al., 1995). BVOCs are a crucial group of plant compounds due to their important role in the ecology of plants (Dicke and Loreto, 2010; Niederbacher et al., 2015). Isoprenoids, which account for >50% of the total emission of BVOCs (Guenther et al., 1995), are the most dominant components of the biosphere-atmosphere exchange of BVOCs (Sharkey and Monson, 2017) because they play important roles in plant responses to both abiotic and biotic stresses (Loreto and Schnitzler, 2010; Holopainen and Gershenzon, 2010; Peñuelas and Llusià, 2003) and in the chemistry of the atmosphere (Dicke and Loreto, 2010; Niederbacher et al., 2015). Plants are essential components of complex communities that include organisms ranging from microorganisms to mammals and have evolved intricate mechanisms for using BVOCs to defend against enemies such as pathogens, parasitic plants and herbivores and for interactions with other plants and beneficial organisms such as pollinators and predators (Dicke and Loreto, 2010; Farré-Armengol et al., 2015). BVOCs are reactive hydrocarbons that contribute to the production of tropospheric ozone in the presence of NO_x compounds and sunlight (Dicke and Loreto, 2010; Tiiva et al., 2017) and to the formation and growth of aerosol particles in the atmosphere (Paasonen et al., 2013; Tiiva et al., 2017).

Most BVOC emissions are associated with photosynthesis (Monson and Fall, 1989) and account for a relevant amount of the carbon fixed by photosynthesis. Under stressed conditions, a larger proportion of fixed carbon is often devoted to isoprenoid emissions (Loreto et al., 2001; Vallat et al., 2005; Blanch et al., 2009), besides, the emission of some terpenes may be limited by stomatal conductance (Niinemets et al., 2002; Harley

et al., 2014) due to strong stomatal sensitivity (Niinemets et al., 2002). An abundant input of N to the soil is usually beneficial for plants due to an increase in photosynthetic activity (Häikiö et al., 2007; Handley and Grulke, 2008), and plants will allocate proportionately more carbon toward growth and less toward carbon-based secondary compounds used for defense (Loreto and Schnitzler, 2010; Peñuelas and Llusià, 2003). Low levels of N addition, however, can increase terrestrial plant productivity (Vitousek and Howarth, 1991; Pivovaroff et al., 2016), and high levels exceeding critical loads may decrease productivity and render plants more susceptible to environmental stressors (Cardoso-Vilhena and Barnes, 2001; Yuan et al., 2017), such as excess of ozone, which induce rapid stomatal closure (Kollist et al., 2007; Vahisalu et al., 2008; Li et al., 2017) and low stomatal vitality (Paoletti and Grulke, 2010; Li et al., 2017). Immediate photosynthetic responses during and after stress are primarily driven by modifications in stomatal conductance (Kollist et al., 2007; Vahisalu et al., 2008), so limitations on CO_2 uptake may substantially decrease photosynthesis, which would also tend to affect BVOC emissions. Even though the assimilative response to N deposition, is not uniform and varies among species or even ecosystems (Bobbink et al., 2010; Alvarez-Clare et al., 2013) because of differences in resource availability, acquisition and strategies of use (Vourlitis and Pasquini, 2009; Pivovaroff et al., 2016), it may affect the emission of BVOCs by directly affecting their biosynthesis or by affecting primary physiological metabolism (Loreto and Schnitzler, 2010; Pivovaroff et al., 2016).

Environmental experiments have been widely used to predict potential physiological and phenological profiles, with general considerations of realistic

changed conditions and their interactions to simulate future environmental scenarios (Beier et al., 2012; Leuzinger et al., 2011; Ogaya et al., 2014). Increases in temperature caused by climate change and increases in soil N content are thus two GEC factors that can interact. Climate change is expected to increase the temperature by 1.5–3.7 °C and the frequency and intensity of drought in many parts of the world by 2100, particularly during the summer and normally drier months (IPCC, 2014; Tiiva et al., 2017). Rates of BVOC emission can increase under moderate drought in conjunction with warmer temperatures caused by climate change and help plants to resist stress (Breshears et al., 2005; Allen et al., 2015), but rates can decrease under severe environmental conditions (Gershenzon et al., 1978; Llusià et al., 2011; Llusià et al., 2013). 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 Llusià, 2001; IPCC, 2014). The important interaction between the emission of phytogenic VOCs and climate change has also elicited great interest in detecting the effects of realistic combinations of other GEC factors on the emission of BVOCs from vegetation. Current models predict that soil N content, air temperature and soil moisture are expected to change concurrently, and understanding their interactive influences on BVOC emissions is essential for predicting future BVOC dynamics (Llusià et al., 2014; Zhang et al., 2017; Tiiva et al., 2017). The triple threat of N deposition, drought and warming, representing the most realistic future, on isoprenoid emissions have not been investigated but could provide valuable insights for understanding the dynamics of isoprenoid emissions in the near future. These changing GEC factors, in a long-term

perspective, could also shift the composition and structure of ecosystems (Valolahti et al., 2015), which could also influence emission profiles.

The influence of N deposition on BVOC emissions is poorly understood. Most studies of the effects on isoprenoids have been short-term or conducted in warm seasons or in greenhouses (Blanch et al., 2007; Carriero et al., 2016) or open-top chambers (Llusià, et al., 2014; Yuan et al., 2017), and the results have been inconsistent (Blanch et al., 2007; Peñuelas and Staudt, 2010; Yuan et al., 2017) due to the variations in response to N availability by different plant species. Yuan et al. (2017) reported that a moderate amount of N fertilization (50 kg N ha^{-1}) increased the emission of isoprene in Cathay poplar (*Populus cathayana*), Llusià et al. (2014) reported that the emission of terpenes decreased in two Mediterranean leguminous species (*Ornithopus compressus* and *Trifolium striatum*) at similar N levels (40 kg N ha^{-1}). Isoprene emission was also stimulated in velvet bean (*Mucuna* sp.) (Harley et al., 1994), aspen and white oak (Litvak et al., 1996) with increasing N availability, supporting the existence of links between foliar N status and isoprene synthase activity (Litvak et al., 1996). Blanch et al. (2007) found that a high amount of N fertilization (250 kg N ha^{-1}) decreased the emission of terpenes in *Pinus halepensis* but had no influence on *Quercus ilex*, and moderate drought increased the terpene emissions of fertilized plants for both Mediterranean species in summer. Kivimäenpää et al. (2016) observed that higher N availability increased the emission of some minor terpene compounds in Scots pine and some major terpene compounds in combination with warming in summer. Carriero et al. (2016) reported that individual monoterpenes had a compound-specific response to

N in silver birch (*Betula pendula*), due to different pathways of biosynthetic formation of the emitted compounds (Kesselmeier and Staudt, 1999; Niinemets et al., 2004), but N fertilization did not significantly affect total terpene emissions. Ormeño et al. (2009) suggested that the positive relationship between N fertilization and terpene emissions for two Mediterranean species, *Rosmarinus officinalis* and *Quercus coccifera*, only occurred at optimal soil N conditions.

Erica multiflora and *Quercus ilex* are two widespread species in western and central Mediterranean shrublands and forests, respectively, and both are important isoprenoid emitters, dominated by isoprene and terpenes, respectively (Kesselmeier and Staudt, 1999). Our study was relatively long-term and conducted in fields, which investigated the seasonal and daytime variations of net photosynthetic rate (A), stomatal conductance (g_s) and rate of isoprenoid emissions in these two dominant species at two study sites, a Garraf shrubland and a Prades forest (Llusià et al., 2011; Llusià et al., 2013; Ogaya et al., 2014). The aim of this study was to assess the realistic and systematic response of isoprenoid emissions in these Mediterranean shrubland and forest ecosystems to experimental N deposition and thus to improve the estimations of the emission dynamics expected in the coming decades by models that take GEC into consideration.

2. Material and methods

2.1. Study sites and species description

The study was carried out in the Garraf and Prades Mountains in Catalonia,

northeastern Spain (Figure S1). The climate and vegetation at the two sites are typically Mediterranean. 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.). The vegetation has a coverage of 50–60% and a maximum height of 70 cm. 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 Sea. The Prades sampling site is a holm oak forest with tree heights between 1.5 and 10 m (Bolòs and Vigo, 1990; Llusià et al., 2013; Ogaya et al., 2014). Both sites contain abundant evergreen and deciduous species, and the dominant species are common throughout the western Mediterranean Basin (Bolòs and Vigo, 1990; Llusià et al., 2013; Ogaya et al., 2014). We chose one dominant species at each site: *E. multiflora* in Garraf and *Q. ilex* in Prades.

2.2. Experimental design

Six plots (5 × 4 m) were randomly established in Garraf in three replicate blocks, with each block having one N and one control plot. Four plots (15 × 10 m) were established in Prades at the same altitude along the slope, two as N and two as control plots. The N treatments were fertilized with 60 kg N ha^{-1} using NH_4NO_3 (Fluka, Buchs, Switzerland), and the control treatments were not fertilized. The N-treatment plots were fertilized homogeneously three times each season (total of 15 kg N ha^{-1} $season^{-1}$) in 2015. Emissions were measured in the centers of the plots to reduce edge effects, with the outer 0.5 and 1 m serving as open buffer zones for the plots in Garraf and Prades, respectively. Emissions were measured from spring 2014 to winter 2016 twice a day, morning (9:00–12:00 solar time) and midday (13:00–16:00 solar time), on three

consecutive days in the middle of each season. The N deposition for the two dominant species had thus been manipulated for one year, but we also obtained pre-treatment data for the previous year. 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 (Delta-T Devices Ltd, Cambridge, England), 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 gas-exchange system (LI-COR, Lincoln, Nebraska USA). A and g_s were measured at a photosynthetic quantum flux density (PPFD) of 1000 ± 20 μmol m^{-2} s^{-1} under a controlled CO_2 concentration of 400 ± 2 ppm. One *E. multiflora* needle cluster or one *Q. ilex* leaf was enclosed in a clip-on gas-exchange cuvette with a surface area of 2 cm^2. The emitted isoprenoids were pumped from the cuvette through a stainless-steel tube (89 mm in length and 6.4 mm external diameter) manually filled with adsorbents (115 mg of Tenax® TA and 230 mg of SulfiCarb®) separated by sorbent-retaining springs, fixed using gauze-retaining springs and closed with air-tight caps (Markes International Inc. Wilmington, USA). The flow across the tubes was measured with a Bios Defender 510 flow meter (Bios International Corporation, Butler, USA) and controlled at about 300 mL min^{-1} with a metallic valve. The flow was generated using a Q-MAX air-sampling pump (Supelco, Bellefonte, USA), and the sampling time was 10 min. 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 (α-pinene, β-pinene, 3-carene, limonene, sabinene and dodecane). The tubes were conditioned before isoprenoid sampling with a stream of 100 mL min^{-1} of purified helium at 350 °C for 35 min. The trapping and desorption efficiencies of liquid and volatilized standards such as α-pinene, β-pinene or limonene (the main terpenes accounting for about 65–90% of total emissions) were near 100%. Blank samples of air without leaves in the cuvette were collected for 10 min immediately before each measurement. The sampled parts of the leaves were cut to the perimeter enclosed in the Licor-6400XT cuvette and stored in a portable cooler at 4 °C, taken to the laboratory and oven-dried at 60 °C to constant weights. The metallic tubes (with trapped BVOCs) were stored at 4 °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 samples were applied to a 30 m × 0.25 mm × 0.25 μm film capillary column (HP-5, Crosslinked 5% pH Me Silicone; Supelco, Bellefonte, USA). A detailed description of the chromatographic method is provided by Mu et al. (2018).

The terpenes 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. The concentrations of common terpenes such as α-pinene, 3-carene, β-pinene, myrcene, limonene, and sabinene were determined from calibration curves every five analyses using four terpene concentrations (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

Data variables were analyzed (ANOVA) using STATISTICA v.8.0 (StatSoft, Inc., Tulsa, USA). Statistical differences between treatments were identified with a Student's *t*-test. Differences were considered significant at $P < 0.05$. The significance of the effects of season, treatment and sampling time were determined by a repeated-measures ANOVA. Regression analyses were conducted using Sigma Plot v. 14.0 for Windows (Systat Software, Chicago, USA). The covariance of emissions of individual terpenes (dependent variables, Y) with environmental conditions and physiological activities (independent variables, X) was analyzed by partial least squares (PLS) regression using the plsdepot package in R v. 3.3.3. The PLS model included two components and was validated by cross-validation. Sigma Plot v. 14.0 and R v. 3.3.3 were also used to generate the figures.

3. Results

3.1. Seasonal and daytime variation of air temperature and soil moisture

Annual air temperature and soil moisture were similar between the control and N

treatments, with variation <10% for both sites. Mean air temperature on the sampling dates in Garraf ranged between 10.1 ± 0.84 °C in winter mornings in 2015 and 34.4 ± 0.60 °C at summer middays in 2014. Soil moisture ranged between 5.5 ± 0.39% (v/v) in summer middays in 2015 and 25.8 ± 0.47% (v/v) at winter middays in 2015 (Fig. S2). Mean air temperature on the sampling dates in Prades ranged between 13.6 ± 1.11 °C in winter mornings in 2016 and 32.7 ± 1.23 °C at summer middays in 2014. Soil moisture ranged between 4.6 ± 0.30% (v/v) at summer middays in 2014 and 24.6 ± 1.37% (v/v) in winter mornings in 2015 (Fig. S2).

3.2. Seasonal and daytime variation of A and g_s

A for *E. multiflora* was always highest in spring or autumn and lowest in winter (Fig. S3A). *A* was also notably high in mornings but decreased significantly at middays during spring and summer. G_s was always highest in autumn and lowest at winter. *A* and g_s differed significantly between the nitrogen and control treatments in winter, lower by 49.0% ($P < 0.05$) in mornings for g_s and by 40.5% for *A* ($P < 0.05$) and 40.8% for g_s ($P < 0.01$) at midday. G_s was 41.0% ($P < 0.05$) lower in the N than the control treatments in summer mornings. *A* increased sharply in spring relative to the pre-treatments after the first addition of 15 kg N ha^{-1}. *A* noticeably decreased and g_s increased compared to autumn in 2014 after the third addition of 15 kg N ha^{-1}.

A for *Q. ilex* was always highest in spring and lowest in summer (Fig. S3B). G_s was always highest in spring or winter and lowest in summer. *A* at winter midday was significantly lower by 32.7% ($P < 0.01$) in the N than the control treatments. *A* in autumn and winter was noticeably higher in the N treatments than the pre-treatments,

and g_s first noticeably decreased in spring mornings and then increased steadily in the following seasons, with higher values than the previous year after continual N fertilization.

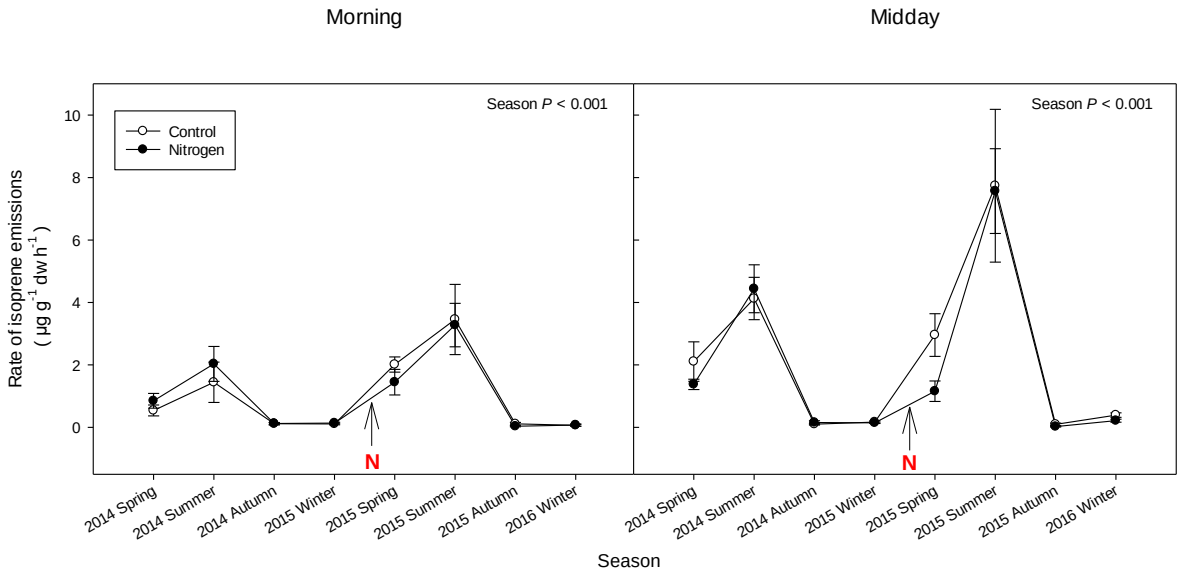
3.3. Seasonal and daytime variation of isoprenoid emissions

Isoprenoid emission rates typically oscillated seasonally in both species, with maxima in summer and minima in cold seasons (Figs. 1 and 2). Isoprenoid emissions were higher for *E. multiflora* in 2015-2016 (Fig. 1), but *Q. ilex* emitted more isoprenoids in 2014-2015 (Fig. 2). Isoprenoid emissions were always higher at midday than in the morning for both species (Figs. 1 and 2). Both species emitted a variety of terpenes in spring and summer, and most terpenes were detected at summer midday (Table S1). α-Pinene and limonene were the two most abundant terpenes for both species and were detected at all sampling times, with trends similar to those for total terpene emissions (Figs. 2 and S4). Large amounts of tricyclene and β-caryophyllene for *E. multiflora* and β-pinene and β-myrcene for *Q. ilex* were also detected in warm seasons (Fig. 3).

E. multiflora emitted both isoprene and terpenes, with isoprene the main compound due to the large emissions in spring and summer (Fig. S5). Isoprene emissions ranged between 0.03 ± 0.02 μg g^{-1} dw h^{-1} at autumn middays in 2015 and 7.74 ± 2.45 μg g^{-1} dw h^{-1} at summer middays in 2015 (Fig. 1A). Isoprene emission rates did not differ significantly between treatments but tended to increase after N fertilization in the same plots, especially at summer middays. Total terpene emissions for *E. multiflora* ranged between 0.10 ± 0.05 μg g^{-1} dw h^{-1} in winter mornings in 2015 and 3.99 ± 0.56 μg g^{-1}

dw h^{-1} at summer middays in 2015 (Fig. 1B). N fertilization increased total terpene emissions in spring and summer relative to the pre-treatments and control treatments, especially at summer midday, with a significant increase of 76.1% ($P < 0.05$). α-Pinene and limonene were emitted mostly at summer middays in 2015, at rates of about 1.3 and 0.8 μg g^{-1} dw h^{-1}, respectively.

A)



B)

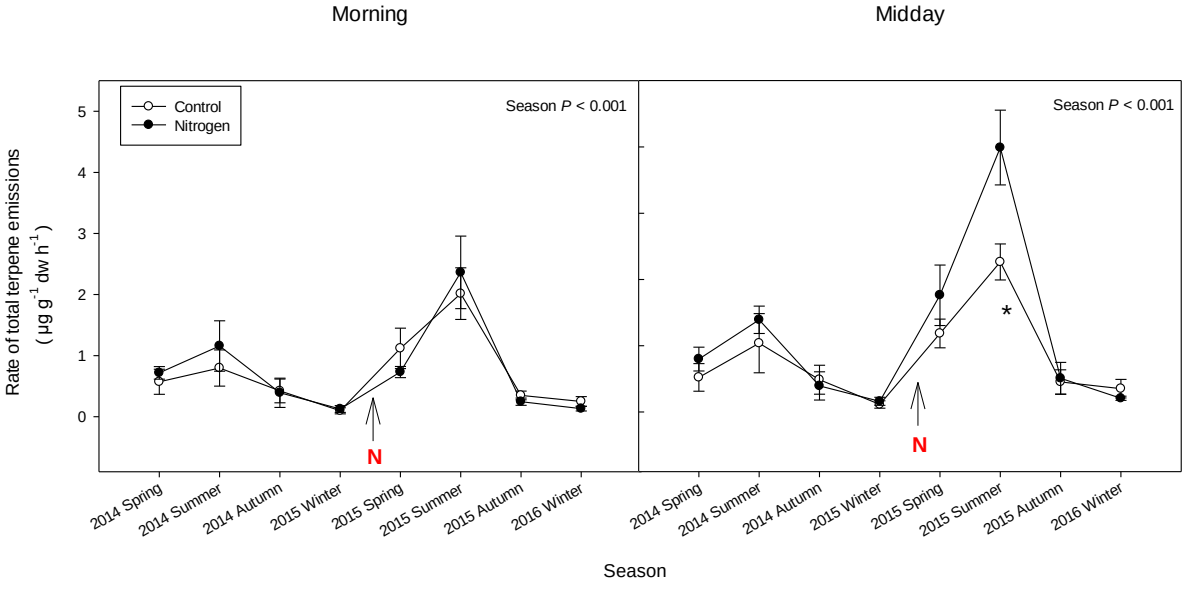


Fig. 1. Seasonal variation of the rates of emission of isoprene (A) and total terpenes (B) for *Erica multiflora*. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means ($n = 6$). Significant differences between treatments identified by Student's *t*-tests are indicated by asterisks (*, $P < 0.05$). The effects of season, treatment and sampling time are depicted in the panels when significant.

Q. ilex did not emit isoprene but was a large terpene emitter. The emission of total terpenes ranged between 0.35 ± 0.08 μg g^{-1} dw h^{-1} at winter middays in 2016 and 23.9 ± 3.23 μg g^{-1} dw h^{-1} at summer middays in 2014 (Fig. 2). *Q. ilex* emitted fewer terpenes in the fertilized year for the same plots, but total terpene emissions still increased significantly relative to the control treatments at summer middays by 83.7% ($P < 0.05$), coinciding with significantly higher limonene ($P < 0.01$). α-Pinene and limonene were emitted mostly at summer middays in 2014, at rates of about 10 and 7 μg g^{-1} dw h^{-1}, respectively.

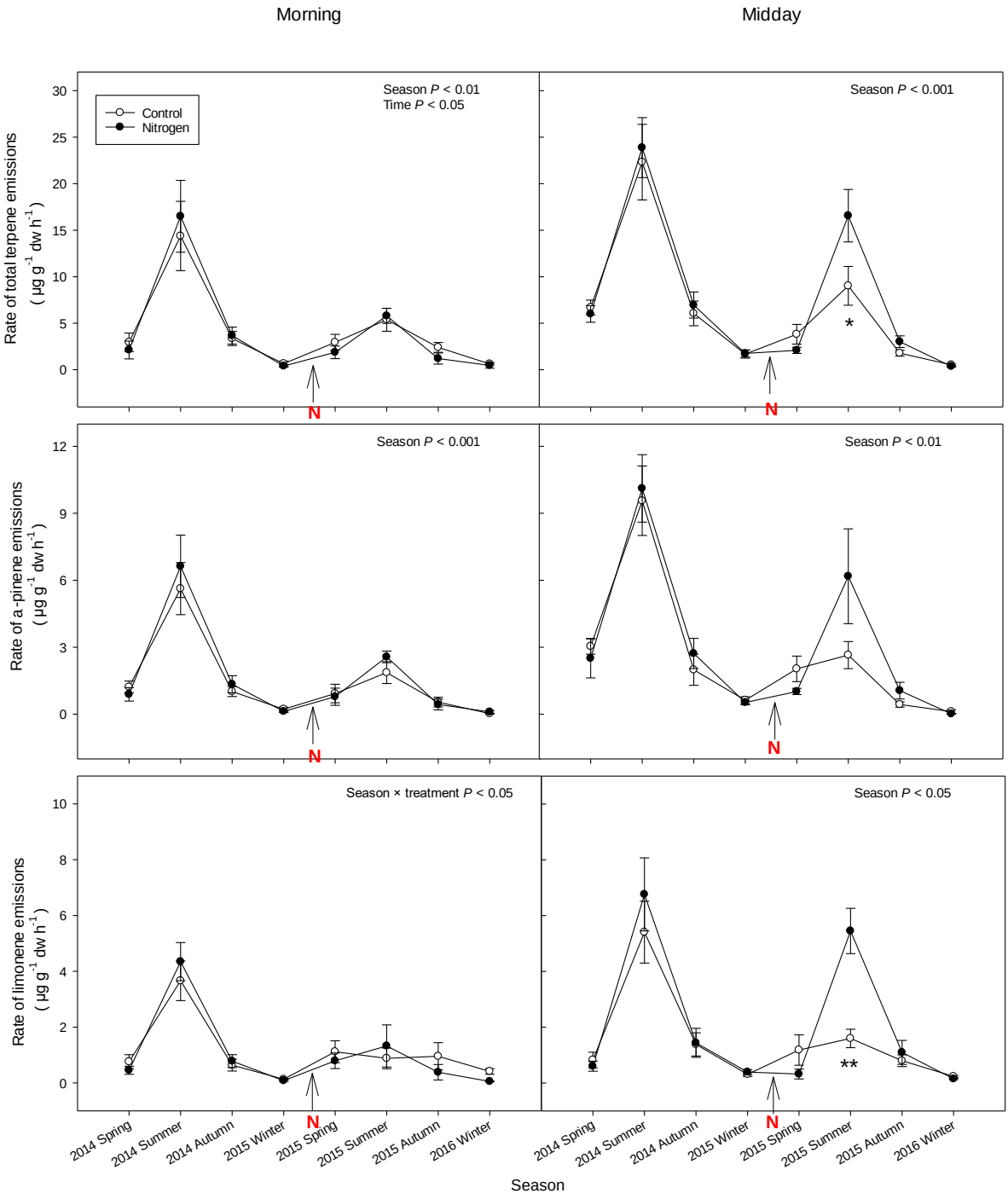
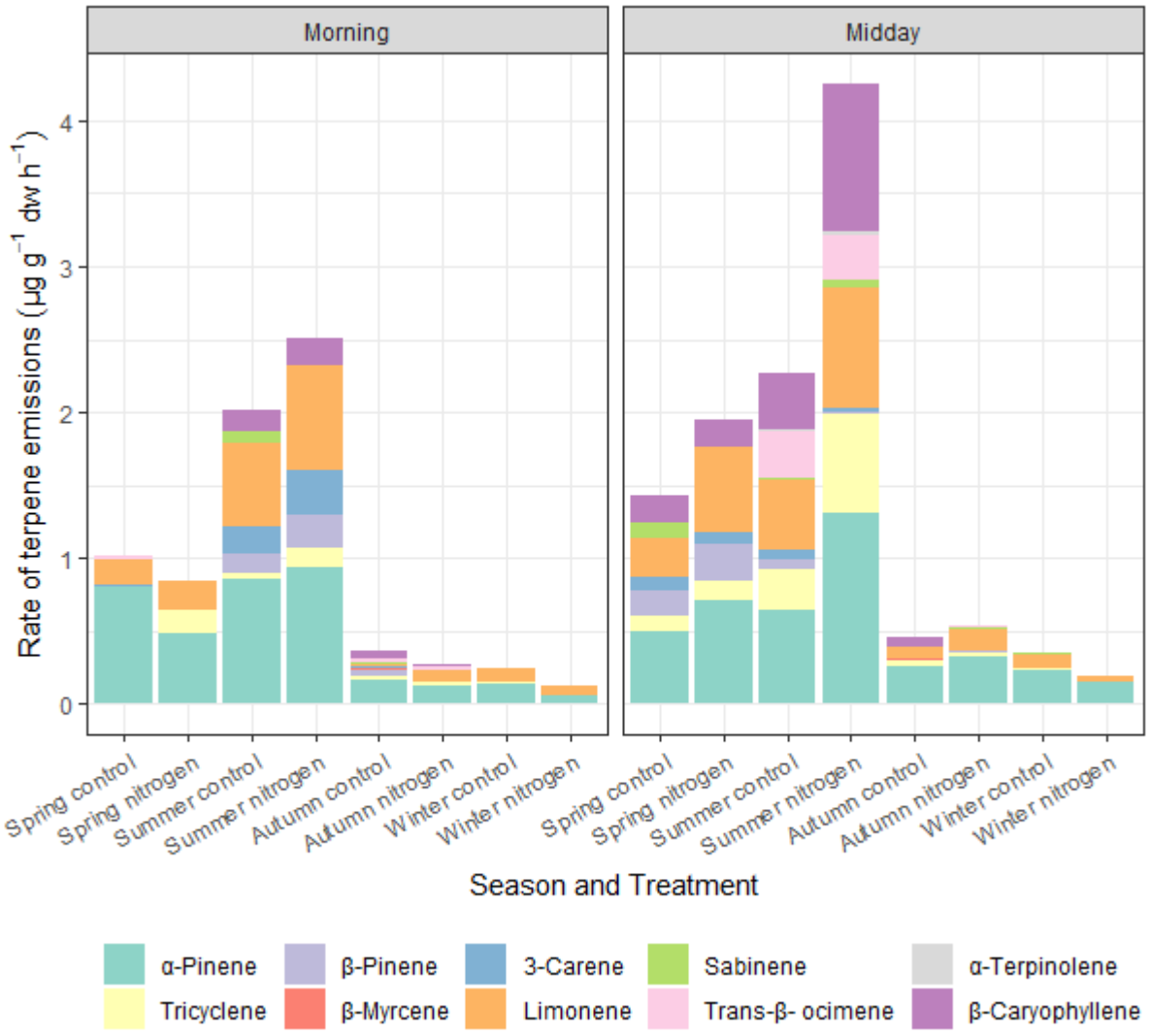


Fig. 2. Seasonal variation of the rates of emission of total terpenes, α-pinene and limonene for *Quercus ilex*. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means (n = 6). Significant differences between treatments identified by Student's t-tests are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$). The effects of season, treatment and sampling time are depicted in the panels when significant.

A)


Morning
Midday
Rate of terpene emissions (µg g⁻¹ dw h⁻¹)
0
1
2
3
4
Spring control
Spring nitrogen
Summer control
Summer nitrogen
Autumn control
Autumn nitrogen
Winter control
Winter nitrogen
Season and Treatment
α-Pinene
β-Pinene
3-Carene
Sabinene
α-Terpinolene
Tricyclene
β-Myrcene
Limonene
Trans-β- ocimene
β-Caryophyllene

B)

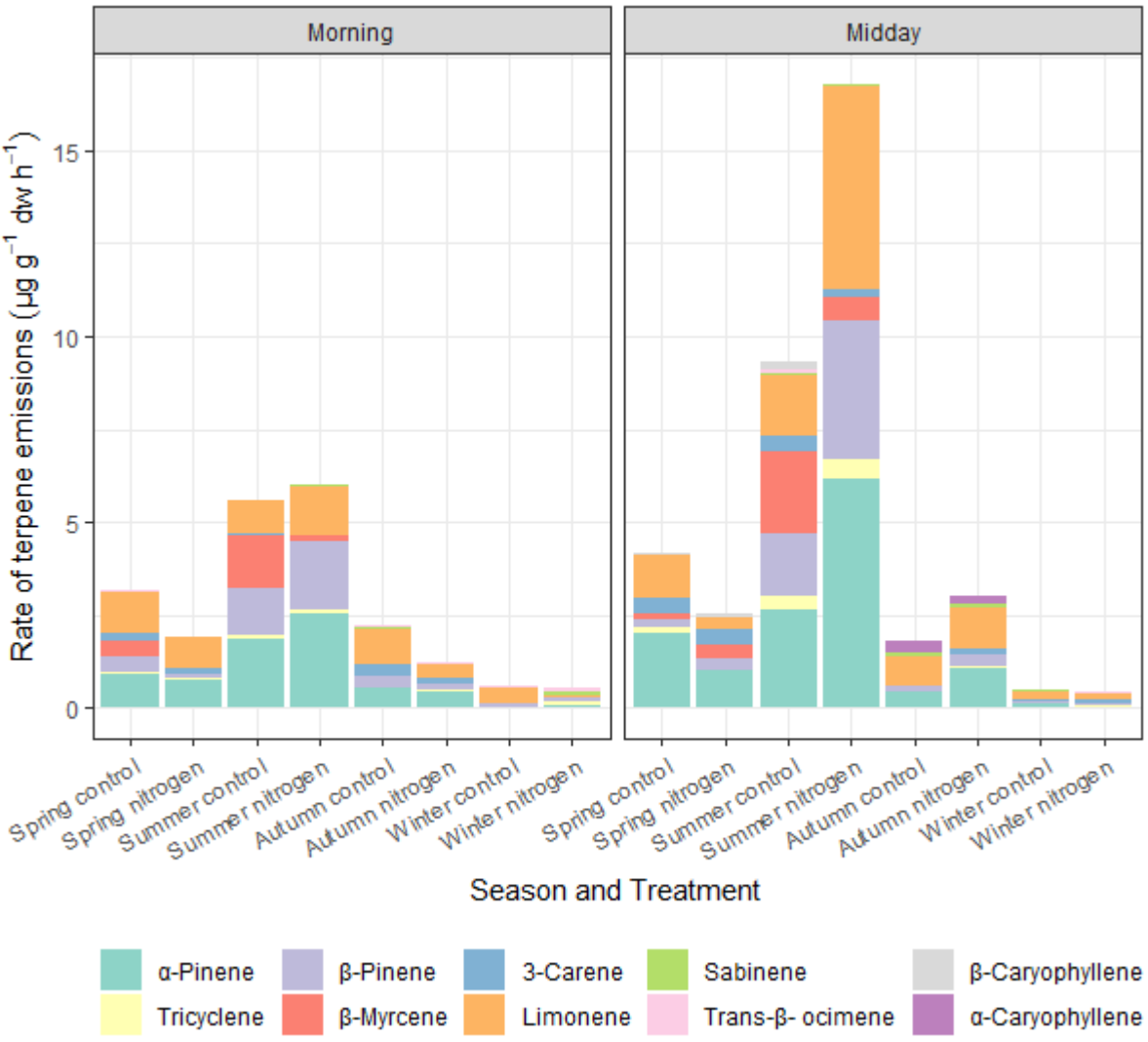


Fig. 3. Distribution of seasonal terpene emissions for *Erica multiflora* (A) and *Quercus ilex* (B) in the morning and at midday for the fertilized year.

4. Discussion

4.1. Seasonal and daytime variations of A, g_s and isoprenoid emissions with N fertilization

Photosynthesis of most plants increases in warm weather if soil moisture and nutrients are not limiting (Wan et al., 2009; Selsted et al., 2012). 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), with A and g_s lowest in winter for *E. multiflora* (Fig. S3A) and in summer for *Q. ilex* (Fig. S3B) in our study. *E. multiflora* was particularly N responsive. *Q. ilex*, however, displayed fewer physiological adjustments with N fertilization, suggesting poor N acclimation (Pivovaroff et al., 2016).

The emission of isoprenoids differed between the species but followed a similar seasonal pattern. 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 (Llusià et al., 2011; Mu et al., 2018). Both species emitted most isoprenoids at summer midday, coinciding with lower A, and the N treatments increased terpene emissions significantly (Figs. 1, 2 and S3). Plants could temporarily decrease photosynthetic activity under drought stress because of the increased resistance to CO_2 in both the stomata and mesophyll (Centritto et al., 2003; Mu et al., 2018), meanwhile, a higher proportion of photosynthetically fixed carbon was used for increasing terpene production to reduce the damage caused by oxidative stress at summer midday (Vallat et al., 2005; Blanch et al., 2009), and plants increased terpene emissions with the higher N deposition under the heat and drought stress, indicating a successful acclimation by adjusting metabolism under environmental stress (Litvak et al., 1996; Loreto et al., 2001). The N treatment favored terpene production (Blanch et al., 2009; Ormeño and Fernandez, 2012), especially under environmental stress. Higher N contents likely translate into higher enzymatic activity and thus higher terpene production (Figs. 1B and 2) in these two non-storing species, whose emission of terpenes depends on short-

term production (Litvak et al., 1996).

4.2. Comprehensive impacts of annual climate and N fertilization on isoprenoid emissions

N fertilization for *E. multiflora* increased *A* in spring 2015 when temperature was suitable and water availability increased. The stimulation also indicated the importance of even a small increase in soil moisture during spring in the warm plots, increasing N availability in this nutrient-limited ecosystem (Figs. S1 and S2A) (Llusià et al., 2014; Zhang et al., 2017; Tiiva et al., 2017). Abundant nutrition helps plants to recover from slow physiological rhythms in winter and to return to growth quickly, especially at midday. The higher temperature at spring midday in 2014, though, may have restrained *A*, although not significantly, because the environmental conditions were sufficiently suitable for assimilation, maintaining *A* at a high level for both treatments and saturating N usage (Gundersen et al., 1998; Chen et al., 2016). N fertilization tended to have the least influence in summer, supported by the similar environmental conditions between the two years, in contrast to previous studies hypothesizing that drought-tolerant evergreen shrubs are favored under a warming climate with increased CO_2 or N levels due to a higher *A* (Fineschi et al., 2013; Tiiva et al., 2017). In autumn and winter, temperature variations may have been the main cause of the fluctuations in *A* between two years, and excessive N began to negatively affect *A* and g_s, especially at winter midday. All fluctuations between the two years for *Q. ilex* were due to the variations in environmental conditions (Figs. S1 and S2B), which was obvious for autumn and winter, indicating that better water-heat interaction led to higher *A* in the fertilized year. *A* even

decreased significantly at winter midday for the N treatments, most likely due to the decrease in both air temperature and soil moisture rather than to excess of available N.

Annual climate clearly played an important role due to the importance of some environmental parameters (water and temperature) in setting the rates of isoprene and terpene emissions (Jardine et al., 2014; Fernández-Martínez et al., 2018) under N-rich conditions. For *E. multiflora*, more isoprenoids were emitted in spring and summer in 2015. Isoprenoid emissions from this temperate heath generally depend on both the current environmental drivers and on the preceding season and weather (Niinemets et al., 2010; Llusià et al., 2013; Tiiva et al., 2017), which may suggest a high emission potential for the ecosystem in the hot season if *A* was high in the previous growing season (Figs. 1 and S3A). Emissions then decreased to stable low levels. Increased *A* in spring stimulated emissions in both spring and summer. *Q. ilex* emitted most terpenes in summer 2014 due to a moderate drought, even though the N treatment significantly increased emissions at summer midday in 2015. *Q. ilex* may thus be much more sensitive to water availability than N content in hot seasons (Figs. 2 and S2).

Taking into account the results and conditions of this study and considering the climate in combination with available N levels could help us to better understand the systematic role of climate as a determinant of the dynamics of isoprenoid emissions (Fernández-Martínez et al., 2018). Species without structures for storing terpenes are more likely to have higher emission rates in N-rich conditions, supporting findings linking high foliar N content to high rates of isoprenoid emission (Litvak et al., 1996; Possell et al., 2004; Blanch et al., 2009; Fernández-Martínez et al., 2018). Our results

thus suggest that N is important for the emission of both isoprene and terpenes, and the variations in emission may be associated with different strategies of N uptake and use (Litvak et al., 2002; Fernández-Martínez et al., 2018), albeit the relationship between them was not very strong, except in summer. This relationship may also be due to the mineralization of soil N or because N absorption by plants is not likely to be limited by water under current conditions (Gundersen et al., 1998; Wan et al., 2009; Chen et al., 2016). These favorable environmental conditions (water and N availability) in the growing season enable high rates of photosynthesis, which in turn have been linked to high rates of isoprenoid emission (Monson et al., 1994; Litvak et al., 1996; Fernández-Martínez et al., 2018) in summer. The positive correlation between soil N content and isoprenoid emission rates may be due to both a direct effect of N on isoprenoid emission rates and an indirect effect from the positive effect of N on photosynthesis (Monson et al., 1994; Fernández-Martínez et al., 2018).

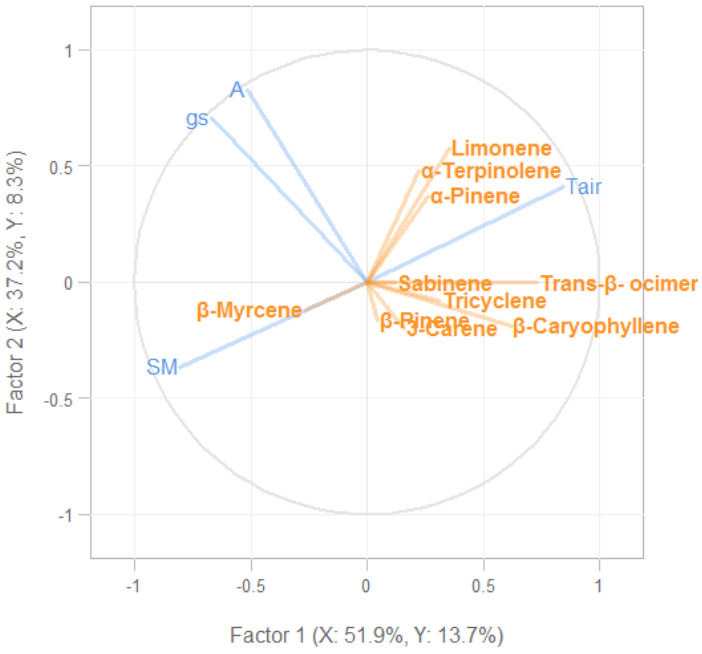
4.3. The influence of N deposition on the relationship between main physiological or environmental parameters and isoprenoid emissions

Environmental conditions such as air temperature and soil moisture are the main factors that determine BVOC emissions (Gershenzon et al., 1978; Breshears et al., 2005; Llusià, et al., 2011; Allen et al., 2015). BVOC emissions, however, are also largely influenced by A and g_s, the two main physiological activities of plants (Eller et al., 2016). The PLS regression analysis did not find a strong correlation between terpene emissions and physiological activities but found a strong correlation with environmental conditions (Fig. 4). All terpene emissions by *E. multiflora* were

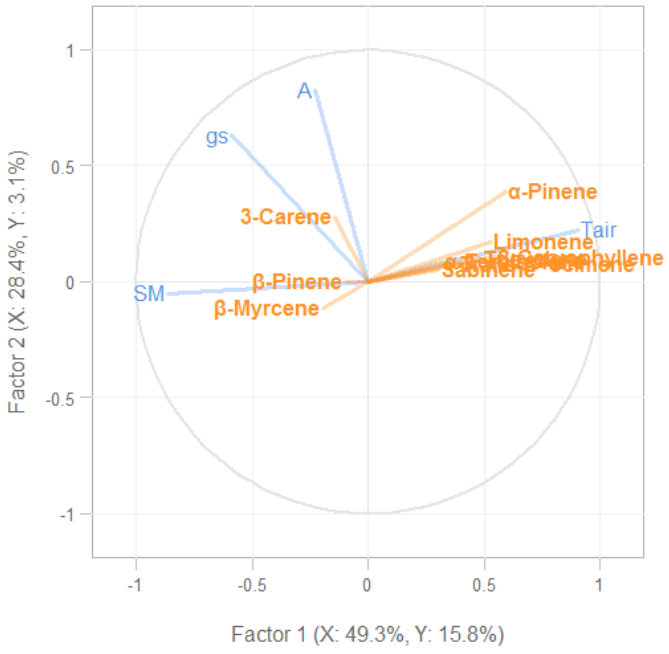
correlated positively with air temperature and negatively with soil moisture, except for β-myrcene, which had the opposite trend. β-Pinene had the same relationship with β-myrcene, and 3-carene emission became strongly correlated with physiological activities in the N plots. Trans-β-ocimene, sabinene and α-caryophyllene emissions by *Q. ilex* were not obviously correlated with any parameter, and other species followed a common trend with environmental conditions. α-Pinene and limonene have recently been reported as the main emitted terpenes; α-pinene is sensitive to temperature, and limonene responds more strongly to water deficits (Mu et al., 2018). The correlation for β-caryophyllene with environmental conditions disappeared, and sabinene emission was slightly positively correlated with g_s in the N plots. N fertilization generally increased the correlation between the most emitted compounds and the environmental conditions but decreased it for the least emitted compounds (Fig. 4), which may indicate that N fertilization increased the rates of terpene emission (Fig. 3) but slightly decreased the diversity of terpenes in the warm seasons (Table S1). *Q. ilex* emitted more terpenes in summer in the N plots, but mainly α-pinene, β-pinene and limonene contributed to the increase, and almost all other terpene emissions decreased, especially for β-myrcene and 3-carene (Fig. 3B) and very-least compounds, such as trans-β-ocimene, β-caryophyllene and α-caryophyllene, tended to disappear (Table S1). Not all terpene emissions, however, were higher in summer. This diversity of responses may have been due to the environmental effects on the activities of synthases, the potential protective roles of the various terpenes under environmental constraints (Blanch et al., 2009) or to the increases in foliar N content (Blanch et al., 2009).

Erica multiflora

A) Control

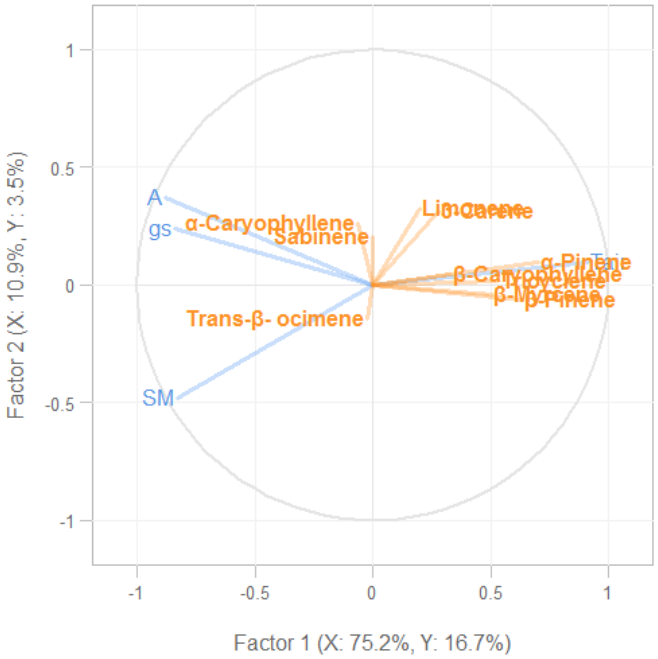


B) Nitrogen



Quercus ilex

C) Control



D) Nitrogen

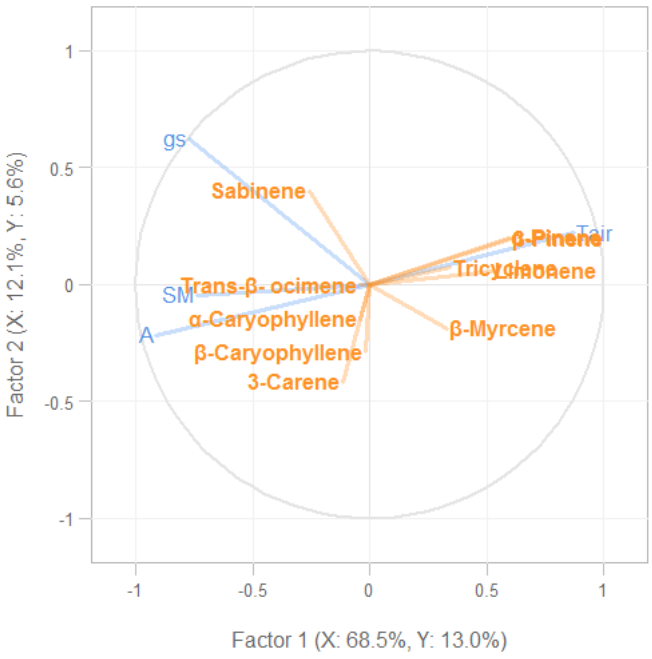
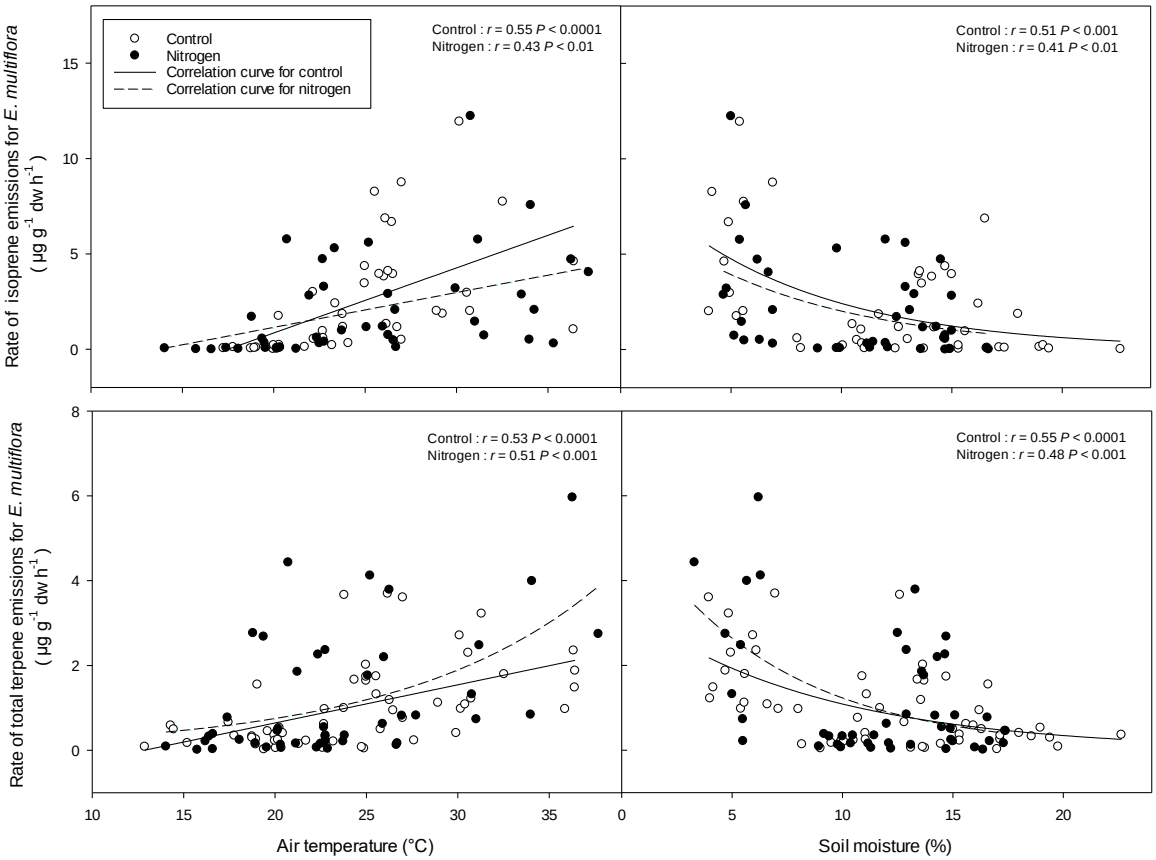


Fig. 4. Partial least squares (PLS) regression between main environmental or physiological parameters and terpene emissions for *Erica multiflora* (A for control treatments, B for nitrogen treatments) and *Quercus ilex* (C for control treatments, D for nitrogen treatments) in the fertilized year. Blue represents environmental or physiological parameters (independent variables, X), and yellow represents emission rates of individual terpenes (dependent variables, Y). Tair, air temperature; SM, soil moisture; A, net photosynthetic rate; gs, stomatal conductance. Individual terpenes: α-pinene, tricyclene, β-pinene, β-myrcene, 3-carene, limonene, sabinene, trans-β-ocimene, α-terpinolene, β-caryophyllene, α-caryophyllene.

We also analyzed the corresponding correlations that can be applied to the modelling of BVOC emissions (Table S2). The emission rates of isoprene and total terpenes were both correlated positively with air temperature and negatively with soil moisture for both species (Fig. 5). N addition slightly decreased isoprene emission, especially at the high-temperature and low-moisture site, and significantly increased total terpene emissions at this same site. The emission rates of isoprenoids were correlated negatively with A and g_s for both species (Fig. S6), which supports the idea that BVOC emissions are mostly influenced by environmental conditions (Gershenzon et al., 1978; Loreto and Schnitzler, 2010; Holopainen and Gershenzon, 2010; Llusià et al., 2011). *Q. ilex* emitted more isoprenoids and A was lowest in summer than in the other seasons (Figs. 2, S3B), and higher percentages of fixed carbon were devoted to isoprenoid emission in summer. The relationships with physiological activities, however, became much weaker for the *E. multiflora* in N plots, also indicating that this species was more sensitive to nutrient availability than *Q. ilex* due to some fluctuations

in A and g_s (Figs. S3A and S6).

A)



B)

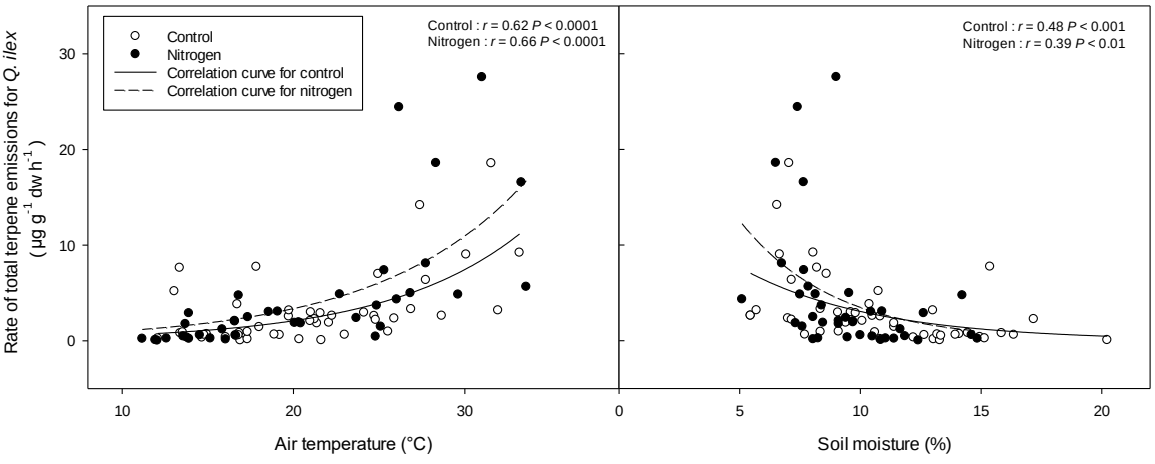


Fig. 5. Relationships for the rate of isoprenoid emissions with main environmental conditions (air temperature and soil moisture) for *Erica multiflora* (A) and *Quercus ilex* (B) in the fertilized year.

In the context of GEC, the combination of environmental factors, such as air temperature, soil moisture and N deposition, mimicked future conditions in this temperate ecosystem. The complex effects among warming, drought and N deposition can decrease the regularity of single-factor effects on isoprenoid emissions, even though the responses varied strongly between seasons, and the emission profile was mainly dominated by synergetic increases in summer. Our results indicate a successful acclimation of plants by increasing isoprenoid emissions under environmental stress, which are expected to be increased by climate change in the Mediterranean region (Litvak et al., 1996; Loreto et al., 2001). N deposition will also further stimulate these emission trends in the warmer and drier conditions projected by climatic and ecophysiological models for the coming decades (Peñuelas and Llusià, 2001; Penuelas and Staudt, 2010; IPCC, 2014). The changes in isoprenoid emissions in this region, however, also thus depend on the species, and therefore on the changes in land covers, for example from forests to shrublands with different emission capacities. Further long-term and quantitative research on the detailed emission mechanisms with multiple factors acting in concert is still warranted.

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Block 2. Soil VOC concentrations in a Mediterranean shrubland and a Mediterranean forest

Chapter 3. Annual and seasonal variations in soil volatile organic compound concentrations in a Mediterranean shrubland and holm oak forest

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Abstract

Soil biogenic volatile organic compounds (VOCs) play an important role in soil ecology and function and may affect atmospheric chemistry. While previous studies of soil VOCs have predominantly measured surface flux exchange rates, VOC concentrations within the surface soil layer are largely unknown, especially in Mediterranean ecosystems. In this study, we measured seasonal and annual concentrations of soil VOCs in a Mediterranean shrubland and a holm oak forest over the period 2014–2016. Soil CO_2 efflux, and soil enzyme and plant activities were measured as explanatory variables of soil VOC concentrations. There were greater total soil VOC concentrations in the shrubland (3.66 ± 1.01 ppb) than the holm oak forest (2.23 ± 0.51 ppb) across the study period. There were the greatest concentrations of monoterpenes (0.85 ± 0.43 ppb) and methanol (0.81 ± 0.20 ppb) in the shrubland and forest, respectively, and concentrations of methanol, acetic acid, formaldehyde, ethanol, and acetaldehyde were the dominant compounds in both ecosystems (>0.1 ppb). Although concentrations of some VOCs in both ecosystems were highest and lowest in spring and winter, respectively, the variability of other VOCs depended on compound and ecosystem. Soil temperature and water content, CO_2 efflux, and enzyme activity were the best explanatory variables for variation in soil VOC concentrations in the two ecosystems: there was a stronger association between concentration of dominant compounds, except formaldehyde, with soil temperature and/or CO_2 efflux than with soil water content. Activity of C- and N-degrading enzymes was positively associated with the concentration of VOCs, depending on ecosystem, and consistently correlated

with high soil water content. In the holm oak forest soils, net photosynthetic rate (*A*) was positively correlated with soil concentration of monoterpenes. These results show that soil VOC concentrations in these Mediterranean ecosystems are driven by soil temperature and water content, and microbial activity, in combination with ecosystem plant activity. It is thus likely that projected climate change increases in temperature increase soil VOC concentrations and lead to increases in emissions to the atmosphere; however, microbial production and consumption of soil VOCs may be modulated by soil water content.

Keywords: Soil VOC concentrations; Isoprenoids; Soil conditions; Soil CO_2 efflux; Photosynthetic rates; PTR-MS; Seasonality; Mediterranean shrubland; Mediterranean holm oak forest

1. Introduction

Biogenic volatile organic compounds (VOCs) are ubiquitous and continuously present in soils, where they play an important role in soil ecology and function and may affect atmospheric chemistry (Greenberg et al., 2012; Mochizuki et al., 2015; Peñuelas et al. 2014; Tang et al., 2019). The key components of soil VOCs are hydrocarbons, such as isoprenoids and benzenoids (Aaltonen et al., 2013; Abis et al., 2018; Asensio et al., 2007b; Ramirez et al., 2010), and oxygenated compounds, such as alcohols and other carbonyl compounds (Asensio et al., 2007b; Abis et al., 2018; Mancuso et al., 2015; Ramirez et al., 2010), and principal biotic sources include microbes (Aaltonen et al., 2013; Leff and Fierer, 2008; Mu et al., 2020; Peñuelas et al., 2014), that may also be consumers (Fall, 2003; Greenberg et al., 2012) by using them as a carbon (C) and energy source (Albers et al., 2018; Kramshøj et al., 2018; Owen et al., 2007), and roots (Gray et al., 2014; Kreuzwieser and Rennenberg, 2013; Tang et al., 2019) that may also indirectly uptake some volatile contaminants of soil solution via accumulation or degradation (Asensio et al., 2007b; Cho et al., 2005). A wide range of soil biological interactions are driven by biogenic soil VOCs, such as communication among soil microbes and plant roots (Svendsen et al., 2018; Wenke et al., 2010) that release carbon (C) rich root exudates consumed by rhizosphere microbes (Leff and Fierer, 2008), promotion of plant growth by bacterial volatiles (Asensio et al., 2007b; Ryu et al., 2003; Tang et al., 2019), and insect repellants and attractants mediated by monoterpenes (Nishida et al., 2005; Nordenhem and Nordlander, 1994).

The role of soil VOC fluxes in atmospheric chemistry has led to a number of studies,

which have shown total ecosystem emissions tend to vary in composition depending on the type of ecosystem, litter, and soil (Mu et al., 2020; Tang et al., 2019), environmental condition (Peñuelas et al., 2014; Rossabi et al., 2018), and season (Mäki et al., 2019; Tang et al., 2019). However, VOC concentrations in soils have rarely been examined, despite their important role in root–soil microbe ecological processes. Soils may function as either sources or and sinks of gaseous VOCs (Asensio et al., 2007c; Leff and Fierer, 2008; Peñuelas et al., 2014; Ramirez et al., 2010), as determined by the balance between their input and output concentrations (Tang et al., 2019) that represent the sum of abiotic processes, such as diffusion between soils and atmosphere, solubility in water, adsorption to soil organic matter (SOM) or soil minerals (Peñuelas et al., 2014; Breus and Mishchenko, 2006), and reaction with soil chemicals (Insam and Seewald, 2010), and biotic processes among roots and microbes. Thus, soil VOC concentrations are dependent on temperature, water availability, and soil physical traits, which affect plant and soil microorganism activities, and atmospheric concentration of VOC compounds near the soil surface (Asensio et al., 2007b; Tang et al., 2019; Greenberg et al., 2012; Peñuelas et al., 2014). For example, Wester-Larsen et al. (2020) found variation in soil VOC concentrations between contrasting species of summer vegetation cover in an arctic heath ecosystem, and there were positive relationships between soil VOC concentrations and soil temperature and moisture, where there was a stronger relationship with soil temperature. These effects of soil moisture and temperature on physical and biological processes associated with soil VOCs have been reported elsewhere (Asensio et al., 2007b; Cho et al., 2005; Peñuelas et al., 2014); for example,

VOC emissions increase with soil temperature and their soil uptake, albeit compound-specific, is greater at higher soil moisture content (Asensio et al., 2007b, 2008b; Mäki et al., 2017, 2019).

Soil carbon dioxide (CO_2) efflux is a good indicator of biological soil processes related to soil VOC dynamics, such as root growth and microbe activity (Asensio et al., 2007b; Leff and Fierer, 2008; Tang et al., 2019), while soil enzyme activity, which reflects nutrient acquisition of soil organisms, is an indicator of plant and microbial nutrient limitation (Baum et al., 2003; Burns et al., 2013), and aboveground processes, such as plant photosynthetic activity that drives availability of photosynthate to roots and associated rhizosphere microbe and enzyme activity, affect soil VOC concentrations (Asensio et al., 2007c; Peñuelas and Llusià, 1999, 2001; Mu et al., 2018, 2019).

Currently, the lack of understanding of drivers of soil VOC concentrations in Mediterranean ecosystems prevents our ability to accurately model and predict soil VOC responses to future climatic conditions. Therefore, we studied seasonal and annual soil VOC concentrations and likely biotic and abiotic drivers in typical Mediterranean calcareous shrubland and holm oak forest ecosystems to test the hypotheses that: 1) quantity and composition of soil VOCs vary with season; 2) soil VOC concentrations vary with environmental variables; and 3) soil VOC profiles differ between Mediterranean ecosystems, because 4) their production and consumption is affected by microbial and plant activities.

2. Material and methods

2.1. Study sites and experimental design

The study was conducted in two natural Mediterranean ecosystems in the northeastern Iberian Peninsula: a shrubland in Garraf Natural Park, south of Barcelona (41°18′N, 1°49′E) and a holm oak forest in the Prades mountains, southwest Catalonia (41°21′N, 1°2′E). The study sites have been described in detail by Mu et al (2018, 2019) and Ogaya et al (2020). Briefly, a typical Mediterranean climate prevails at both locations, where there are mild winters, dry summers, and rainy springs and autumns; soil type of the shrubland (10–40 cm) is a Petrocalcic Calcixerept and vegetation, which is dominated by evergreen species *Erica multiflora* L., *Pinus halepensis* L., *Rosmarinus officinalis* L. and *Globularia alypum* L., is <1.5 m in height, while soil type of the holm oak forest (35–90 cm) is a Dystric Cambisol and vegetation, dominated by *Quercus ilex* L. with abundant *Phillyrea latifolia* L., *Arbutus unedo* L. and other evergreen species, is >4 m in height. Twelve plots (5 × 4 m) were established at random in the shrubland and six (10 × 15 m) in the holm oak forest.

2.2. VOC sampling and analysis

Seasonal concentrations of soil VOCs were measured at two and three randomly selected locations per plot, ensuring minimal disturbance to plant roots, in the shrubland and holm oak forest, respectively, from spring 2014 to winter 2016, on three consecutive days at the mid-point of each season, from 09:00 to 12:00 hrs solar time. During 2014, VOC concentrations were sampled from all twelve shrubland and six holm oak forest plots, and during 2015 and 2016, VOC concentrations from six and

four plots, respectively, were sampled. Samples were collected using a micro-perforated Teflon probe (20 mm outer diameter, 19 mm inner diameter) inserted 25 cm into the soil; prior to probe insertion to the soil, a pilot hole was carefully made at the selected sampling location, using a metal bar with the same dimensions as the probe. Once inserted into the soil, the exposed end of the probe was connected to a membrane pump (Deluxe 224-PCMTX8, SKC Limited, Dorset, UK) that extracted air from the soil into 3-L Tedlar® polyvinyl fluoride (PVF) bags (SKC Inc., Eighty Four, PA) at a flow rate of 300 mL min^{-1}, to collect a total soil gas volume of 2.4 L. Degradation of VOCs by solar radiation in the field was minimized by enclosing the Tedlar bags inside a dark-colored bag that was then placed in shaded conditions prior to transportation to the laboratory; then, gas sample bags were stored at 4 °C in a darkened chamber prior to analysis, within 2 d of sample collection.

Collected gas was pumped from the Teflon bags to a proton-transfer-reaction mass spectrometer (PTR-MS) inlet (PTR-MS-FTD hs, Ionicon Analytik, Innsbruck, Austria) and analyzed for VOC concentrations as described by Asensio et al. (2007b, 2008b). The PTR-MS drift tube was operated at 2.1 mbar and 40 °C, with a drift field of 600 V cm^{-1}, and parent ion signal was maintained at c. 3×10^6 counts s^{-1} during measurement. Instrument calibration and transmission efficiency of the detection system were obtained using an aromatic mix standard gas (TO-14A, Restek, Bellefonte, PA, USA), with mass identification based on calibration standards and previous studies; using this approach, we conducted scans of all compounds with masses of between 22 and 205, and of 30 masses that have previously been identified with high certainty, 22

compounds were identified tentatively and three remained unknown (Table 1). We estimated concentrations of acetic acid, p-cymene, monoterpenes, and sesquiterpenes as the sum concentration of ions with several masses (checked with an α-pinene standard), because fragmentation of these compounds occurred during ionization in the drift tube.

2.3. Soil CO_2 efflux and temperature

Soil CO_2 and soil temperature were measured from 09:00 to 15:00 hrs, as described by Zuccarini et al. (2020). Briefly, 5-cm tall soil collars were inserted 2 cm into the soil (five and three per plot in the shrubland and holm oak forest, respectively) and remained in situ for the duration of the study. Soil CO_2 efflux was measured using a SRC-1 soil chamber (closed system) placed atop the collars and connected to an EGM-4 portable measurement system (PP-systems, Amesbury, USA) and soil temperature (10 cm depth) was measured at a single point proximate to the soil collars, using a digital soil thermometer (TO 15, Jules Richard instruments, Argenteuil, France).

Table 1 Tentative identification of main volatile organic compound (VOC) masses detected during the period 2014–16 and previous studies in which PTR-MS has been used to identify VOC masses.

Mass	Tentative identification	Soil studies that used PTR-MS analysis
M31	Formaldehyde	Asensio et al. (2007b), Asensio et al. (2007a), Mancuso et al. (2015)
M33	Methanol	Mancuso et al. (2015), Aaltonen et al. (2013), Asensio et al. (2007a), Ramirez et al. (2010), Abis et al. (2018)
M43	Acetic acid	Asensio et al. (2007a)
M45	Acetaldehyde	Asensio et al. (2007a), Asensio et al. (2007b), Aaltonen et al. (2013), Mancuso et al. (2015), Abis et al. (2018)
M47	Ethanol	Asensio et al. (2007a), Mancuso et al. (2015), Abis et al. (2018)
M55	1,3-Butadiene	Asensio et al. (2007a)
M57	(E)-2-hexenal	Asensio et al. (2007a), Asensio et al. (2007b)
M59	Acetone	Aaltonen et al. (2013), Asensio et al. (2007a), Ramirez et al. (2010), Abis et al. (2018)
M61	Acetic acid	Mancuso et al. (2015)
M69	Isoprene	Asensio et al. (2007b), Aaltonen et al. (2013), Mancuso et al. (2015), Abis et al. (2018)
M79	Benzene	Aaltonen et al. (2013), Asensio et al. (2007a)
M81	Monoterpene fragment	Asensio et al. (2007b), Aaltonen et al. (2013), Ramirez et al. (2010), Abis et al. (2018)
M83	Methylfuran	Mancuso et al. (2015)
M85	Cyclopentanone	Mancuso et al. (2015)
M87	Hexanol	Asensio et al. (2007b)
M93	Toluene	Asensio et al. (2007a), Asensio et al. (2007b), Ramirez et al. (2010), Abis et al. (2018)
M97	Heptanal	Asensio et al. (2007b)
M101	Unknown	
M103	Hexenol	Asensio et al. (2007a)
M107	Xylene	Asensio et al. (2007a)
M109	Sesquiterpenes	Asensio et al. (2007b)
M123	Sesquiterpenes	Asensio et al. (2007a), Asensio et al. (2007b)
M135	p-Cymene	Asensio et al. (2007a), Abis et al. (2018)
M136	p-Cymene	Asensio et al. (2007a), Asensio et al. (2007b)
M137	Monoterpenes	Asensio et al. (2007a), Ramirez et al. (2010), Aaltonen et al. (2013), Mancuso et al. (2015), Abis et al. (2018)
M138	Ethyl-3-methylbutanoate	Ramirez et al. (2010)
M147	Unknown	
M149	Unknown	
M155	Linalool	Asensio et al. (2007a)
M205	Sesquiterpenes	Asensio et al. (2007b)

2.4. Soil sampling and analyses

During each season, from spring 2014 to winter 2016, we collected three and five soil cores (5 cm diameter × 10 cm depth) per plot in the shrubland and holm oak forest, respectively; soil samples were sieved (2-mm gauge) in the laboratory and stored at 4ºC prior to analysis. A subsample of each sample was tested for gravimetrical soil water content by measuring weight loss after drying at 105 °C for 24 h. Additional subsamples were used for colorimetric analysis of soil enzyme activity, as described by Zuccarini et al. (2020): activity of acid and alkaline phosphomonoesterase (acp and akp), *β*-glucosidase (bgl), protease (prot), and urease (ure) was measured, except in 2015-16, leucine and glycine aminopeptidases (leu and gly) were measured instead of prot and ure, using *p*-nitroaniline derivate chromogenic substrates, as described in Peguero et al. (2019). Enzyme activity was expressed as µmol of substrate released $gram^{-1}$ of dry soil h^{-1} of incubation time and these values were used as enzymatic indicators of biogeochemical cycling of C (bgl), N (prot + ure or leu + gly), and P (acp + akp), that were named as enzC, enzN, and enzP respectively; stoichiometry of the enzymatic indicators was calculated as enzC/enzN, enzC/enzP, and enzN/enzP.

2.5. Plant photosynthetic and conductance activity

Mean seasonal net photosynthetic rate (A) and stomatal conductance (g_s) of two *E. multiflora* and three *Q. ilex* individuals per plot in the shrubland and holm oak forest, respectively, were measured concurrently with soil VOC sampling, using a portable

photosynthesis system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) as described in Mu et al. (2018, 2019).

2.6. Statistical analyses

Ecosystem differences in soil temperature, water content, CO_2 efflux and VOC concentration were tested using one-way analysis of variance (ANOVA), and season and year effects were tested using repeated-measures ANOVA; differences were tested at $P<0.05$. A heatmap, based on values of log10-transformed mean concentrations of compounds, was made using the pheatmap package in R (v. 4.0.3). The association between isoprenoid concentration and *A* was tested using linear correlation analysis in Sigma Plot (v. 14.0 for Windows, Systat Software, Chicago, USA) and covariance in concentrations of individual compounds with plant activity, and soil moisture, temperature, CO_2 efflux, and enzyme activity was analyzed at $P<0.1$, using redundancy analysis (RDA) in the vegan package and visualized with ggplot2 and ggrepel package of R.

3. Results

3.1. Seasonal and annual variation in soil temperature, water content, and CO_2 efflux

Average soil temperature over the sampling period spring 2014 to winter 2016 was higher in the shrubland (15.79 ± 2.91 °C) than in the holm oak forest (13.22 ± 1.71 °C) ($P<0.05$), with greater differences in spring and summer ($P<0.001$, Fig. 1, Table S1). In contrast, there were no ecosystem differences in soil water content (shrubland: 14.86

± 2.45%; holm oak forest: 16.10 ± 3.65%) (Table S1). Overall, soil water content was greatest in winter 2015, when soil temperatures were lowest; maximum soil temperatures were recorded in summer 2015 (Fig. 1). Average soil CO_2 efflux was higher in the holm oak forest (1.96 ± 0.30 μmol m^{-2} s^{-1}) than in the shrubland (1.29 ± 0.16 μmol m^{-2} s^{-1}) (P<0.001, Table S1) and highest levels of CO_2 efflux were recorded in the holm oak forest in spring 2014 and summer 2015 (Fig. 1).

At the study sites, there were seasonal variations in soil temperature (P<0.001), soil water content (shrubland: P<0.001; holm oak forest: P<0.01), and CO_2 efflux (P<0.001) (Fig. 1); variations in soil temperature and water content, and soil CO_2 efflux depended on year (season × year: P<0.001 and P<0.01, respectively) (Fig. 1). There were no annual variations in soil temperature, moisture, or CO_2 efflux (Fig. 1).

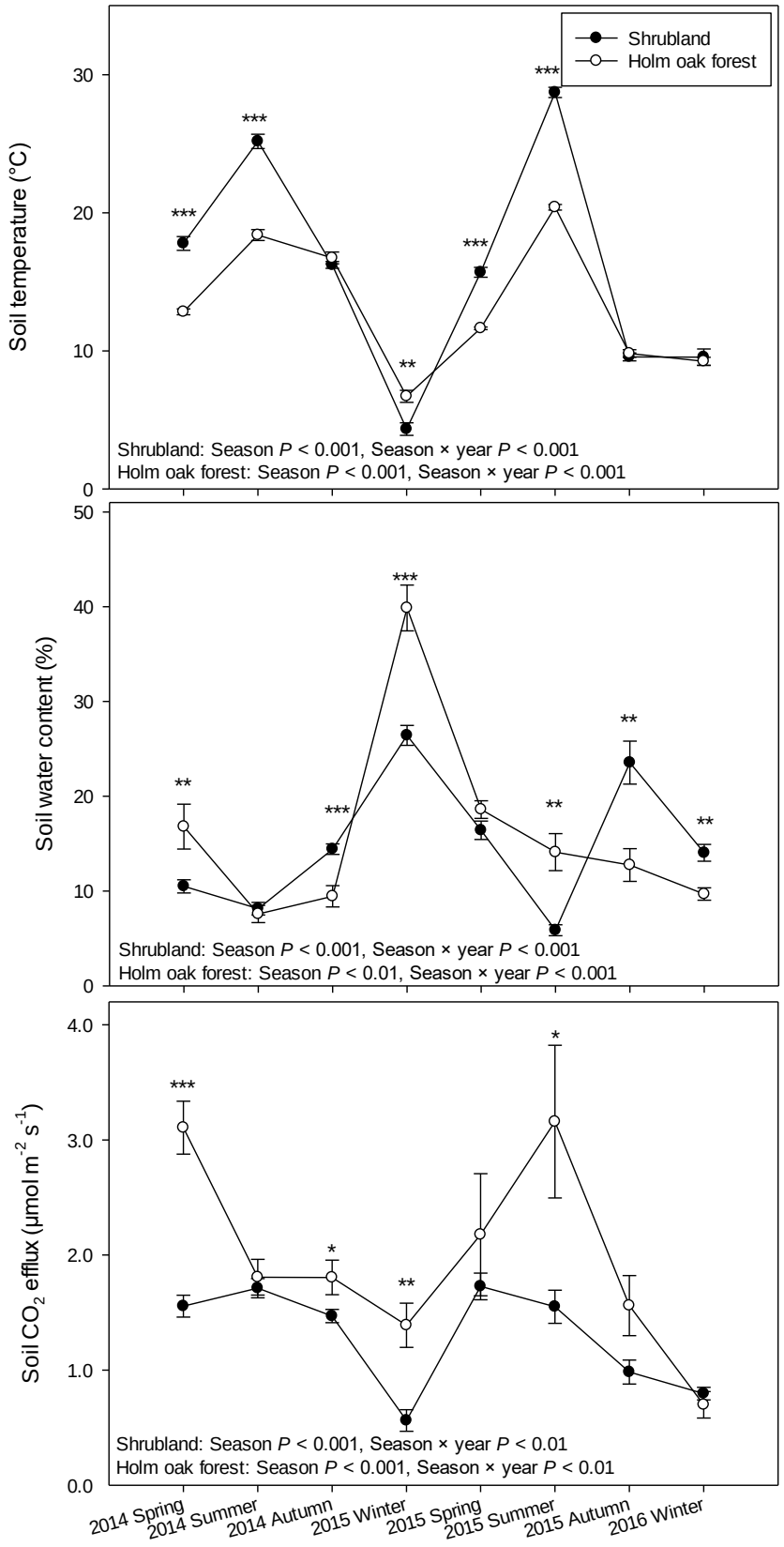

Shrubland
Holm oak forest
Soil temperature (°C)
Shrubland: Season P < 0.001, Season × year P < 0.001
Holm oak forest: Season P < 0.001, Season × year P < 0.001
Soil water content (%)
Shrubland: Season P < 0.001, Season × year P < 0.001
Holm oak forest: Season P < 0.01, Season × year P < 0.001
Soil CO2 efflux (µmol m-2 s-1)
Shrubland: Season P < 0.001, Season × year P < 0.01
Holm oak forest: Season P < 0.001, Season × year P < 0.01
2014 Spring
2014 Summer
2014 Autumn
2015 Winter
2015 Spring
2015 Summer
2015 Autumn
2016 Winter
Season

Fig. 1. Differences in mean seasonal soil temperature, water content, and CO_2 efflux in shrubland and holm oak forest ecosystems (Student's *t*-test, **P*<0.05, ***P*<0.01, and ****P*<0.001). Errors bars are ±SEM.

3.2. Soil VOC concentrations

3.2.1. Ecosystem differences over the period 2014–16

Soil VOC concentrations over the study period were 1.6 times greater in the shrubland (3.66 ± 1.01 ppb) than in the holm oak forest (2.23 ± 0.51 ppb) (Table 2). Concentrations of 11 of the 25 compounds were greater in the shrubland than in the holm oak forest (*P*<0.05) and that of a single compound (methanol) was lower (*P*<0.01); there were no ecosystem differences in concentrations of the remaining 13 compounds (Table 2).

Monoterpenes were the most dominant compounds in the shrubland (0.85 ± 0.43 ppb), followed by methanol, p-cymene, acetic acid, acetaldehyde, toluene, formaldehyde, ethanol, 1, 3-butadiene, and (E)-2-hexenal, the means of which were also above 0.1 ppb, while in the holm oak forest, the most dominant compound was methanol (0.81 ± 0.20 ppb), followed by acetic acid, acetaldehyde, ethanol, and formaldehyde (Table 2). Mean concentrations of xylene, hexenol, M101, methylfuran, heptanal, benzene, linalool, and cyclopentanone were <0.01 ppb in the two ecosystems (Table 2).

Table 2 Mean (± SEM) seasonal concentrations of detected volatile organic compounds (nmol mol^{-1}; ppb), classified as 'high' (>0.1 ppb), 'moderate' (0.01–0.1 ppb), or 'low' (<0.01 ppb), from shrubland and holm oak forest soils over the study period 2014–16. Ecosystem differences tested by Student's *t*-tests (**P*<0.05, ***P*<0.01 and ****P*<0.001).

Compound	Concentration (nmol mol^{-1})		Ecosystem difference	Concentration class
	Shrubland	Holm oak forest		
Methanol	0.530 ± 0.142	0.813 ± 0.200	**	
Acetic acid	0.483 ± 0.136	0.308 ± 0.069	**	
Formaldehyde	0.341 ± 0.051	0.299 ± 0.035		High
Ethanol	0.193 ± 0.054	0.148 ± 0.041		
Acetaldehyde	0.183 ± 0.042	0.114 ± 0.026	*	
Monoterpenes	0.845 ± 0.432	0.041 ± 0.018	*	
p-Cymene	0.362 ± 0.153	0.046 ± 0.027	*	
Toluene	0.186 ± 0.080	0.025 ± 0.006	**	
1, 3-Butadiene	0.104 ± 0.021	0.086 ± 0.015	*	
(E)-2-hexenal	0.104 ± 0.034	0.044 ± 0.008	***	
Sesquiterpenes	0.083 ± 0.023	0.089 ± 0.038		
Ethyl-3-methylbutanoate	0.062 ± 0.029	0.017 ± 0.011	*	Moderate
Unknown (M149)	0.047 ± 0.027	0.067 ± 0.047		
Acetone	0.037 ± 0.008	0.021 ± 0.005	***	
Unknown (M147)	0.018 ± 0.010	0.031 ± 0.023		
Isoprene	0.014 ± 0.004	0.013 ± 0.003		
Hexanol	0.013 ± 0.002	0.012 ± 0.003		
Xilene	0.009 ± 0.002	0.007 ± 0.003	*	
Hexenol	0.009 ± 0.002	0.010 ± 0.005		
Unknown (M101)	0.008 ± 0.003	0.009 ± 0.005		
Methylfuran	0.007 ± 0.002	0.007 ± 0.003		Low
Heptanal	0.007 ± 0.002	0.009 ± 0.005		
Benzene	0.007 ± 0.002	0.004 ± 0.002	*	
Linalool	0.006 ± 0.003	0.008 ± 0.006		
Cyclopentanone	0.005 ± 0.002	0.006 ± 0.003		
Total	3.663 ± 1.013	2.233 ± 0.507		

We tentatively identified five isoprenoids: isoprene (M69), p-cymene (M135 + M136), monoterpenes (M81 + M137), linalool (M155), and sesquiterpenes (M109 + M123 + M205) (Tables 1 and 2). Of these, concentrations of monoterpenes were

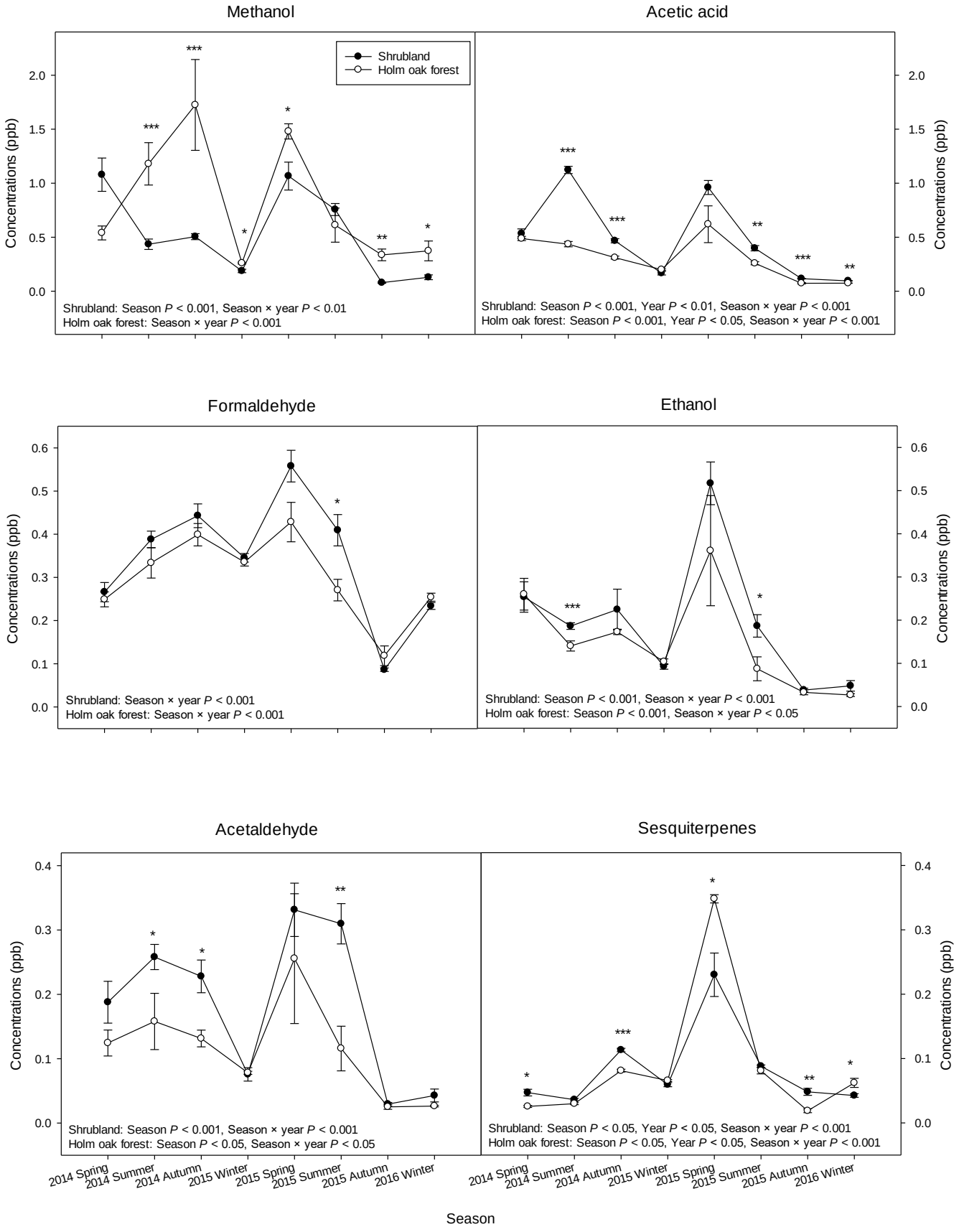
greatest in the shrubland, followed by p-cymene and sesquiterpenes, whereas in the holm oak forest, concentrations of sesquiterpenes were greatest, followed by p-cymene and monoterpenes; concentrations of isoprene and linalool were low for both ecosystems (Table 2). In general, there were greater concentrations of isoprenoids, particularly of monoterpenes and p-cymene (P<0.01), in shrubland soils (Table 2).

3.2.2. Seasonal and annual ecosystem variations

The concentrations of studied compounds broadly showed seasonal variations in both ecosystems, and showed more annual variations in the holm oak forest than in the shrubland (Table S2). There were season × year interactions for all 25 compounds in the holm oak forest, and for 17 compounds in the shrubland, indicating that seasonal differences in compound concentrations varied with year (Table S2).

Of the five dominant compounds (methanol, acetic acid, formaldehyde, ethanol, and acetaldehyde) and three isoprenoids (monoterpenes, p-cymene and sesquiterpenes), there were seasonal variations in concentrations in the two ecosystems, with the exception of formaldehyde and methanol in the holm oak forest (P<0.05); seasonal variations in concentrations depended on year, except for monoterpenes and p-cymene in the shrubland (season × year interaction: P<0.05) (Fig. 2). Concentrations of acetic acid in the two ecosystems (shrubland: P<0.01; holm oak forest: P<0.05) were highest during 2014, while concentrations of p-cymene in the holm oak forest (P<0.01) and of sesquiterpenes in both ecosystems (P<0.05) were highest in 2015 (Fig. 2).

A)


Methanol
Acetic acid
Formaldehyde
Ethanol
Acetaldehyde
Sesquiterpenes
Shrubland
Holm oak forest
Concentrations (ppb)
Shrubland: Season P < 0.001, Season × year P < 0.01
Holm oak forest: Season × year P < 0.001
Shrubland: Season P < 0.001, Year P < 0.01, Season × year P < 0.001
Holm oak forest: Season P < 0.001, Year P < 0.05, Season × year P < 0.001
Shrubland: Season × year P < 0.001
Holm oak forest: Season × year P < 0.001
Shrubland: Season P < 0.001, Season × year P < 0.001
Holm oak forest: Season P < 0.001, Season × year P < 0.05
Shrubland: Season P < 0.001, Season × year P < 0.001
Holm oak forest: Season P < 0.05, Season × year P < 0.05
Shrubland: Season P < 0.05, Year P < 0.05, Season × year P < 0.001
Holm oak forest: Season P < 0.05, Year P < 0.05, Season × year P < 0.001
2014 Spring
2014 Summer
2014 Autumn
2015 Winter
2015 Spring
2015 Summer
2015 Autumn
2016 Winter
Season

B)

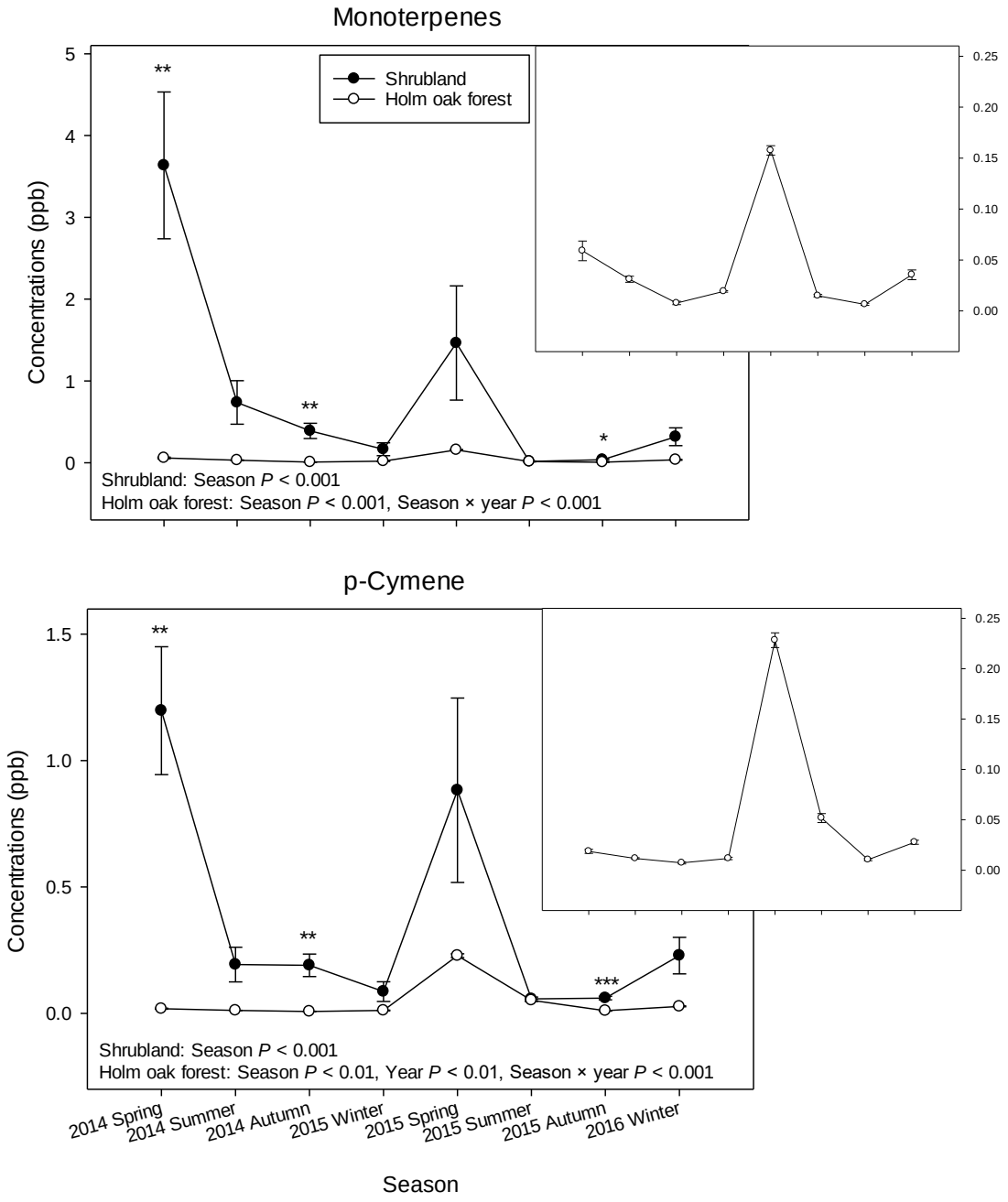


Fig. 2. Differences in mean seasonal concentrations of (A) methanol, acetic acid, formaldehyde, ethanol, acetaldehyde, and sesquiterpenes, and (B) monoterpenes and p-cymene in shrubland and holm oak forest soils (Student's *t*-test, **P*<0.05, ***P*<0.01, and ****P*<0.001).

There were similar seasonal patterns in VOC concentrations in the two ecosystems, with the exception of methanol during 2014, where lowest concentrations of most compounds were recorded during winter 2015, followed by autumn 2015 (Fig. 2). Highest levels of VOC concentrations depended on compound, ecosystem, and year: between spring 2014 and winter 2015, highest concentrations of formaldehyde, acetaldehyde, sesquiterpenes (shrubland and holm oak forest), acetic acid (shrubland), and methanol (holm oak forest) were recorded from summer to autumn, while highest concentrations of ethanol, monoterpenes, p-cymene (shrubland and holm oak forest), methanol (shrubland), and acetic acid (holm oak forest) were recorded in spring, whereas from spring 2015 to winter 2016, concentrations of all compounds were greatest in spring, with those of formaldehyde, ethanol, acetaldehyde, sesquiterpenes (shrubland and holm oak forest), acetic acid, monoterpenes, and p-cymene (holm oak forest) being the maximum recorded over the study period (Fig. 2). There were ecosystem differences in methanol, acetic acid, and acetaldehyde concentrations during summer and autumn and for isoprenoids in spring and autumn (Fig. 2).

3.3. Ecosystem drivers of soil VOC concentrations

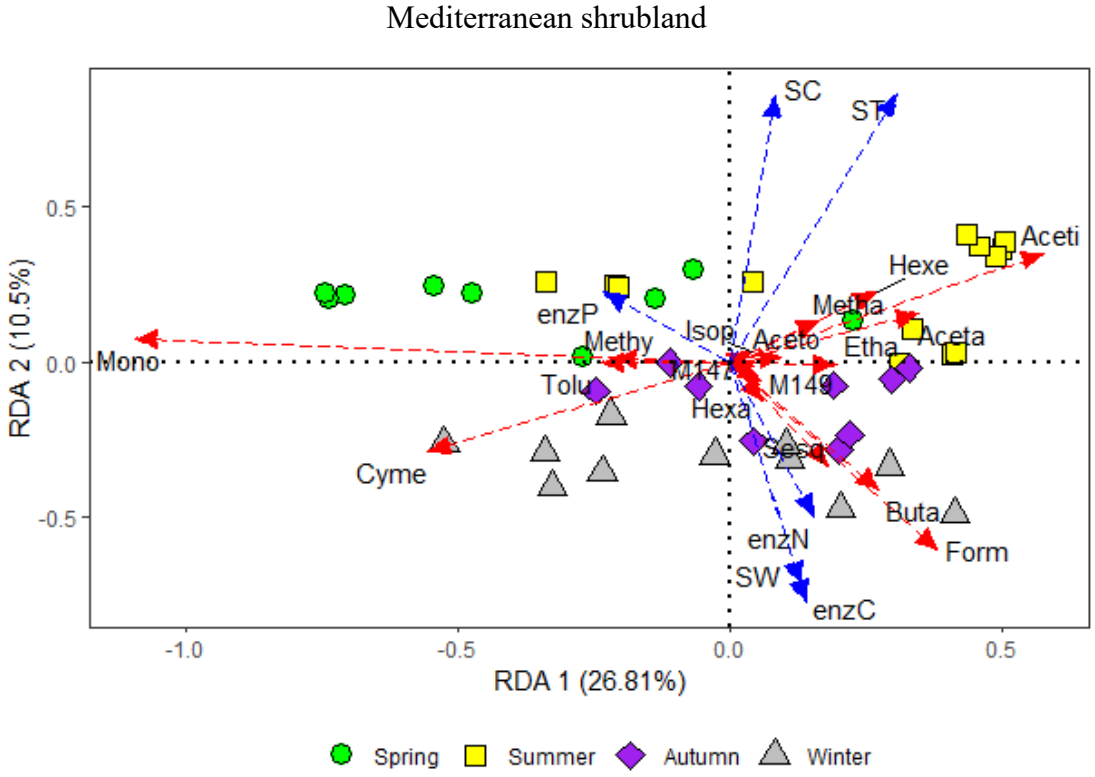
Redundancy analysis of 17 compounds with mean soil concentrations >0.01 ppb showed that soil temperature and moisture were correlated with soil VOC concentrations in the two ecosystems, while in the holm oak forest, *A* was marginally correlated with soil VOC concentrations (Table S3). Variation in mean soil VOC concentrations was best explained by levels of CO_2 efflux in the two ecosystems (shrubland: r^2=0.46, P<0.001; holm oak forest: r^2=0.41, P<0.001; Table S3).

In the shrubland, soil CO_2 efflux, soil enzyme activity, and soil conditions explained 26.81 and 10.5% of variation in soil VOC concentrations along axes 1 and 2, respectively, particularly of monoterpenes, p-cymene, sesquiterpenes, ethyl-3-methylbutanoate, toluene, formaldehyde, acetic acid, acetaldehyde, (E)-2-hexenal, and 1, 3-butadiene (axis 1) and 1, 3-butadiene, formaldehyde, and sesquiterpenes (axis 2) (Fig. 3A). Seasonal variation in soil VOC concentrations (winter, autumn and spring-summer) was separated along the second axis by soil temperature, soil moisture, and CO_2 efflux, where concentrations in spring-summer were positively associated with higher levels of soil respiration and temperature and those in winter were positively associated with higher soil water content (Fig. 3A). Overall, dominant soil VOCs in spring-summer were monoterpenes, methanol, (E)-2-hexenal, acetaldehyde, and acetic acid, whereas sesquiterpenes were more abundant between autumn and winter, and 1,3-butadiene and formaldehyde were more abundant in winter. Soil temperature and CO_2 efflux were positively correlated with soil concentrations of methanol, (E)-2-hexenal, acetaldehyde, ethanol and acetic acid, and negatively correlated with concentrations of monoterpenes, p-cymene, ethyl-3-methylbutanoate, and toluene; soil water content was positively associated with soil concentrations of sesquiterpenes, 1,3-butadiene, and formaldehyde, and activity of C- and N-degrading enzymes, and negatively associated with activity of P-degrading enzymes (Fig. 3A).

In the holm oak forest, soil CO_2 efflux, soil enzyme activity, plant activity, and soil conditions explained 25.77 and 18.89% of variation in soil VOC concentrations along axes 1 and 2, respectively, particularly of acetic acid, methanol, ethanol, M149, M147,

monoterpenes, p-cymene, sesquiterpenes, and ethyl-3-methylbutanoate (axis 1) and acetaldehyde, acetic acid, methanol, and ethanol (axis 2) (Fig. 3B). Concentrations were strongly defined by soil temperature and enzymatic activity (enzC, enzC/enzN, and enzC/enzP) along axis 1 and by CO_2 efflux along axis 2. There was no clear separation of soil VOC concentrations by season, and in general, concentrations of acetic acid, ethanol, and p-cymene were dominant in spring, while those of methanol were dominant in autumn (Fig. 3B). There were positive correlations between soil CO_2 efflux and concentrations of acetic acid, ethanol, and acetaldehyde, between soil temperature and concentrations of acetic acid, ethanol, (E)-2-hexenal, and methanol. There were positive correlations for soil water content and A with enzyme activities, and concentrations of acetaldehyde, isoprenoids, M149, M147, ethyl-3-methylbutanoate, toluene, hexanal, and formaldehyde. In contrast, there were negative associations between soil water content and concentrations of methanol, and between soil temperature and isoprenoids, M149, M147, ethyl-3-methylbutanoate, toluene, hexanal, and formaldehyde (Fig. 3B).

A)



B)

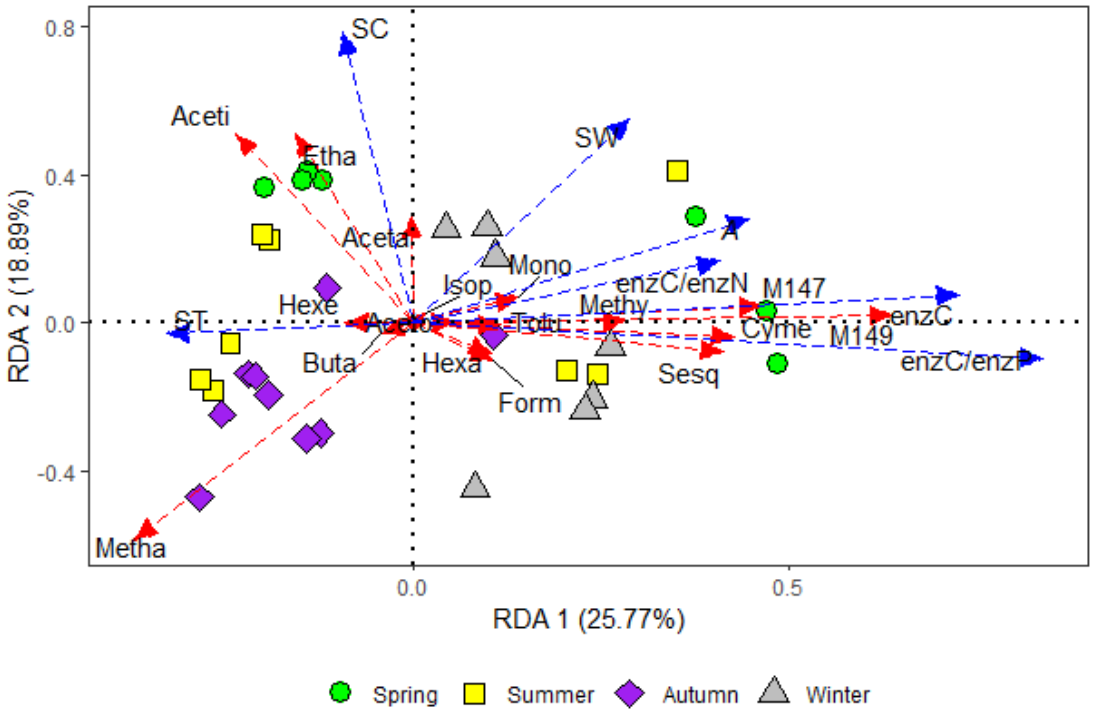


Fig. 3. Redundancy analysis of seasonal VOC compound concentrations, soil CO_2

efflux, soil enzyme activity, plant activity, and soil conditions in shrubland (A) and holm oak forest (B) soils. Metha: methanol; Aceti: acetic acid; Form: formaldehyde; Etha: ethanol; Aceta: acetaldehyde; Mono: monoterpenes; Cyme: p-cymene; Tolu: toluene; Buta: 1,3-butadiene; Hexe: (E)-2-hexenal; Sesq: sesquiterpenes; Methy: ethyl-3-methylbutanoate; Aceto: acetone; Isop: isoprene; Hexa: hexanol; M147: unknown mass M147; M149: unknown mass M149; SC: soil CO_2 efflux; enzC: C-degrading enzymes; enzN: N-degrading enzymes; enzP: P-degrading enzymes; *A*: net photosynthetic rate; ST: soil temperature; SW: soil water content.

4. Discussion

The results provide quantitative evidence for seasonal variation in VOC concentrations in two types of Mediterranean ecosystem soils; these variations within the shrubland and holm oak forest were driven by soil biological activity (plant roots and microorganisms), and soil water content and temperature, while differences between the two ecosystems were linked to the contrasting plant species composition. These results support current understanding of the factors that govern soil VOC fluxes in natural ecosystems (Tang et al., 2019) and demonstrate seasonal fluctuations in concentrations of specific soil VOCs in water-limited Mediterranean ecosystems.

4.1. Possible sources of dominant soil VOCs in Mediterranean soils

Soil VOCs were dominated by concentrations of methanol, acetic acid, formaldehyde, ethanol, and acetaldehyde in the two ecosystems (>0.1 ppb), compared with the lower concentrations of other VOCs, such as like xylene, hexenol, M101, methylfuran, heptanal, benzene, linalool, and cyclopentanone (<0.01 ppb) (Table 2). The dominant compounds recorded in this study are generally ubiquitous in the

atmosphere, so the relatively high soil concentrations we measured might reflect an immission process, driven by the concentration gradient created between VOC-rich air just above the soil surface and lower levels within soil air spaces (Asensio et al., 2007b, 2008b). For example, the greater concentrations of methanol and acetic acid in the air immediately above the soil surface (Asensio et al., 2008b; Peñuelas et al., 2013) may explain their greater fluxes to the soil compared with other compounds reported in previous studies (Asensio et al., 2007b). Methanol is involved in a variety of belowground processes, including the emission of methanol by roots through physiological activities (Folkers et al., 2008; Tang et al., 2019) and by SOM through physicochemical processes (Schade and Custer, 2004) and microbial activities (Asensio et al., 2008a; Bourtsoukidis et al., 2018), and its consumption by aerobic methylotrophic bacteria that frequently inhabit the rhizosphere and phyllosphere (Asensio et al., 2007a; Boyd et al., 2000; Trotsenko et al., 2001), leading to high concentrations of formaldehyde as an oxidation product of methanol and other VOCs (Mancuso et al., 2015; Seco et al., 2007). Ethanol is a typical product of anaerobic fermentation and is easily oxidized to acetic acid via acetaldehyde (Tang et al., 2019); its production from roots, along with that of acetaldehyde, is increased under flooded conditions (Kreuzwieser and Rennenberg, 2013; Seco et al., 2007). Acetic acid is the most prominent volatile acid emitted by plants (Kesselmeier and Staudt, 1999), is characterized by high water solubility, and is present in some root exudates (Strobel, 2000) making it easily used by soil microbes (Asensio et al., 2007a; Evans, 1998).

In general, there were greater accumulations of VOCs in shrubland soils than in the

holm oak forest soils, as supported by the ecosystem differences in concentrations of 11 compounds, especially isoprenoids (Table 2); methanol was the only exception, with greater concentrations in holm oak forest soils. These results contrast with emission patterns characterized by similarly low isoprenoid emission rates and higher methanol emission rates previously recorded in the Garraf shrubland (2007b, 2008b). Although the overall higher levels of soil VOC concentrations in the shrubland may be related to the higher temperatures (Fig. 1, Table S1), the ecosystem differences may reflect differences in plant species composition (Asensio et al., 2008b; Wester-Larsen et al., 2020). For example, VOC emissions from leaf, litter, and roots are species-specific (Gray and Fierer, 2012; Ramirez et al., 2010; Svendsen et al., 2018) and emissions from litter and roots are simultaneously and similarly affected by the associated microbial community composition (Gray et al., 2010; Svendsen et al., 2018). Plants modify the rhizosphere environment, such as soil porosity, pH, and moisture content (Bardgett, 2005; Wester-Larsen et al., 2020), and plant species composition affects soil microbial community composition (Carney and Matson, 2006; Mitchell et al., 2010) and microbial consumption of VOCs and mineralization activities (Asensio et al., 2008b). Thus, complex variations in dynamic VOC processes in soil that are affected by plant species composition (Dicke et al., 2003; Peñuelas and Llusià, 2001; Wester-Larsen et al., 2020) may explain the differences in soil VOC concentrations between the two ecosystems in this study.

We found that concentrations of monoterpenoids differed between the two ecosystems as expected from having different plant species composition. The shrubland

soils were characterized by greater concentrations of monoterpenoids than the soils of the holm oak forest (Table 2), likely as a result of species differences in root isoprenoid emissions. For example, *P. halepensis* and *R. officinalis*, which are abundant in the shrubland (Peñuelas and Llusià, 2001; Asensio et al., 2008b; Mu et al., 2018), are known to emit large amounts of terpenes (Blanch et al., 2007; Chetehouna et al., 2009; Ormeño et al., 2007; Owen et al., 1997) and *Pinus* roots emit high concentration levels of monoterpenes and sesquiterpenes (Lin et al., 2007; Smolander et al., 2006), likely at higher levels of monoterpene emissions than *Q. ilex* that is the dominant plant species in the holm oak forest. Interestingly, our results indicate that roots may also be the dominant source of terpenoids in the holm oak forest, because here, we found a positive relationship between concentration of soil terpenoids (monoterpenes, p-cymene, linalool, and sesquiterpenes) and *A*, especially for monoterpenes (Table S4). Although little is known about terpenoid emissions from *Q. ilex* roots, our data indicate a direct link between *Q. ilex* root activity and soil terpenes.

Concentrations of methanol were higher in the holm oak forest than in the shrubland soils, possibly due to greater amounts of root biomass and levels of plant and/or microbial activity, as supported by the higher rates of soil respiration during the studied period (Fig. 1, Table S1). However, in the shrubland, methanol concentrations were more linked to biological activity, as indicated by the positive correlations with soil temperature and CO_2 efflux, compared with the positive association between methanol concentrations with soil temperature, negative association with soil water content, and lack of association with CO_2 efflux in the holm oak forest soils (Fig. 3). These data

indicate that the build-up of methanol concentrations in the soil atmosphere in the holm oak forest soils was more directly linked to the thermal desorption of methanol adsorbed to the rhizosphere or soil particles and subsequent release of dissolved methanol than to its immediate biological production, and is a preferentially abiotic mechanism of emission, as has been demonstrated in soil and litter (Schade and Custer, 2004; Gray et al., 2010).

4.2. Abiotic and biotic controls of soil VOC concentrations in Mediterranean soils

Increases in soil temperature may change the partition coefficient between adsorbed and/or dissolved VOCs and gaseous VOCs, leading to greater concentrations of VOCs in soil air (Tang et al., 2019), due to the increased volatility of all VOCs (Insam and Seewald, 2010). Strong positive relationships between soil temperature and soil VOC concentrations have been demonstrated in cold arctic soils (Wester-Larsen et al., 2020); however, our results show that rises in soil temperature may elicit stronger impacts on hydrophilic than hydrophobic VOCs, as indicated by the stronger positive relationship between soil temperature and hydrophilic VOCs, such as methanol, acetic acid, and ethanol than with hydrophobic VOCs, such as terpenes, ethyl-3-methylbutanoate, and toluene (Fig. 3), which were probably more tightly bounded to SOM.

As expected, soil CO_2 efflux was positively correlated with temperature, especially in the shrubland soils, showing that increases in temperature are linked to increases in plant and microbial activity that, in turn, may contribute to increases in the concentrations of some VOCs in soil. This was true for compounds, such as (E)-2-hexenal, methanol, acetic acid, and acetaldehyde in the shrubland soils and acetic acid

and ethanol in the holm oak forest soils (Fig. 3). These positive associations between soil VOC concentrations and soil CO_2 efflux and soil temperature in the Mediterranean soils contrasts with those reported for arctic soils, where only a clear correlation between soil VOC concentrations and temperature, but not with soil respiration was found (Wester-Larsen et al., 2020). It is likely that in the warmer Mediterranean ecosystems, biological activity has a stronger effect on soil VOC concentrations than in colder arctic ecosystems; however, increases in temperature lead to increasing VOC consumption by microbes (Asensio et al., 2007a; Ramirez et al., 2010) and associated decreases in soil atmosphere concentrations of VOCs. Terpenoids in the two ecosystems were negatively correlated with soil temperature (Fig. 3) and the lack of correlation between soil terpene concentrations and CO_2 efflux does not directly imply lack of association with microbial activity, because CO_2 efflux also includes root respiration that may hinder microbial activity-VOC relationships; nevertheless, these relationships tended to be concealed by the stronger relationships between terpenes and *A* in the holm oak forest soils. Thus, the negative correlation between soil terpene concentration and temperature in the ecosystem soils indicates that increases in temperature could promote terpenoid consumption to a greater degree than production.

Soil moisture is a key driver of physiological processes in plants and microorganisms (Asensio et al., 2007a; Cho et al., 2005; Mu et al., 2020). We found a high dependence of C- and N-degrading enzyme activities on soil water content (Fig. 3) in the two Mediterranean ecosystems; thus, the increases in concentrations of VOCs, such as 1, 3-butadiene, formaldehyde, and sesquiterpenes in the shrubland soils and

acetaldehyde and monoterpenes in the holm oak forest soils, which were associated with high levels of soil water content (Fig. 3), may reflect increased microbial activity or root exudation during the growing period that typically occurs during the wet seasons in these ecosystems. In contrast, soil concentrations of methanol in the holm oak forest soils were negatively correlated with soil water content, possibly due to its greater uptake through microbial activity or/and an increase of physical passive storage at high soil moisture levels (Asensio et al., 2007b, 2008b; Tang et al., 2019). It is likely that our results reflect the complex biophysical interactions in soils: roots release exudates that enhance microbial VOC consumption activities under higher levels of soil water content (Asensio et al., 2007a, b; Walker et al., 2003), indirectly leading to decreases in the concentrations of some compounds in soil air.

The expected increase in temperature and decrease in water availability in the next three or four decades, as predicted by climatic and ecophysiological models (IPCC, 2014; Peñuelas and Staudt, 2010; Sabaté et al., 2002), may also affect Mediterranean semi-arid soil respiration and VOC concentrations and will more than likely also affect soil VOC fluxes with the atmosphere in these ecosystems. The potential significance of climate change effects on soil VOC emissions, atmospheric processes, and C budgets may be low for this region (Asensio et al., 2007b, 2008b; Mu et al., 2020), but climate change effects on soil VOC concentrations and soil function may be greater, albeit currently unclear; thus, further research in this area is warranted.

4.3. Seasonal and annual variability in soil VOC concentrations

Although seasonality of the concentration of individual VOCs differed, depending

on compound, ecosystem, and year, the most consistent pattern in concentrations was characterized by a spring maximum and winter minimum. (Fig. S1). The concentrations of the studied compounds tended to be greatest during the warm seasons (spring and summer), with some compounds showing greatest values in autumn 2014, coinciding with high soil temperatures (Figs. 1 and S1). These results indicate that temporal variations in VOC concentrations were driven by soil temperature.

Soil VOC concentrations throughout the study period tracked seasonal variations in soil temperature, water content, and biological activity, and some seasonal variations in these factors were similar across the two ecosystems (Fig. 1), affecting soil VOC concentrations similarly. The increases in soil temperature and biological activity from winter to spring (the growing season) were reflected by increases in CO_2 efflux and moderate levels of soil water content (Fig. 1) that probably drove the increase in almost all VOC concentrations in the two ecosystems during this period (Fig. S1) and coincided with high foliar (Mu et al., 2018, 2019) and soil (Asensio et al., 2007b, 2008b) VOC emission rates in spring. We found that the low levels of activities of C- and N-degrading enzymes (in spring in the shrubland and in autumn in the holm oak forest) (Fig. 3), due to decreasing availability of soil nutrients, such as ammonium and nitrate, corresponded to the high rates of photosynthesis in the plant growing seasons (shrubland: autumn maxima; holm oak forest: spring minima), and may have been related to growing season increases in soil VOC concentrations and increased root emissions and exudates (Asensio et al., 2008b; Sardans et al., 2007; Walker et al., 2003). These results could explain the high concentrations of methanol, formaldehyde, ethanol

and sesquiterpenes recorded in the warm autumn of 2014, following the slight decrease in CO_2 efflux from summer to autumn of that year.

The rise in soil temperatures in 2015, from spring to summer, in parallel with the decrease in soil water content during the summer drought (Fig. 1) was associated with a strong decrease in the concentration of eight typical soil VOCs in the two ecosystems (Fig. 2). Given the levels of soil CO_2 did not decrease (there was an increase in the holm oak forest) and there was a decrease in rates of photosynthesis (by 20.8 and 50.7% in the shrubland and holm oak forest, respectively) during this period (Fig. 1), it is possible that the decrease in soil VOC concentrations was the result of increased microbial uptake and decreased root emissions during the summer drought. However, the pattern of levels of soil concentrations observed in spring-summer 2015 was inconsistent among the VOCs and between the ecosystems in 2014, and was dependent on compound (levels of formaldehyde and acetaldehyde increased; ethanol and monoterpenoids decreased; sesquiterpenes did not change) and ecosystem (levels of methanol decreased in the shrubland and increased in the holm oak forest; levels of acetic acid increased in the shrubland and did not change in the holm oak forest). This variation in responses could be the result of multiple abiotic and biotic factors specific to each season, ecosystem, and VOC sampled.

Seasonal concentrations and patterns of change of formaldehyde and ethanol were similar between the two ecosystems, where concentrations were only higher in the shrubland than in the holm oak forest in summer, probably due to higher soil temperatures (Figs. 1 and 2A). There were higher soil concentrations of acetic acid and

acetaldehyde in the shrubland in almost all seasons, particularly in summer and autumn, probably due to the multiple effects of ecosystem differences in plant species, summer soil temperatures, and autumn soil water content (Figs. 1 and 2A). Soil concentrations of methanol were inconsistent and variable between the two ecosystems, and although they tended to be greater in the holm oak forest and in most seasons, there were contrasting seasonal patterns of change between spring and autumn 2014 (Fig. 2A). These variations could be a result of the effects of multiple abiotic and biotic factors on methanol dynamic processes in soil air. For example, similar seasonal concentrations and patterns of change of these common dominant compounds between the ecosystems may be driven by similar concentration gradients between air and soil air in this region, where shifts in concentration tend to occur in warmer and growing seasons, due to changes in soil property caused by impacts of the contrasting plant phenologies on abiotic and biotic factors (Asensio et al., 2007b, c, 2008b; Wester-Larsen et al., 2020).

There were higher levels of soil concentrations of monoterpenes and p-cymene in the shrubland than in the holm oak forest that coincided with differences in growing season (Table 2, Fig. 2B), indicating the role of soil biological activity in monoterpenoids soil concentrations. We found there were no annual differences in soil sesquiterpene concentrations, but there were irregular seasonal patterns and ecosystem differences (Table 2, Fig. 2A). Sesquiterpenes are highly reactive compounds that are easily be consumed or oxidized (Tang et al., 2019; Horváth et al., 2012; Mäki et al., 2017), possibly including in soil air that may lead to their concentrations being highly variable. There was a positive association between sesquiterpene concentration and soil

water content (Fig. 3), indicating likely sensitivity to temporal differences in soil water content, as supported by similar seasonal patterns with soil water content; we also suggest that high levels of soil water content in the preceding winter of 2014 may have contributed to the largest concentrations in spring 2015 (Figs. 1 and 2A).

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Block 3. Ground level BVOC exchanges associated with plants

Chapter 4. Ground level isoprenoid exchanges associated with Pinus pinea trees in a Mediterranean turf

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Abstract

The emissions of isoprenoids, a kind of biogenic volatile organic compounds (BVOCs), from soils is not well characterized. We quantified the exchange of isoprenoids between soil with litter and atmosphere along a horizontal gradient from the trunks of the trees, in a Mediterranean Pinus pinea plantation with dry and green needle litter to open herbaceous turf during mornings at mid-summer. Further, potential associated drivers were identified. Isoprenoid emissions were greatest and most diverse, and also can be roughly estimated by litter dry weight near the trunk, where the needle litter was denser. The composition of emitted isoprenoid by needle litter was different than the composition previously described for green needles. Low exchange rates of isoprenoids were recorded in open turf. Isoprenoid exchange rates were correlated positively with soil temperature and negatively with soil moisture. Given the variations in ground emissions with soil, vegetation, microorganisms, and associated interactions, we recommend widespread extensive spatio-temporal analysis of ground level BVOC exchanges in the different ecosystem types.

Keywords: Isoprenoid exchanges; Ground; Litter emissions; Soil; *Pinus pinea*; Distance gradient; Mediterranean turf

1. Introduction

Biogenic volatile organic compounds (BVOCs) represent 90% of total volatile organic compound (VOC) emitted into the atmosphere [1], impacting the atmospheric chemistry and climate processes [2–4]. BVOC emission profiles from terrestrial ecosystems tend to be driven by plant species composition [5] which is linked to phenology and climate [2,6] and are usually dominated by isoprenoids [5] with blends of other carbon-based compounds, such as alkanes, alkenes, carbonyls, alcohols, esters, ethers, and acids [5,6]. This key role of terrestrial plants in BVOCs has received much research attention [7–10]; however, there is emerging evidence that a wide range of BVOCs are also released from terrestrial ecosystem ground [10] regardless of level of vegetation [8,10,11].

Ground level emission of BVOCs from natural and semi-natural ecosystems may derive from organic litter and soil where plant root systems and microorganisms are major sources [11,12] and sometimes also by understory vegetation [13,14]. Most of the ground measurements do not distinguish the emissions from plant roots, decomposing litter, or the microbes themselves [15,16]. Litter has often been suggested as the main BVOC source in the forests besides vegetation [14,17–19], in fact, the decomposing litter has been assumed to be the main BVOCs source in the forest ground [8,14,20,21]. It is evident that both decomposers and the decomposing material affect the quantities and types of VOC productions [21], and also that VOCs released through the decomposition processes are strongly dependent on litter type, climate and soil microbial composition [21,22]. Differentiating each of the soil component responsible

for these emissions is very complex [10,18,23]. For example, the assessment of the contribution of root emissions to the overall soil VOC fluxes is difficult because of their linkage with soil microbes owing to root exudates can boost microbial activity, which can either increase the production or consumption of VOCs [10]. Some soil microbes, particularly fungi, are capable of producing terpenoid compounds [24], but plant roots are likely to be the dominant source of these compounds [8,23,25]. Isoprenoids are commonly emitted from litters and soils [8,12,22], and are likely adsorbed on the living leaf surfaces which are covered by a lipophilic cuticle layer [14]. Soil and litter microbes can also modify VOC emissions by metabolizing plant-emitted VOCs [26], which may cause low isoprenoid fluxes measured from soil with dense understorey vegetation cover [15]. Some understorey vegetation (grasses, shrubs, mosses, lichens, and other vegetation) [13,14] can also contribute to the exchange of BVOCs by emitting them [7].

Ground level emissions to the atmosphere are often 1–2 orders of magnitude lower than those from aboveground vegetation [10]. Moreover, they may represent up to 50% of net canopy BVOC flux, depending on the type of ecosystem, litter and soil [19], environmental conditions [10,27] and season of the year [19], particularly in coniferous forests that produce large amounts of litter [4,10]. Nevertheless, some studies suggest that these emissions play an insignificant role because they constitute a very low fraction with respect to the total ecosystem emissions [12,19,20,28]. In addition, soil VOCs also have important ecological roles [8,10,29], affecting microbial process such as methane oxidation, nitrification, nitrogen mineralization, and aerobic respiration

[8,23,25] and biological interactions as key compounds in communication among soil microorganism and plant roots [4] that release carbon-rich root exudates and thus feed associated populations of bacteria, fungi, arthropod and nematode within the rhizosphere [8].

Soils are considered to be sources and sinks of BVOCs [8,10,12,22] with very low exchanges in Mediterranean-type ecosystems [17,29,30]. Maybe microbial processes play most important roles in atmosphere-soil exchanges of BVOCs [7,12,19]. In this line, there are studies showing the lower VOC emission rates in the litter plus soil treatments indicating many litter VOCs appear to be metabolized in soil [8,12,22], meanwhile, litter VOCs represent an important carbon source to soil and elevate soil microbial activity [22]. In addition, the consumption of some specific VOCs in soils result from microbial activities [7,22] depending on the type of compound and soil [7]. Besides, abiotic processes like adsorption to soil particles [19,31], dissolution in soil water [19], and reactions with soil chemicals [32] are also the mechanism behind the soil uptake [7,19].

Deposition and emission of ground level BVOCs is strongly influenced by environmental conditions [19,29]. Soil temperature and moisture seems to be the most important factors since they control physiological processes both in plants and microorganisms [30]. Temperature affects VOC production [10,12] through the temperature dependence of enzyme production and activity in VOC synthesis [2,21], while soil water content can determine which microbial groups are most active [21,33], which means both the physiological activity and community composition of

decomposer microorganisms can be affected by environmental conditions [4,34]. Soil temperature and moisture affect, moreover, soil BVOC physical processes, including dissolution in soil water [7], and physico-chemical processes [2,21], such as diffusion and volatility. The over-arching effects of climate warming on increasing soil temperature and decreasing soil moisture will contribute higher BVOC volatilization from soil into the atmosphere [31], and may influence composition of vegetation and distribution of the associated soil microorganisms, and cause further variations in BVOC exchange profiles [4,8,34].

Isoprenoids are produced by all conifers and are stored in the needles [35] where they readily volatize from needle storing tissues [22]. The distance to the conifer tree can be a qualitative and quantitative determinant of ground level BVOC exchange profiles [17]. Here, we aimed to quantify emissions and exchange of isoprenoids and its potential drivers along a horizontal gradient from dense *Pinus pinea* litter to open herbaceous turf to improve understanding of spatial-temporal differences in ground level BVOC exchange to the atmosphere.

2. Material and Methods

2.1. Study Site and Experimental Design

We selected four isolated, similar sized (mean trunk circumference at breast height: 1.20 ± 0.06 m) *Pinus pinea* L. trees in a managed herbaceous turf on a silty-clay Typic Calcixerept soil, with a high proportion of carbonates (pH: 8) [17], near the campus of the Autonomous University of Barcelona (41°30′ N, 2°6′ E). Ground vegetation was

dominated by legumes, such as *Trifolium repens* L., *Psoralea bituminosa* L., *Medicago minima* (L.) Bartal, with other herbs, such as *Plantago lanceolata* L. and grasses (*Lolium perenne* L., *Brachypodium phoenicoides* R. and S., and *Bromus intermedius* Guss.). Sampling points (N = 11) were arranged every meter along a single 10 m transect from the trunk of each tree, and avoided canopy effects of other trees, where point 1 was as close to the trunk as possible. Leaf litter was present within 4 m, and most dense within 2 m from the trunk where litter covered ground totally.

2.2. Isoprenoid Sampling

Sampling was carried out during the summer when climate warming effects are most pronounced and BVOCs emissions are greatest at this region [9,29], from 18 July to 8 August of 2018, on sunny or slightly cloudy days, between 09:00 and 13:00 hrs. Emitted isoprenoids were collected with a Teflon® soil VOC chamber and retained in stainless steel tubes (89 mm in length with 6.4 mm external diameter, Markes International Inc. Wilmington, USA) manually filled with adsorbents (115 mg of Tenax TA and 230 mg of SulfiCarb, Markes International Inc. Wilmington, USA) separated by sorbent-retaining springs that were fixed using gauze-retaining springs and closed with air-tight caps. Flow was generated using a Q-MAX air-sampling pump (Supelco, Bellefonte, USA) and measured using a Bios Defender 510 flow meter (Bios International Corporation, Butler, USA) and sampling time was 20 min. This dynamic system was also connected to ambient air with a Teflon® tube of 3 mm of inner diameter and air inside chamber was homogenized using a small fan. The flow rate across the sampling cartridges was adjusted at around 200 mL min^{-1} [17]. Although the studied

emission could be slightly influenced by an addition from ambient air under this situation, it can be counteracted by the subtraction of blank measurements whose emissions were collected prior to the measurement of each sample using Tedlar® PVF film between ground and the chamber (Figure S1). Each point cost around 1 h including twice measurements (blank and sample), the time for operation and movement to next point, and the order of sampling at points along the transect was randomized for every tree and varied with sampling period (Table S1). Soil temperature and moisture content around the soil chamber were measured using a Pt100 4.5 × 150 mm probe (Jules Richard Instruments-ICT, SL, Fesches-le-Chatel, France) and a ML3 ThetaProbe sensor connected to a ML3 ThetaKit (Delta-T Devices, Cambridge, UK), respectively. The litter below the soil chamber was collected after sampling at 0, 1, 2 and 3 m from tree trunks and oven-dried at 60 °C to a constant weight. The sampled cartridges were stored at 4 °C until analysis.

2.3. GC-MS Analyses of BVOCs

BVOCs were analyzed using a GC-MS system (7890A GC-system interfaced with a 5975C VL MSD and a Triple-Axis detector; Agilent Technologies, Palo Alto, USA). An automated thermal desorption unit (Ultra 2 and Unity 2; Markes International Ltd, Llantrisant, UK) was used for desorption of sampled cartridges. Desorbed BVOCs were cryofocused at −25 °C for 2 min, then, the cryotrap was rapidly heated to 320 °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). Carrier gas was helium and column flow was 1 mL min^{-1}. Total run time was 30 min, where initial oven temperature was

held at 35 °C for 5 min, then programmed to increase by 15 °C min^{-1} to 150 °C for 5 min, then by 15 °C min^{-1} to 250 °C for another 3 min, and finally by 30 °C min^{-1} to 280 °C for 2 min [17]. Terpenes were identified by comparing retention times with those of standards from Fluka (Buchs, Switzerland) and published spectra from the Wiley275 and NIST05a mass-spectral library using GCD ChemStation G1074A HP. Isoprenoid concentrations were determined by reference to trapped standards of α-pinene, 3-carene, β-pinene, limonene and sabinene every five analyses, and their calibration curves were made using three terpene concentrations (relationship between signal and terpene concentrations: $r^2 > 0.99$) [9,12]. The most abundant isoprenoids, such as α-pinene, 3-carene, β-pinene, limonene and sabinene, had similar sensitivities, with <5% differences among calibration factors.

The exchange rates were expressed as differences between the emission rates of the sample and the corresponding blank in μg m^{-2} h^{-1}. When the values are positive, they indicate BVOC emission from ground to atmosphere, and the exchange rates are then referred to be "emission rates". When the values are negative, they indicate BVOC adsorption to ground, and the exchange rates are then referred to be "adsorption rates".

2.4. Statistical Analyses

Differences in terpene exchange along the horizontal gradient were tested using one-way analysis of variance at $P < 0.05$ in Statistica v.8.0 (StatSoft, Inc., Tulsa, USA) and covariance in terpene exchanges with soil environmental conditions was analyzed using partial least squares (PLS) regression using the plsdepot package in R v. 3.3.3. A comparison of emission profile was made between the ground covering dense litter and

green needle of *P. pinea* according to the data from this study and Staudt et al (2000) [36].

3. Results

3.1. Soil Environmental Conditions

Mean soil temperatures along the transects of the four trees were 28.5 ± 0.80°C, 28.8 ± 1.16 °C, 33.1 ± 1.01 °C and 34.3 ± 1.46 °C, and soil moisture (v/v) was 12.2 ± 1.06%, 16.7 ± 0.91%, 4.7 ± 0.70% and 2.2 ± 0.39%. There was some variation in soil environmental conditions among the trees due to precipitation, but within-transect variation was lower owing to the randomization of sampling. Mean soil temperature ranged between 28.4 ± 1.00 °C (at 1 m) and 33.2 ± 1.88 °C (at 8 m), and mean soil moisture (v/v) ranged between 7.1 ± 2.23% (at 8 m) and 10.5 ± 3.09% (at 6 m) (Figure S2). The BVOC exchanges at same distance can be considered paralleled in terms of similar environmental conditions, which makes the average value can represent exchange at the distance; the conditional environment for all samplings was concentrated in certain scope which is also optimum to analyse its relationship with exchange.

3.2. Terpene Exchange

There were no detectable isoprene emissions, while the terpene emissions varied greatly in terms of amount and composition along the transects. Terpene emissions varied most significantly along the horizontal gradient under litter, where they were greater at 1 m (371.4 ± 71.1 μg m^{-2} h^{-1}) than at 0 m (135.2 ± 22.9 μg m^{-2} h^{-1}; $P < 0.05$)

and 2 m (13.1 ± 1.8 μg m^{-2} h^{-1}; $P < 0.01$), and greater at 0 m than at 2 m ($P < 0.01$) (Figure 1); emissions were 7.3 ± 2.0 μg m^{-2} h^{-1} at 3 m. Litter was absent from 4 m along the transect and terpene exchange became irregular, where terpenes were emitted or adsorbed at low rates (average < 2 μg m^{-2}·h^{-1}); greatest adsorption rates were recorded at 4m (1.6 μg m^{-2} h^{-1}) (Figure 1).

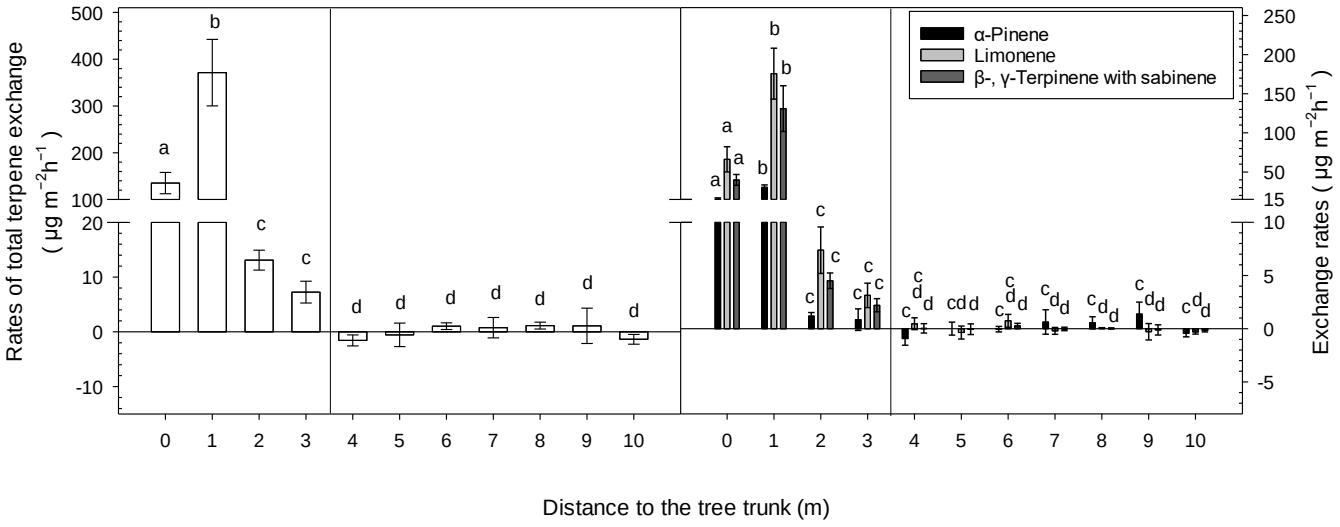


Figure 1. Rates of total terpene, α-pinene, limonene, β- and γ-terpinene with sabinene exchange along the transects. Data are means ± SE; n = 4. Different letters indicate differences among distances ($P < 0.05$).

Nine monoterpenes and two sesquiterpenes were detected from the transects, all of which were detected for emission at only 0 and 1 m and, with the exception of camphene, tended to be emitted in greater quantities at 1 m than at 0 m (Figure 2). Limonene, β- and γ-terpinene with sabinene, and α-pinene together account for around 90% of total emissions at dense litter zone, while the spectrum of emission of green

needles from *P. pinea* is dominated by trans-β-ocimene, followed by limonene, linalool, and 1,8-cineole, that together, accounted for around 90% (Table S2; Figure 3) of all emissions in summer mornings [36]; α-pinene and limonene account for higher proportion at dense litter zone than green needles, while myrcene showed similar proportion (Table S2; Figure 3).

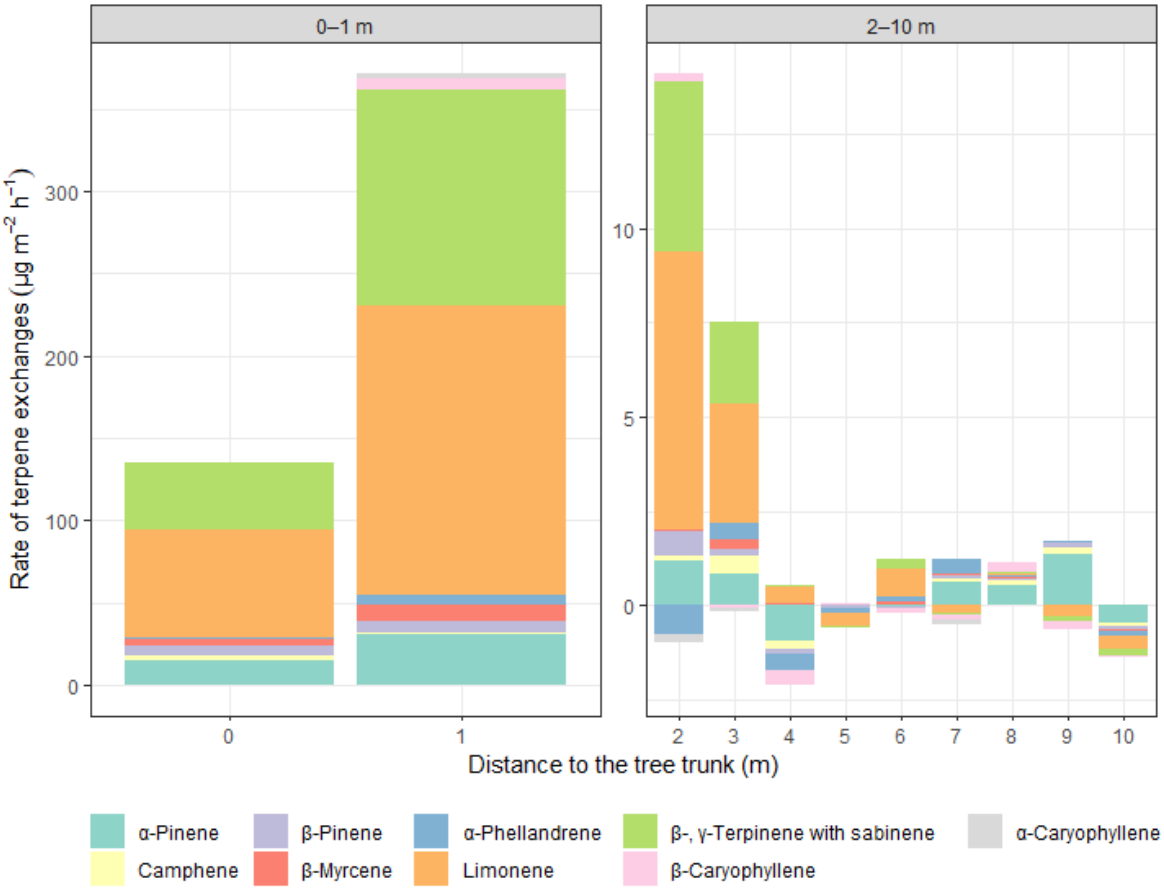


Figure 2. Distribution of terpene exchange along the transects.

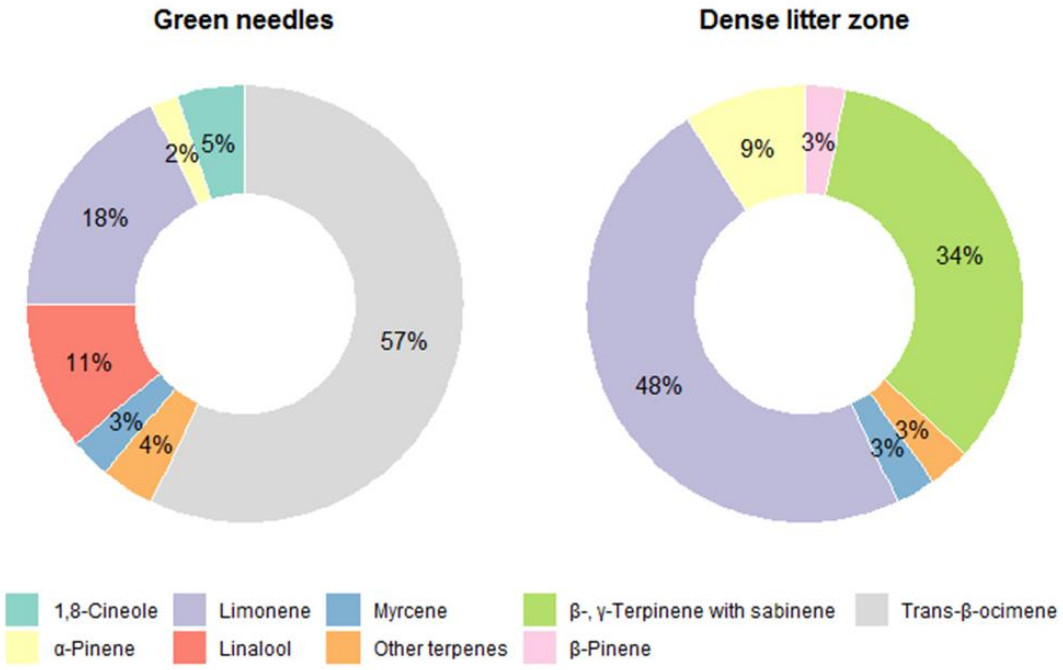
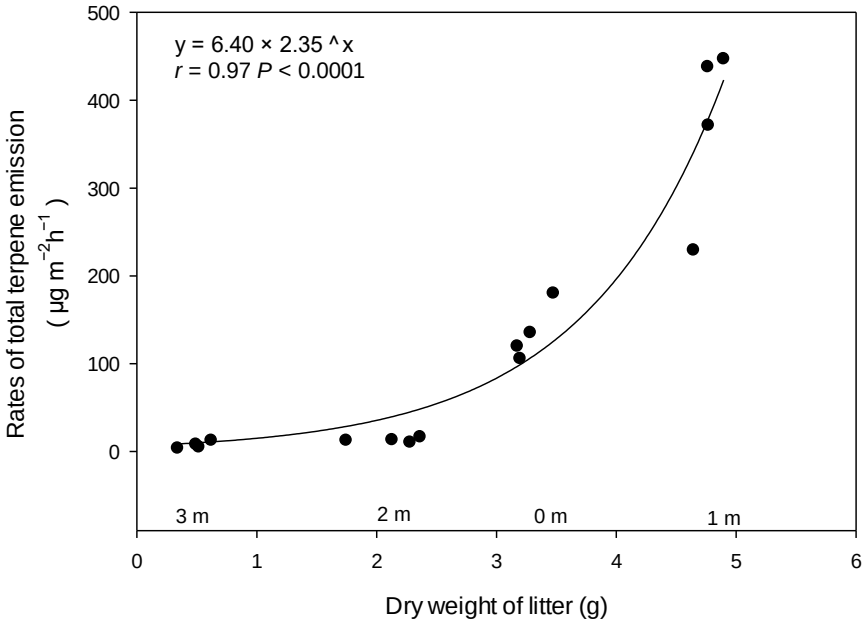
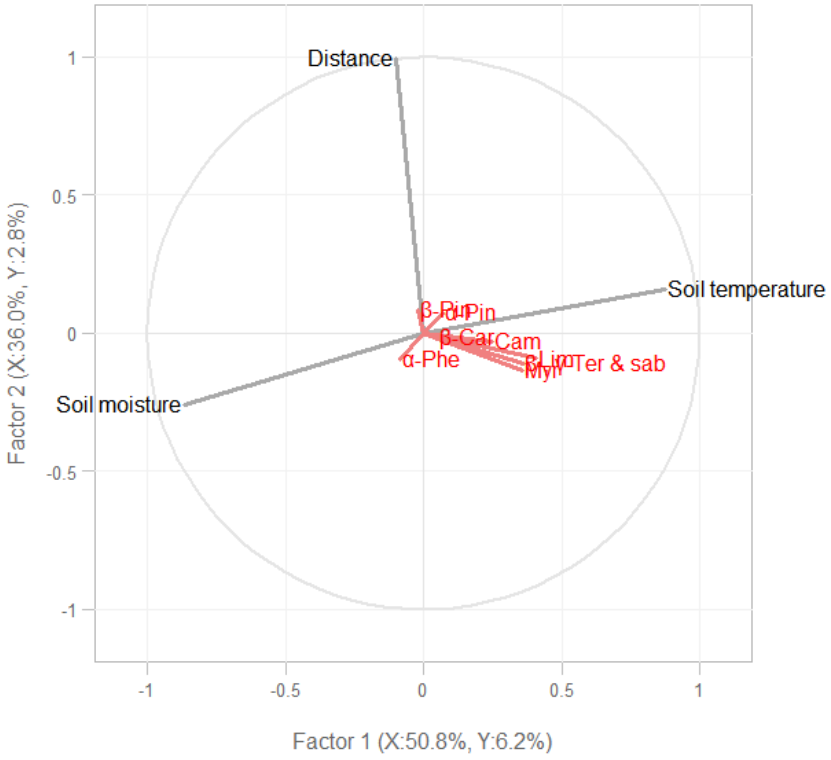


Figure 3. The composition of terpene emissions for green needles [36] and dense litter zone of our study (≤1 m) in summer mornings.

Limonene was the most dominant compound, followed by β- and γ-terpinene with sabinene, and α-pinene (Figure 1), and their emissions along the transect were similar to those of total terpenes in the litter zone (greater concentrations at 1 m than 0 and 2m, and greater at 0 m than at 2 m; $P < 0.05$) (Figure 1), and there were relatively high emissions of other terpenes at 0 and 1 m (<10 μg m^{-2} h^{-1}), but lower emissions (<1 μg m^{-2} h^{-1}) at the other distances (Figure 2). While two sesquiterpenes β-caryophyllene and α-caryophyllene were emitted at around 7.5 and 2.5 μg m^{-2} h^{-1} at 1 m, they were emitted at <0.5 μg m^{-2} h^{-1} at other distances, and were barely detected after 4 m (Figure 2). Almost all terpenes were adsorbed at 4 and 10 m, particularly α-pinene (Figure 2).



(a)



(b)

Figure 4. Relationship between total terpene emissions and litter dry weight within 4

m of the study trees (a). Partial least squares (PLS) regression between soil temperature or soil moisture content or distance to the trees and terpene exchange along the transects in turf zone (≥4 m) (b). Black represents soil temperature, soil moisture content and distance to the trees as independent variables (X), red represents exchange rates of individual terpenes as dependent variables (Y). α-Pin, α-pinene; Cam, camphene; β-Pin, β-pinene; Myr, myrcene; α-Phe, α-phellandrene; Lim, limonene; β-, γ-ter and sab, β-, γ-terpinene with sabinene; β-Car, β-caryophyllene.

The litter dry weight was largest at 1 m (4.768 g), followed by 0 m (3.282 g), 2 m (2.128 g) and 3 m (0.492 g), and it showed strong exponential relationship with terpene emission (Figure 4a). The detected compounds were correlated positively with soil temperature and negatively with soil moisture content and distance to the tree (Figure 4b)

4. Discussion

Terpene exchanges varied with the distance to the trunk of *Pinus pinea*, a storage species for these compounds [35]. The ground showed totally different emission pattern due to litter coverage or not, with different magnitude, and it is possible to divide the exchange profiles into three groups: short distances (<2 m) covering dense litter, medium distances covering moderate litter (2–4 m), turf (≥4 m), where emission rates were greatest at <2 m and lowest at ≥4 m, reflecting the degree of canopy coverage of the ground. Previous studies also found evidence of a gradient from high levels of monoterpenes in the vicinity of the tree trunk to lower levels at the farthest distance [16,17,37]. These studies suggested that the large source of volatiles result

predominantly from a large amount of litter or roots/rhizosphere activity in the soil near trunks [16,19].

The BVOC emissions at short and medium-distances were dominated by litter (Figure 4a), and also probably released by microbial metabolism and sparse ground vegetation, especially by roots owing to emissions decreasing with increasing distance from the tree trunk. However, the points at 0m with less litter, maybe owing to uneven ground near trunk, also showed obviously less emissions compare to that of the points at 1 m, where is supposed to be farther from and have less quantities of underground roots for the species of taproot system. The roots were reported to increase [38] or decrease [29] soil emissions, both with low fluxes indicating root-rhizosphere activity [10,30,39] would be a much smaller source compared to litter in this study. However, roots may represent a strong terpene source for *Pinus* spp. as well [17,37] and are a non-negligible source of VOCs for some species, like Arabidopsis [30,39]. Soil microbial activity has been shown to correlate with VOC emissions over a range of different forest soils [8,12,16], however, the soil moisture recorded were very low which has probably strongly hampered microbial metabolism [33]. Although activity of soil microorganisms and roots were both decreased in summer [29], the changes towards a decrease in the ratio of microorganisms/roots activities in the rhizosphere was found [30], which may suggest the emission from microbe activity may be a smaller source compared to roots.

The strongly positive relationship between emission rate and litter dry weight (Figure 4a) also indicated that the aboveground plant litter was likely the dominant

terpene source [8,16,18] as also reported for aboveground litter of other species storing terpenes [28,34,40]. Turf is a “simpler” ecosystem compared to other ecosystems because it has less vegetation mass and associated interactions, than forests, grasslands or croplands [28,41]. The strong relationship (Figure 4a) may actually indicate that the aboveground plant litter was the most dominant terpene source, while other biotic sources like roots and microorganism, and abiotic factors like soil properties and environmental conditions may play less important roles in ground level isoprenoid emissions. This makes that the emissions can be estimated by litter dry weight in this type of ecosystem while it may be instead unrealistic for other ecosystems.

The quantity of terpenes is thus dominantly linked to the amount of needle litter at short and medium-distances which might mask the variation caused by environmental conditions, although effects of temperature on emissions elicit changes in transport resistance along the diffusion path from the litter [5] and temperature and humidity are always supposed to be main factors acting on terpene emission in Mediterranean summer daytime [29,42]. However, litter emissions associated with microbial decomposition of organic matter have been reported to be quantitatively more dominant than emissions caused only by abiotic factors as temperature and humidity [8,34,40] as suggested by the strong correlation between VOC production and microbial CO_2 production [8].

The emitted compounds found in this study follow a pattern similar to other studies of ground VOC emissions which consist of very few abundant compounds associated with several less representative ones (Figure 3) [11,22,29]. The spectrums of emission

vary significantly between dense litter zone and green needles from *P. pinea* (Figure 3), but both contain high amounts of limonene [36]. Emission rates of trans-β-ocimene, linalool and 1,8-cineole are light-dependent, and carbon dioxide exerts a particularly positive influence on the emission rates of trans-β-ocimene [35]. Trans-β-ocimene is directly synthesized in chloroplasts and follow a different metabolic path to other monoterpenes which are stored in resin ducts [35], this can be proved by its presence only in the sampling of green needle. However, this variation was also found in another typical Mediterranean pine species *Pinus halepensis* whose litter showed remarkably high sesquiterpenes (β-caryophyllene, followed by α-caryophyllene) emissions [17] which represent less than 5% of the total emissions of green needles [43,44]. Although the relative composition of terpenes in needle litter is related to that of green needles, terpene concentrations may change with time during decomposition processes [17]. The increased proportion of limonene and α-pinene and similar proportion of myrcene emission in dense litter zone compared with green needles may showed soil microbes readily consume a diverse array of BVOCs with different ability of utilization which also varied from distinct microbial communities [8,24,34], representing an important sink of BVOCs in terrestrial ecosystems dominated by plants that store terpenes [8]. On the other hand, the high proportion of β- and γ-terpinene with sabinene emissions in dense litter zone could indicate soil microbes producing terpenes that are not emitted by plants [8,17].

We found that terpene exchanges were very low (Figure 1) which was in agreement with the previous studies [17,20,29,30] and not correlated with the distance from the

trunk overall (Figure 4b) at the greatest distance from the trees in the herbaceous turf, where there was a lack of needle litter and too far to be influenced by roots as well. Despite potential terpene content in grasses [17,45], there was a negligible impact on exchange rates owing to the small biomass compared with the pine, supporting research that shows terpene emissions from ground in close proximity to trees derives from litter and plant roots [17]. Much less research has been directed towards the more intensively anthropogenic managed turf soils than forest, grassland or cropland systems that have been studied [28,40]. Our findings showed that turf soils produced negligible BVOC emissions, which were much lower than forest soil in Mediterranean summer [30]. Further, BVOC exchange profiles depend on soil type [10,28], and influenced by environmental conditions [10,27]. However, most of the measured fluxes from forest soil probably originated from understory vegetation [21,34]. Previous research suggests that biotic factors affecting the emissions of VOCs from soil are 5–10 times stronger than the abiotic ones [8,27], and soil environmental conditions affect both sources by altering volatility of VOCs and the activity and community composition of microorganisms [34]. In this study, α-caryophyllene was lacking at the greatest distances from the trunks where turf dominated, and emissions of β-pinene were not related to soil temperature or moisture content but positively related to the distance (Figure 4b). However, the emission of the rest of emitted compounds including the most abundant compound, limonene, along with β- and γ-terpinene, sabinene, and α-pinene were correlated positively with soil temperature and negatively with soil moisture content except for α-phellandrene which showed an opposite trend (Figure 4b). Soils in this

study emitted a variety of terpenes that varied as a function of soil temperature and moisture [4,32], and slight trend can be found for total terpene exchange (Figure S3). The positive correlation with temperature and negative correlation with moisture of BVOC emissions are also in agreement with previous study in Mediterranean holm oak forest soil [29] and high arctic soil [6]. The diversity of compounds found in this study, although not very high, gives an idea, of the various factors for VOC emissions that can be taken into account, such as temperature, moisture and their interaction. In addition, the type of soil and low vegetation also influences. Mediterranean soil behaves more as a sink than as a source of BVOCs since total soil BVOC adsorption overcame emission over the year [29,30]. However, our results show that soil VOC exchange with the atmosphere might greatly change in response to climate change, with likely increased emissions under the warmer and drier summers expected for the coming decades in the Mediterranean region [41]. Given the variations in ground emissions with soil, vegetation, microorganisms, and associated interactions, we recommend further spatio-temporal analysis of ground level BVOC exchanges in a wider range of ecosystem types.

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General conclusions

1. Isoprenoid emissions increased with air temperature and also generally increased as the amount of soil moisture decreased in drought treatments. The production of isoprenoids has been linked to an increased tolerance to water stress for *E. multiflora* and *Q. ilex*. Higher isoprenoid emissions can be expected in the warmer and drier conditions predicted for the coming decades in the Mediterranean region. The improvement of current models is required to better predict the actual emission rates in response to drought, since that algorithm only predicts reduction in emissions and not possible increases at mild drought conditions.

2. N deposition did not significantly affect isoprene emission but significantly increased total terpene emissions and decreased the diversity of terpenes. The complex effects of air temperature, soil moisture and N deposition was mainly dominated by synergetic increases in summer. The plants showed successful acclimation by increasing isoprenoid emissions under environmental stress. N deposition will further stimulate the emission trends in the warmer and drier conditions projected for the coming decades.

3. Soil VOCs differed between the Mediterranean ecosystems characterized by contrasting dominant plant species. The difference was driven by contrasting plant activities, soil temperature and water content, and microbial activity. Seasonal concentrations of most VOCs were greatest in spring, as soil VOC concentrations are positively associated with soil temperature and the corresponding increased activity of roots and microbes. These data on soil concentrations, which are scarce in the literature, will allow to identify fundamental mechanisms that control soil VOC dynamics thus

facilitating future advances in the modelling of soil VOC fluxes.

4. The presence of aboveground litter was the dominant source of ground level terpene emissions in the proximity of *Pinus pinea* trees, to the point that the ground emission rates can be estimated by litter dry weight. Soils act as a source or sink of terpenes in managed Mediterranean turf environments with often negligible terpene exchanges. These exchanges correlate positively with soil temperature and negatively with soil moisture. The soil terpene emissions are thus expected to increase in response to climate change in the Mediterranean region.

Supplementary material

Chapter 2. Profile of foliar isoprenoid emissions from Mediterranean dominant shrub and tree species under experimental nitrogen deposition

Table S1. Distribution of seasonal terpene emissions (μg g^{-1} dw h^{-1}) for *Erica multiflora* (A and B) and *Quercus ilex* (C and D) in the morning and at midday for the fertilized year. ND, not detected.

A)

	Morning							
	Spring		Summer		Autumn		Winter	
	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen
α-Pinene	0.81 ± 0.26	0.48 ± 0.14	0.86 ± 0.31	0.94 ± 0.26	0.17 ± 0.02	0.13 ± 0.06	0.14 ± 0.05	0.06 ± 0.03
Tricyclene	ND	0.16 ± 0.09	0.04 ± 0.02	0.13 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0.01	ND
β-Pinene	ND	ND	0.14 ± 0.05	0.22 ± 0.08	0.04 ± 0.02	<0.01	ND	ND
β-Myrcene	ND	ND	ND	ND	0.01	<0.01	ND	ND
3-Carene	0.01	ND	0.18 ± 0.05	0.31 ± 0.11	0.02 ± 0.01	ND	ND	ND
Limonene	0.18 ± 0.03	0.21 ± 0.04	0.58 ± 0.18	0.71 ± 0.30	0.02 ± 0.01	0.07 ± 0.01	0.1 ± 0.04	0.07 ± 0.02
Sabinene	ND	ND	0.08 ± 0.05	<0.01	<0.01	ND	<0.01	<0.01
Trans-β-ocimene	0.02 ± 0.01	ND	ND	0.01	0.04 ± 0.02	0.04 ± 0.02	ND	ND
α-Terpinolene	ND	ND	ND	ND	ND	ND	ND	ND
β-Caryophyllene	0.01	ND	0.14 ± 0.08	0.19 ± 0.10	0.05 ± 0.03	<0.01	ND	ND

B)

	Midday							
	Spring		Summer		Autumn		Winter	
	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen
α-Pinene	0.50 ± 0.09	0.71 ± 0.19	0.65 ± 0.14	1.31 ± 0.27	0.25 ± 0.02	0.33 ± 0.14	0.23 ± 0.09	0.16 ± 0.04
Tricyclene	0.10 ± 0.03	0.14 ± 0.06	0.28 ± 0.06	0.68 ± 0.23	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	ND
β-Pinene	0.17 ± 0.06	0.25 ± 0.13	0.07 ± 0.01	0.01	0.01	0.01	ND	ND
β-Myrcene	ND	ND	ND	ND	0.01	ND	ND	ND
3-Carene	0.10 ± 0.04	0.07 ± 0.04	0.06 ± 0.03	0.03 ± 0.01	<0.01	0.01	ND	ND
Limonene	0.27 ± 0.11	0.60 ± 0.20	0.49 ± 0.16	0.82 ± 0.21	0.09 ± 0.05	0.15 ± 0.09	0.09 ± 0.01	0.04 ± 0.01
Sabinene	0.10 ± 0.06	ND	0.01	0.05± 0.02	ND	0.01	0.02 ± 0.01	ND
Trans-β-ocimene	ND	ND	0.32 ± 0.09	0.30± 0.18	ND	0.01	ND	ND
α-Terpinolene	ND	ND	<0.01	0.03 ± 0.01	ND	ND	ND	ND
β-Caryophyllene	0.20 ± 0.05	0.19 ± 0.10	0.38± 0.10	1.01± 0.40	0.06 ± 0.04	ND	ND	ND

C)

	Morning							
	Spring		Summer		Autumn		Winter	
	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen
α-Pinene	0.93 ± 0.42	0.78 ± 0.38	1.87 ± 0.49	2.57 ± 0.26	0.54 ± 0.22	0.44 ± 0.24	0.03 ± 0.02	0.10 ± 0.07
Tricyclene	0.03 ± 0.02	0.01	0.11 ± 0.06	0.06 ± 0.02	0.01	0.04 ± 0.01	ND	0.06 ± 0.03
β-Pinene	0.43 ± 0.17	0.14 ± 0.02	1.24 ± 0.25	1.86 ± 0.21	0.32 ± 0.07	0.16	0.10 ± 0.05	0.10 ± 0.06
β-Myrcene	0.42 ± 0.24	ND	1.44 ± 0.58	0.15 ± 0.02	ND	ND	ND	ND
3-Carene	0.21 ± 0.03	0.15 ± 0.04	0.04 ± 0.02	ND	0.29 ± 0.10	0.17 ± 0.10	ND	<0.01
Limonene	1.12 ± 0.39	0.8 ± 0.28	0.88 ± 0.37	1.33 ± 0.76	0.95 ± 0.49	0.38 ± 0.28	0.41 ± 0.10	0.05 ± 0.02
Sabinene	ND	ND	0.02 ± 0.01	0.04 ± 0.02	0.08 ± 0.05	0.01	0.02 ± 0.01	0.14 ± 0.04
Trans-β-ocimene	0.03 ± 0.02	0.04 ± 0.02	0.01	ND	0.02 ± 0.01	0.01	0.06 ± 0.03	0.07 ± 0.04
β-Caryophyllene	ND	ND	ND	ND	ND	ND	ND	ND
α-Caryophyllene	ND	ND	ND	ND	ND	ND	ND	ND

D)

	Midday							
	Spring		Summer		Autumn		Winter	
	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen	Control	Nitrogen
α-Pinene	2.04 ± 0.57	1.02 ± 0.14	2.65 ± 0.61	6.18 ± 2.12	0.43 ± 0.12	1.06 ± 0.38	0.12 ± 0.08	0.02 ± 0.01
Tricyclene	0.12 ± 0.04	0.01	0.35 ± 0.08	0.50 ± 0.18	ND	0.05 ± 0.03	ND	0.06 ± 0.04
β-Pinene	0.23 ± 0.06	0.29 ± 0.04	1.72 ± 0.26	3.72 ± 1.20	0.16 ± 0.08	0.34 ± 0.17	0.08 ± 0.04	0.06 ± 0.04
β-Myrcene	0.19 ± 0.12	0.38 ± 0.12	2.21 ± 1.19	0.66 ± 0.30	ND	ND	ND	ND
3-Carene	0.37 ± 0.07	0.44 ± 0.03	0.43 ± 0.13	0.20 ± 0.08	ND	0.14 ± 0.08	0.02 ± 0.01	0.09 ± 0.06
Limonene	1.18 ± 0.55	0.32 ± 0.18	1.60 ± 0.33	5.45 ± 0.81	0.80 ± 0.21	1.10 ± 0.43	0.24 ± 0.03	0.15 ± 0.02
Sabinene	ND	ND	0.06 ± 0.04	0.04 ± 0.02	0.08 ± 0.05	0.13 ± 0.03	0.03 ± 0.02	0.01
Trans-β-ocimene	0.01	ND	0.07 ± 0.05	ND	0.04 ± 0.02	0.01	0.02 ± 0.01	0.08 ± 0.03
β-Caryophyllene	0.05 ± 0.02	0.07 ± 0.03	0.24 ± 0.15	ND	ND	ND	ND	ND
α-Caryophyllene	ND	ND	ND	ND	0.28 ± 0.15	0.18 ± 0.08	ND	0.01

Table S2. Relationships for the rate of isoprenoid emissions with main environmental conditions (air temperature and soil moisture) and physiological activities (net photosynthetic rate and stomatal conductance) for *Erica multiflora* (A for isoprene and B for total terpenes) and *Quercus ilex* (C for total terpenes) in the fertilized year (SE, Standard Error).

A)

		Control		Nitrogen	
		Linear	Exponential	Linear	Exponential
Air temperature	*R*	**0.5540**	0.453	**0.4299**	0.3969
	P	**<0.0001**	0.0018	**0.0045**	0.0093
	SE	**2.3937**	2.5634	**2.3847**	2.4243
Correspondent equation		**y = -5.745 + 0.342x**		**y = -2.504 + 0.183x**	
Soil moisture	*R*	0.4682	**0.5086**	0.3899	**0.4059**
	P	0.0012	**0.0004**	0.0107	**0.0076**
	SE	2.5406	**2.4756**	2.4323	**2.4139**
Correspondent equation		**y = 9.360*0.873 ^x**		**y = 7.686*0.874 ^x**	
Net photosynthetic rate	*R*	**0.4151**	0.3937	**0.0711**	0.0636
	P	**0.0046**	0.0074	**0.6545**	0.6893
	SE	**2.6159**	2.6431	**2.6346**	2.636
Correspondent equation		**y = 5.780 - 0.650x**		**y = 2.590 - 0.261x**	
Stomatal conductance	*R*	0.4893	**0.5177**	**0.3110**	0.2993
	P	0.0006	**0.0003**	**0.0450**	0.0541
	SE	2.5076	**2.46**	**2.5103**	2.5202
Correspondent equation		**y = 7.718*8.277E-010 ^x**		**y = 3.532 - 22.24x**	

B)

		Control		Nitrogen	
		Linear	Exponential	Linear	Exponential
Air temperature	*R*	**0.5260**	0.4861	0.4887	**0.514**
	P	**<0.0001**	0.0001	0.0006	**0.0003**
	SE	**0.8429**	0.8661	1.2814	**1.2599**
Correspondent equation		**y = -1.155 + 0.090x**		**y = 0.117*1.097 ^x**	
Soil moisture	*R*	0.5199	**0.5496**	0.4272	**0.4832**
	P	<0.0001	**<0.0001**	0.0031	**0.0007**
	SE	0.8466	**0.828**	1.3279	**1.2859**
Correspondent equation		**y = 3.423*0.892 ^x**		**y = 5.669*0.858 ^x**	
Net photosynthetic rate	*R*	**0.0712**	0.0704	**0.2844**	0.2674
	P	**0.6021**	0.6062	**0.0527**	0.0692
	SE	**0.9886**	0.9887	**1.4024**	1.4096
Correspondent equation		**y = 0.835 - 0.037x**		**y = 0.107 + 0.226x**	
Stomatal conductance	*R*	**0.1552**	0.1537	**0.0808**	0.0766
	P	**0.2769**	0.2816	**0.6066**	0.6252
	SE	**1.0017**	1.0019	**1.4908**	1.4913
Correspondent equation		**y = 1.419 - 5.228x**		**y = 1.065 - 3.286x**	

C)

		Control		Nitrogen	
		Linear	Exponential	Linear	Exponential
Air temperature	*R*	0.5373	**0.6177**	0.6304	**0.6572**
	P	0.0001	**<0.0001**	<0.0001	**<0.0001**
	SE	3.2277	**3.0096**	5.1507	**5.0009**
Correspondent equation		**y = 0.165*1.135 ^x**		**y = 0.317*1.125 ^x**	
Soil moisture	*R*	0.4611	**0.4831**	0.3836	**0.392**
	P	0.0013	**0.0007**	0.0114	**0.0089**
	SE	3.3960	**3.3508**	6.1275	**6.1042**
Correspondent equation		**y = 19.18*0.832 ^x**		**y = 45.42*0.773 ^x**	
Net photosynthetic rate	*R*	0.4064	**0.5558**	0.5205	**0.531**
	P	0.0051	**<0.0001**	0.0008	**0.0006**
	SE	3.4968	**3.1816**	5.6657	**5.6225**
Correspondent equation		**y = 15.45*0.753 ^x**		**y = 17.44*0.769 ^x**	
Stomatal conductance	*R*	0.3069	**0.4334**	0.4407	**0.5144**
	P	0.0380	**0.0026**	0.0056	**0.0013**
	SE	3.6424	**3.4489**	5.9563	**5.6901**
Correspondent equation		**y = 13.47*6.120E-011 ^x**		**y = 17.36*6.628E-011 ^x**	

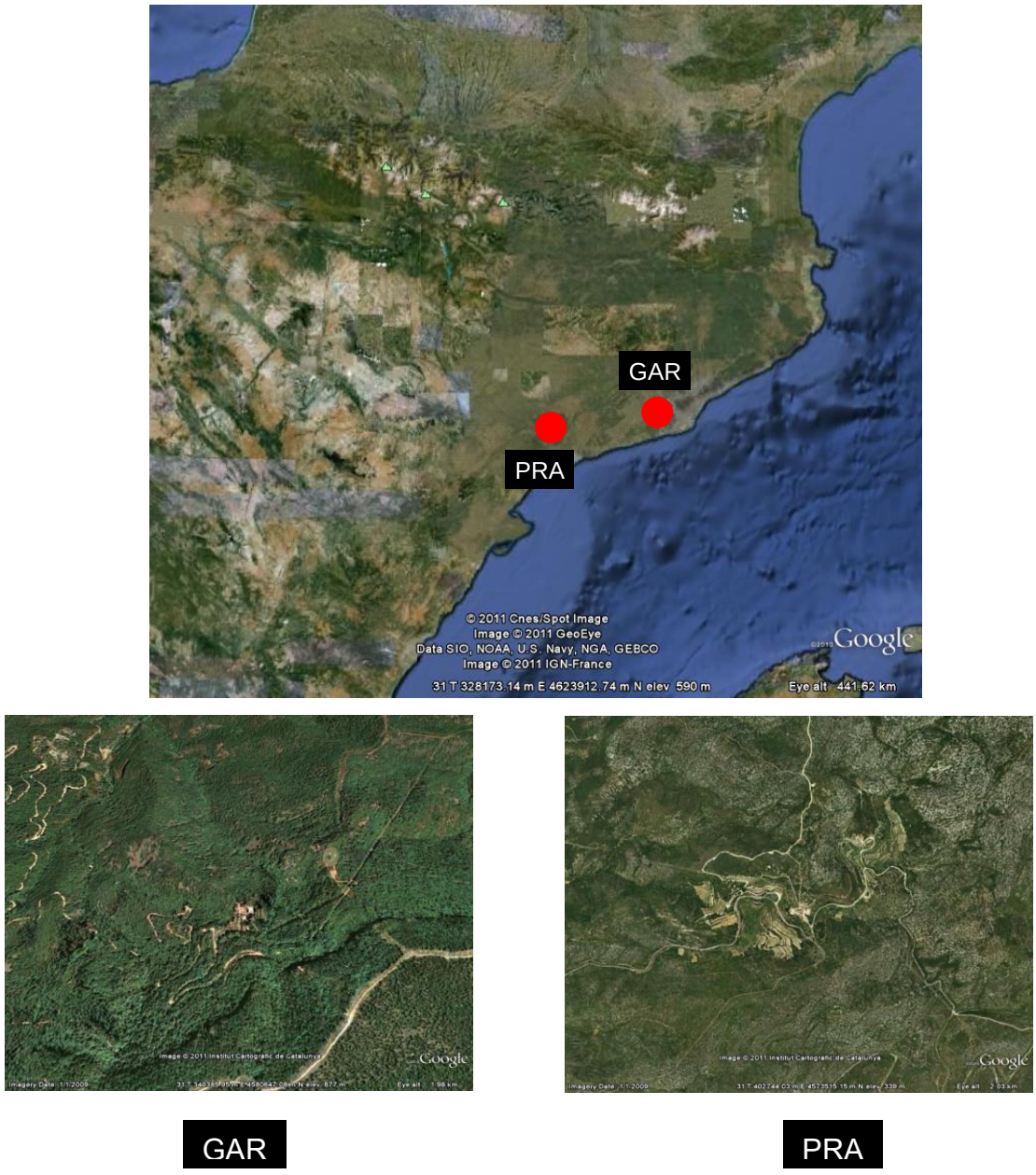


Fig. S1. Location and landform of experimental sites. GAR, Garraf; PRA, Prades.

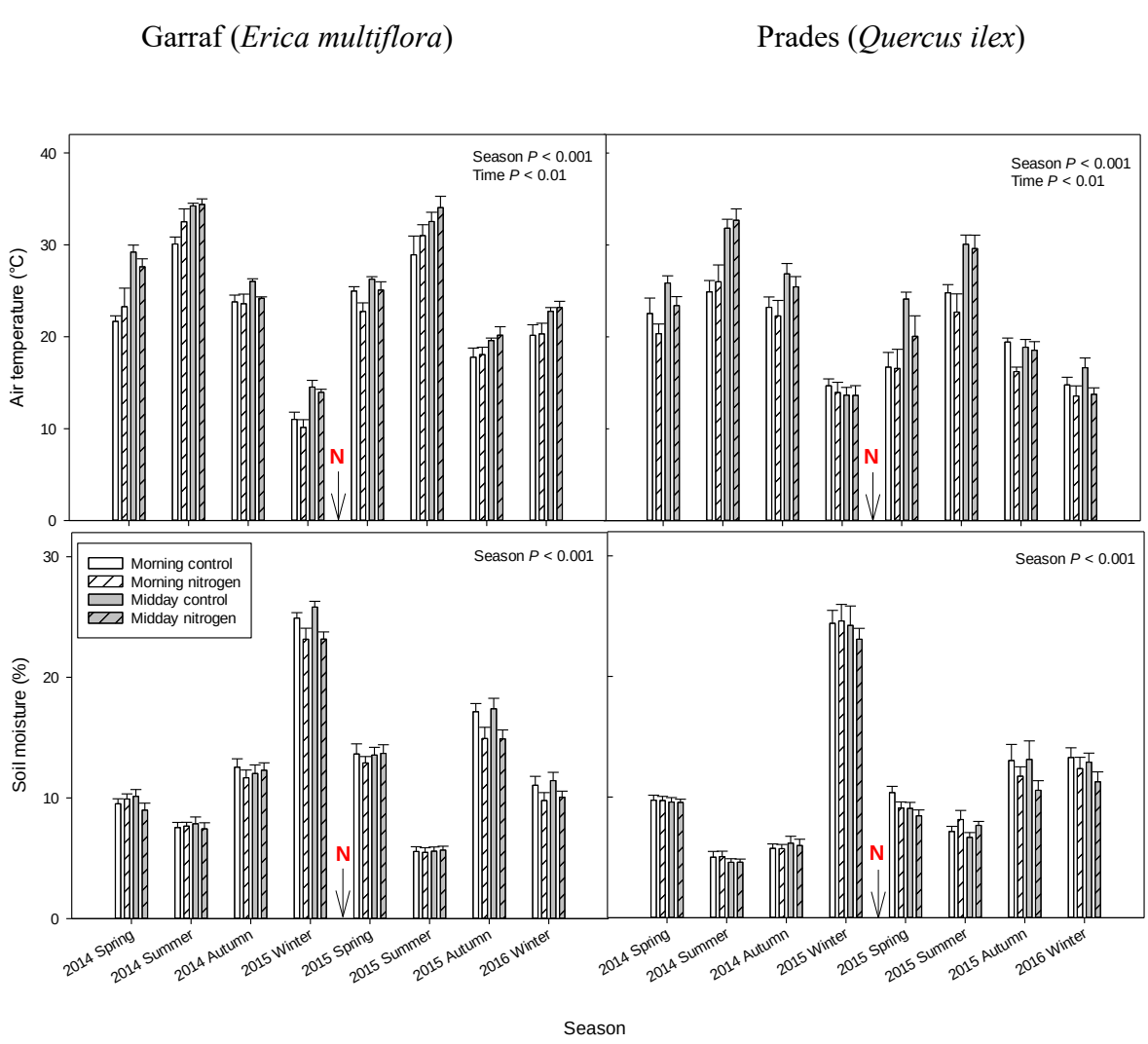
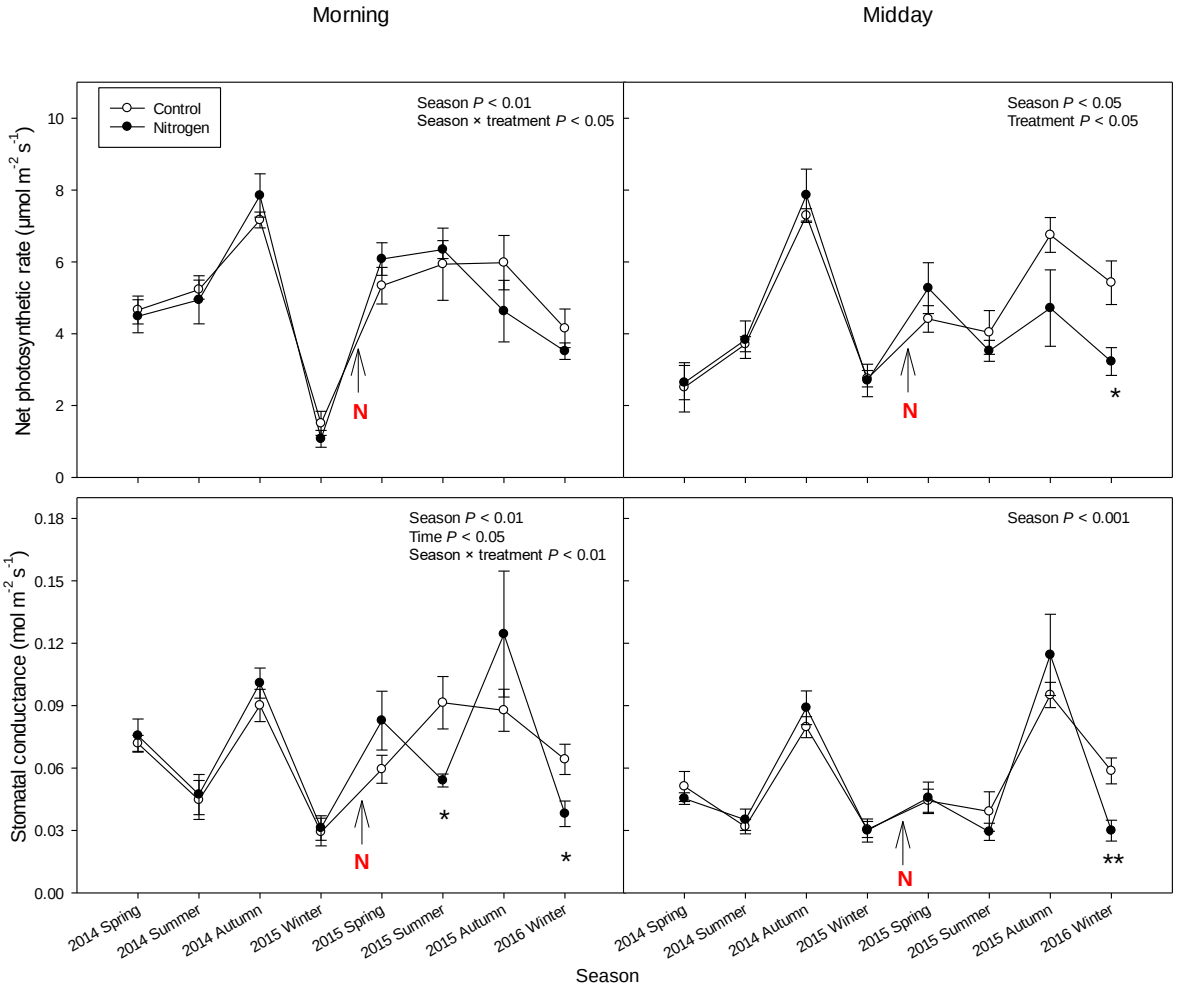


Fig. S2. Seasonal morning and midday variation of mean air temperature and soil moisture in Garraf and Prades. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means (n = 6). The significance of the effects of season and sampling time (repeated-measures ANOVA) is depicted in the panels.

A) *Erica multiflora*



B) *Quercus ilex*

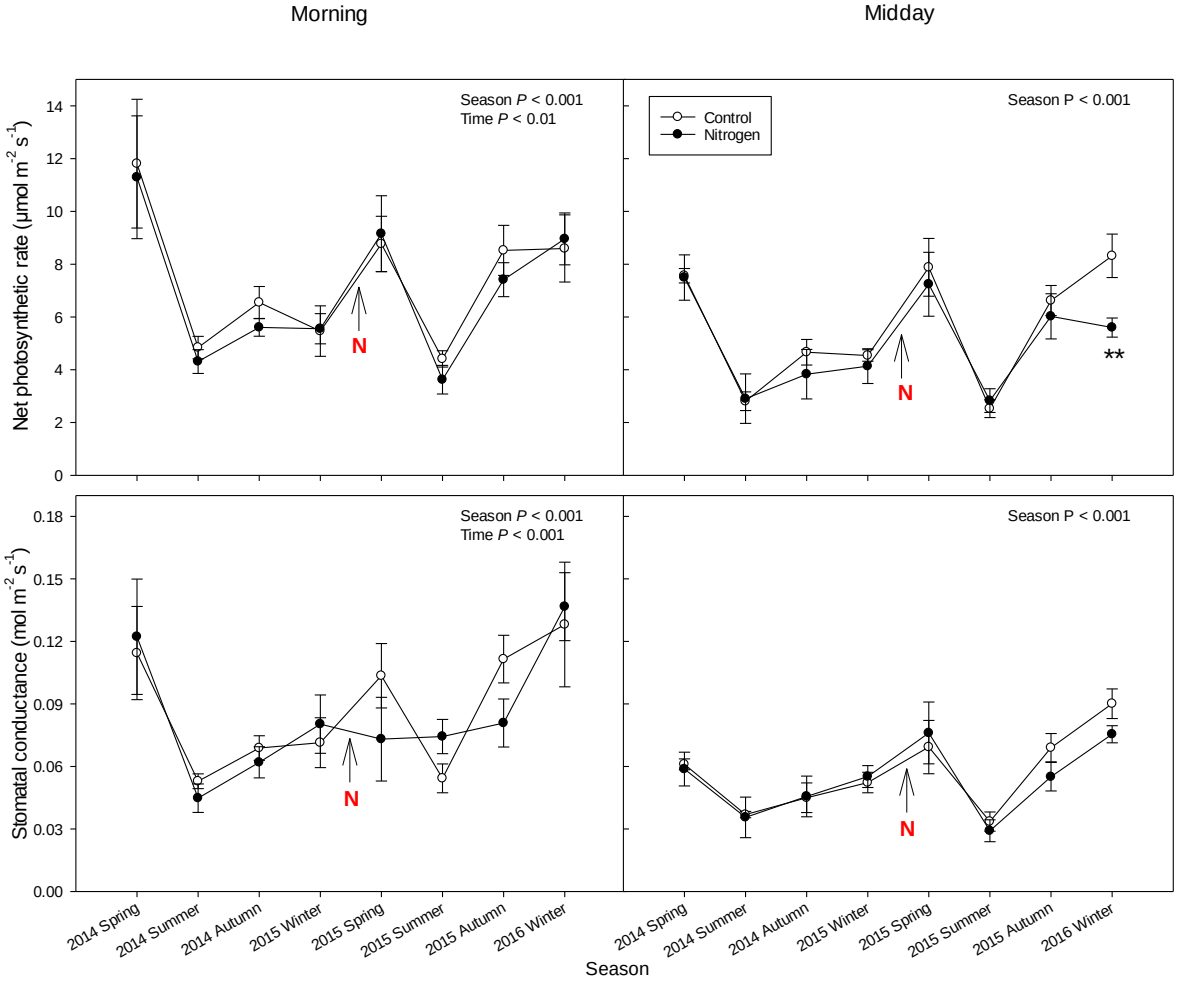


Fig. S3. Seasonal net photosynthetic rates and stomatal conductances for *Erica multiflora* (A) and *Quercus ilex* (B) in the morning and at midday. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means (n = 6). Significant differences between treatments identified by Student's t-tests are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$). The significance of the effects of season, treatment and sampling time (repeated-measures ANOVA) is depicted in the panels.

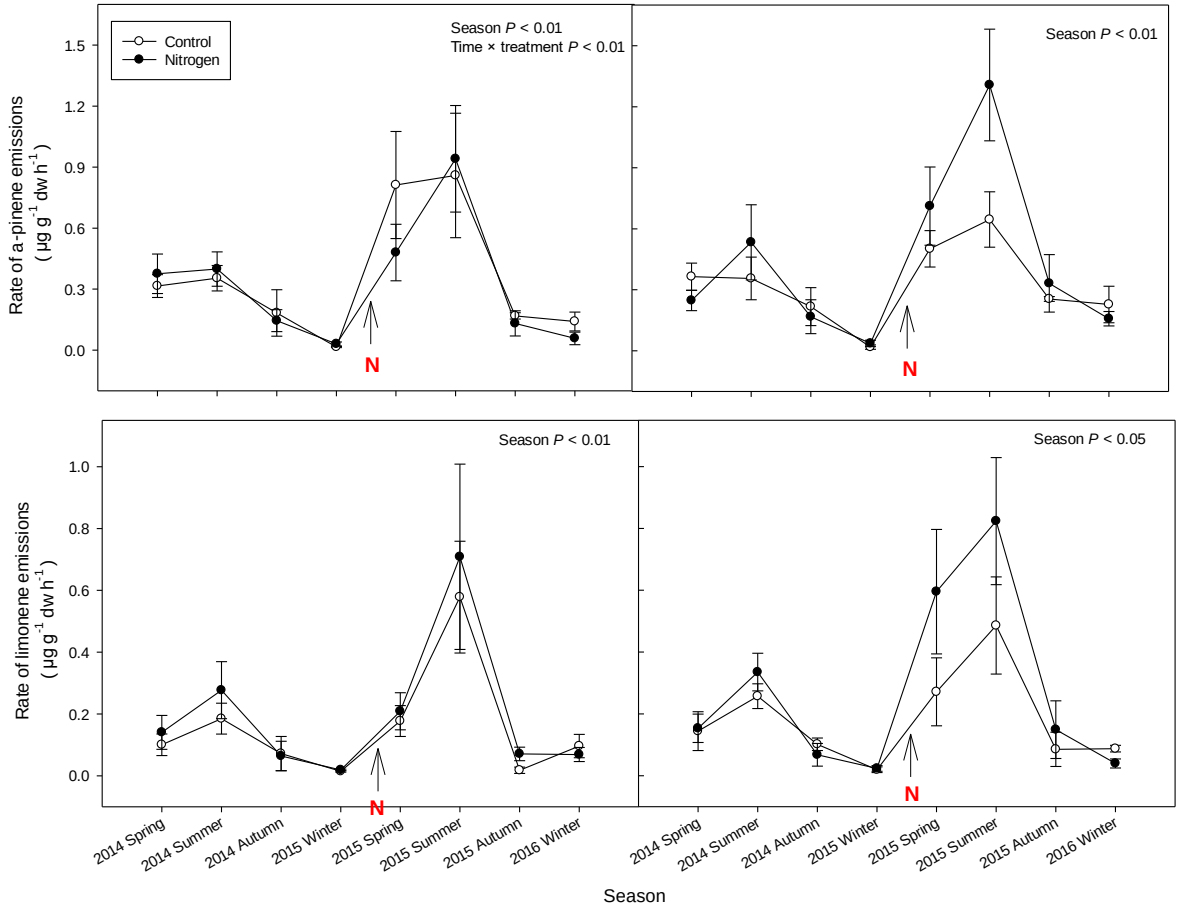


Fig. S4. Seasonal variation of the rates of emission of α-pinene and limonene for *Erica multiflora*. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means (n = 6). The effects of season, treatment and sampling time are depicted in the panels when significant.

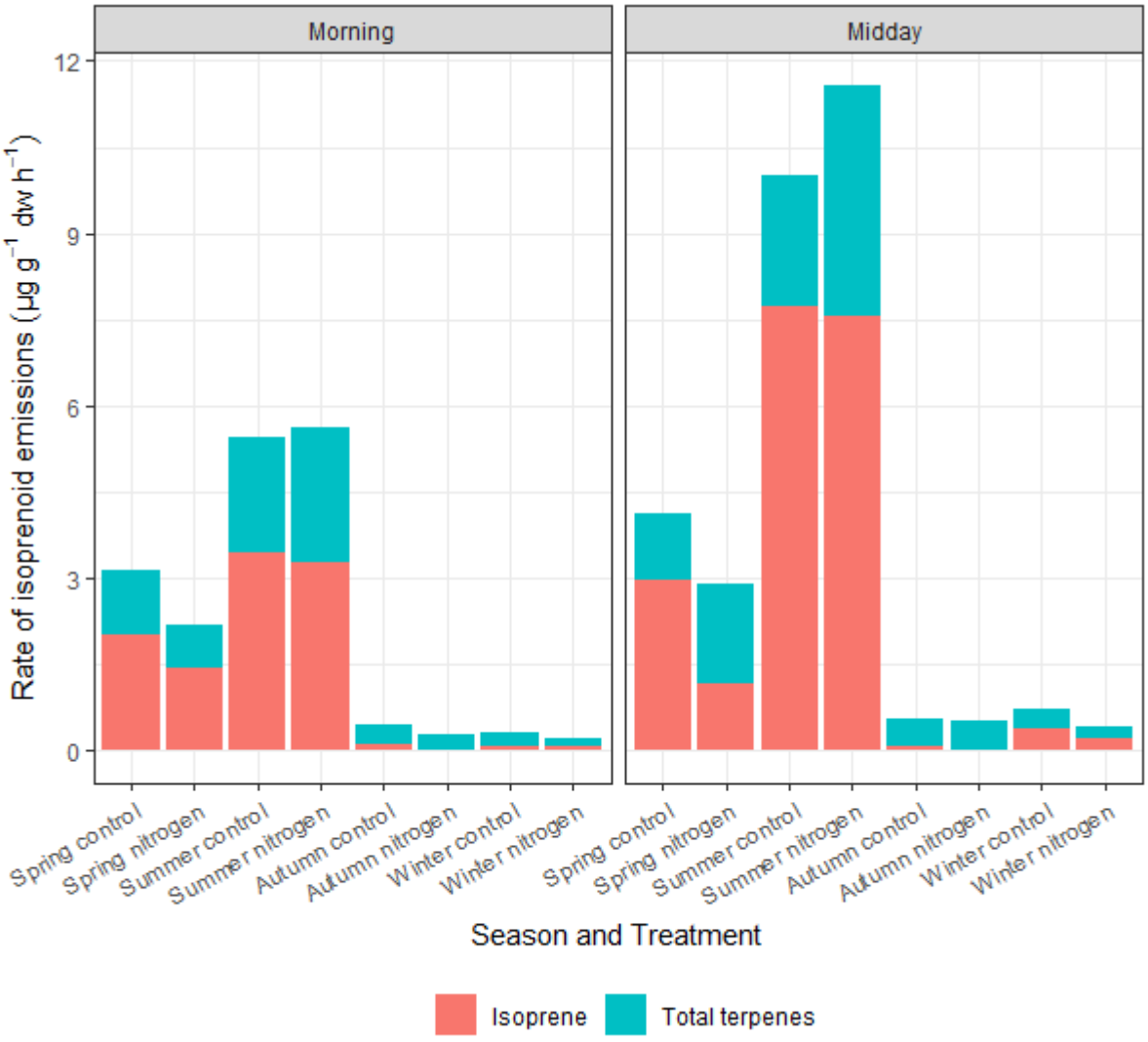
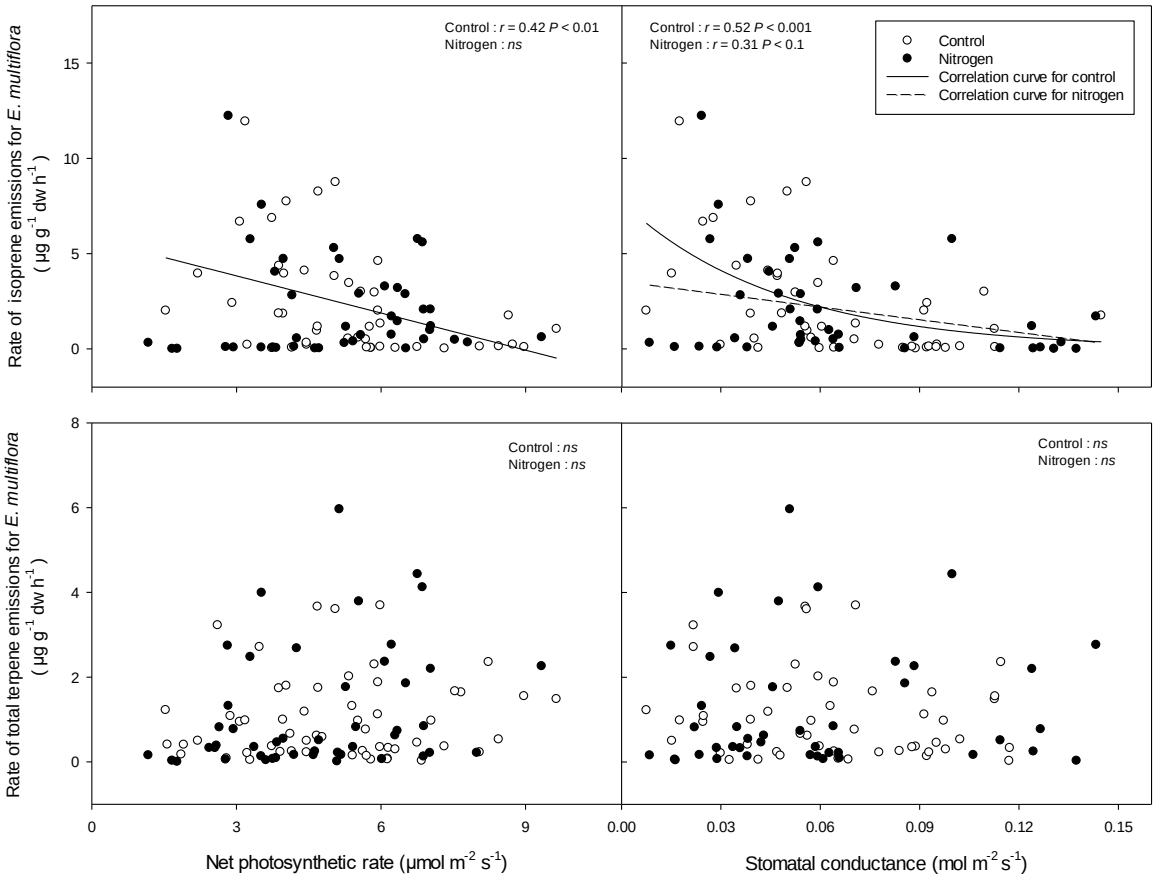


Fig. S5. Distribution of seasonal isoprenoid emissions for *Erica multiflora* in the morning and at midday for the fertilized year.

A)


Control : r = 0.42 P < 0.01
Nitrogen : ns
Control : r = 0.52 P < 0.001
Nitrogen : r = 0.31 P < 0.1
Control
Nitrogen
Correlation curve for control
Correlation curve for nitrogen
Rate of isoprene emissions for E. multiflora
(μg g-1 dw h-1)
Control : ns
Nitrogen : ns
Control : ns
Nitrogen : ns
Rate of total terpene emissions for E. multiflora
(μg g-1 dw h-1)
Net photosynthetic rate (μmol m-2 s-1)
Stomatal conductance (mol m-2 s-1)

B)

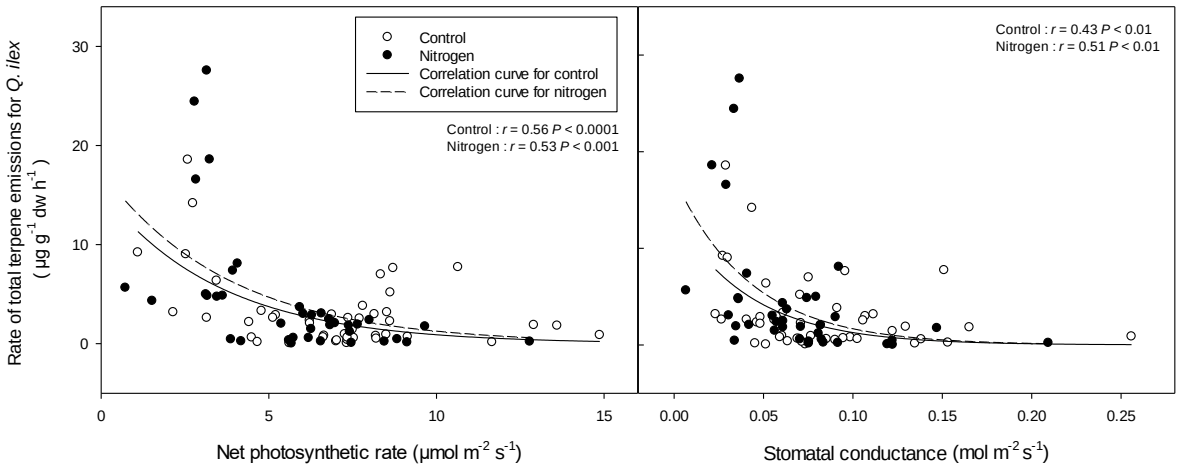


Fig. S6. Relationships for the rate of isoprenoid emissions with main physiological activities (net photosynthetic rate and stomatal conductance) for *Erica multiflora* (A) and *Quercus ilex* (B) in the fertilized year. ns, not significant.

Chapter 3. Annual and seasonal variations in soil volatile organic compound concentrations in a Mediterranean shrubland and holm oak forest

Table S1 Differences in mean (±SEM) seasonal soil temperature (°C), water content (%), and CO_2 efflux (μmol m^{-2} s^{-1}) recorded from shrubland and holm oak forest ecosystems during the period 2014–16 (Student's *t*-tests, **P*<0.05, ****P*<0.001).

Soil variable	Shrubland	Holm oak forest	Ecosystem difference
Temperature	15.79 ± 2.91	13.22 ± 1.71	*
Water content	14.86 ± 2.45	16.10 ± 3.65	
CO_2 efflux	1.29 ± 0.16	1.96 ± 0.30	***

Table S2 Seasonal and annual differences in detected organic volatile compounds during the period 2014–16 in shrubland and holm oak forest ecosystems (repeated measures ANOVA, **P*<0.05, ***P*<0.01, and ****P*<0.001).

Compound	Mass Fragment	Shrubland			Holm oak forest		
		Season	Year	Season × Year	Season	Year	Season × Year
Formaldehyde	M31			***			***
Methanol	M33	***		**			***
Acetaldehyde	M45	***		***	*		*
Ethanol	M47	***		***	***		*
1,3-Butadiene	M55	***	***	***	*	**	***
(E)-2-hexenal	M57	***	***	***	***	**	***
Acetone	M59	***	*	***	**		***
Acetic acid	M43 + M61	***	**	***	***	*	***
Isoprene	M69	***			*		***
Benzene	M79	***			**	**	***
Methylfuran	M83	***			**	*	***
Cyclopentanone	M85	***		***	**	**	***
Hexanol	M87		***	***		**	***
Toluene	M93	**			**		***
Heptanal	M97	***		***	**	**	***
Unknown (M101)	M101		**	***	*	*	***
Hexenol	M103	**		***	**	*	***
Xylene	M107	***			*	**	***
p-Cymene	M135 + M136	***			**	**	***
Monoterpenes	M81 + M137	***			***		***
Ethyl-3-methylbutanoate	M138	**			**	**	***
Unknown (M147)	M147	*	***	***	**	**	***
Unknown (M149)	M149		***	***	**	**	***
Linalool	M155	*	***	***	**	**	***
Sesquiterpenes	M109 + M123 + M205	*	*	***	*	*	***

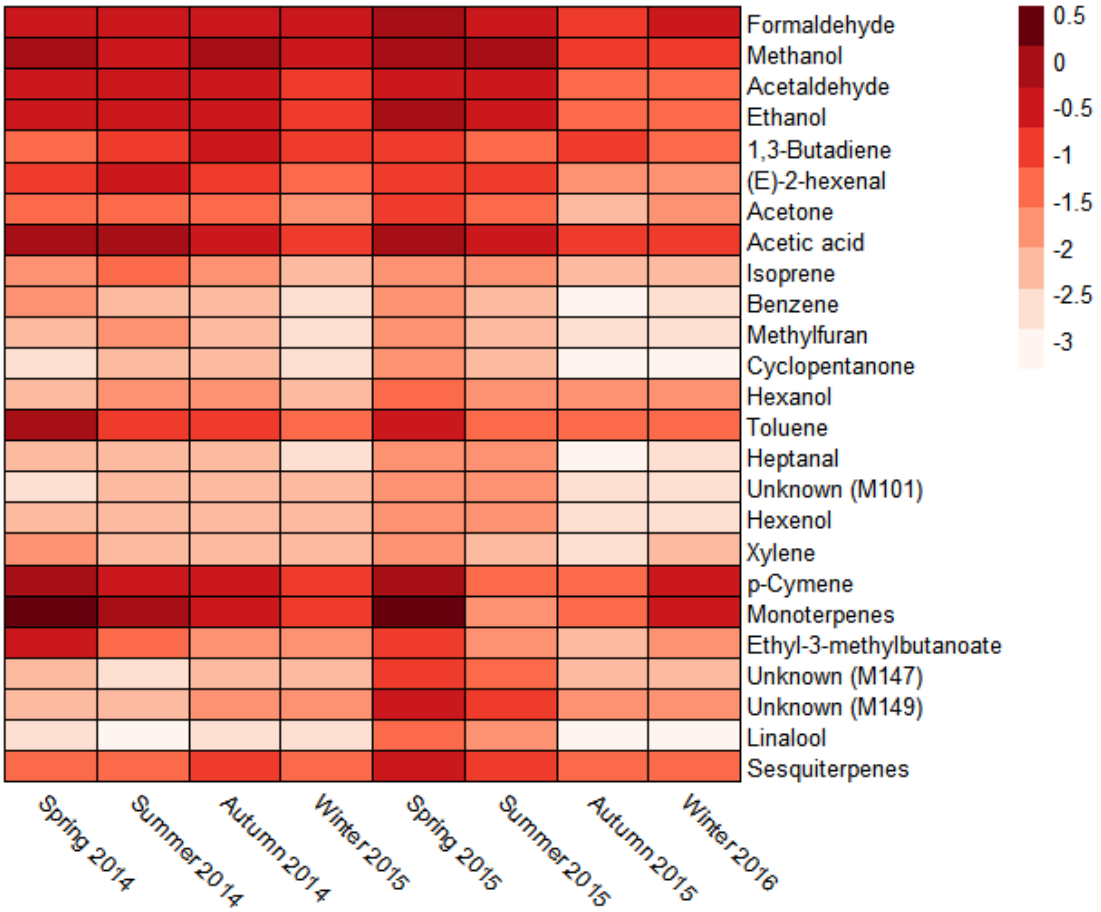
Table S3 Correction of R squares and significance test for the constrained axes (permutations = 999) in the RDA of parameters of shrubland and holm oak forest soils (• P<0.1, *P<0.05, **P<0.01, and ***P<0.001).

Ecosystem	R^2	Corrected R^2	Constrained axes	r^2	P
Shrubland	0.40	0.30	Soil temperature	0.234	**
			Soil water content	0.206	*
			Soil CO2 efflux	0.457	***
			C-degrading enzymes	0.384	***
			N-degrading enzymes	0.267	**
			P- degrading enzymes	0.187	*
Holm oak forest	0.59	0.47	Soil temperature	0.307	**
			Soil water content	0.315	**
			Soil CO_2 efflux	0.408	***
			C-degrading enzymes	0.254	*
			C-degrading enzymes/ N-degrading enzymes	0.282	**
			C-degrading enzymes/ P-degrading enzymes	0.209	*
			Net photosynthetic rate	0.155	•

Table S4 Linear correlation analysis between isoprenoid concentration and *A* of dominant species (*E. multiflora* in shrubland, *Q. ilex* in holm oak forest). +: positive; -: negative.

Isoprenoid		Shrubland	Holm oak forest
Isoprene	*r*	0.0948	0.2212
	P	0.5125	0.1819
	Correlation	+	+
Monoterpenes	*r*	0.0295	**0.6442**
	P	0.8404	**<0.0001**
	Correlation	-	**+**
p-Cymene	*r*	0.0433	**0.4924**
	P	0.7677	**0.0017**
	Correlation	+	**+**
Linalool	*r*	0.0430	**0.4644**
	P	0.7600	**0.0033**
	Correlation	-	**+**
Sesquiterpenes	*r*	0.2919	**0.4181**
	P	0.0358	**0.0090**
	Correlation	+	**+**

A)


Formaldehyde
Methanol
Acetaldehyde
Ethanol
1,3-Butadiene
(E)-2-hexenal
Acetone
Acetic acid
Isoprene
Benzene
Methylfuran
Cyclopentanone
Hexanol
Toluene
Heptanal
Unknown (M101)
Hexenol
Xylene
p-Cymene
Monoterpenes
Ethyl-3-methylbutanoate
Unknown (M147)
Unknown (M149)
Linalool
Sesquiterpenes
Spring 2014
Summer 2014
Autumn 2014
Winter 2015
Spring 2015
Summer 2015
Autumn 2015
Winter 2016
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3

B)

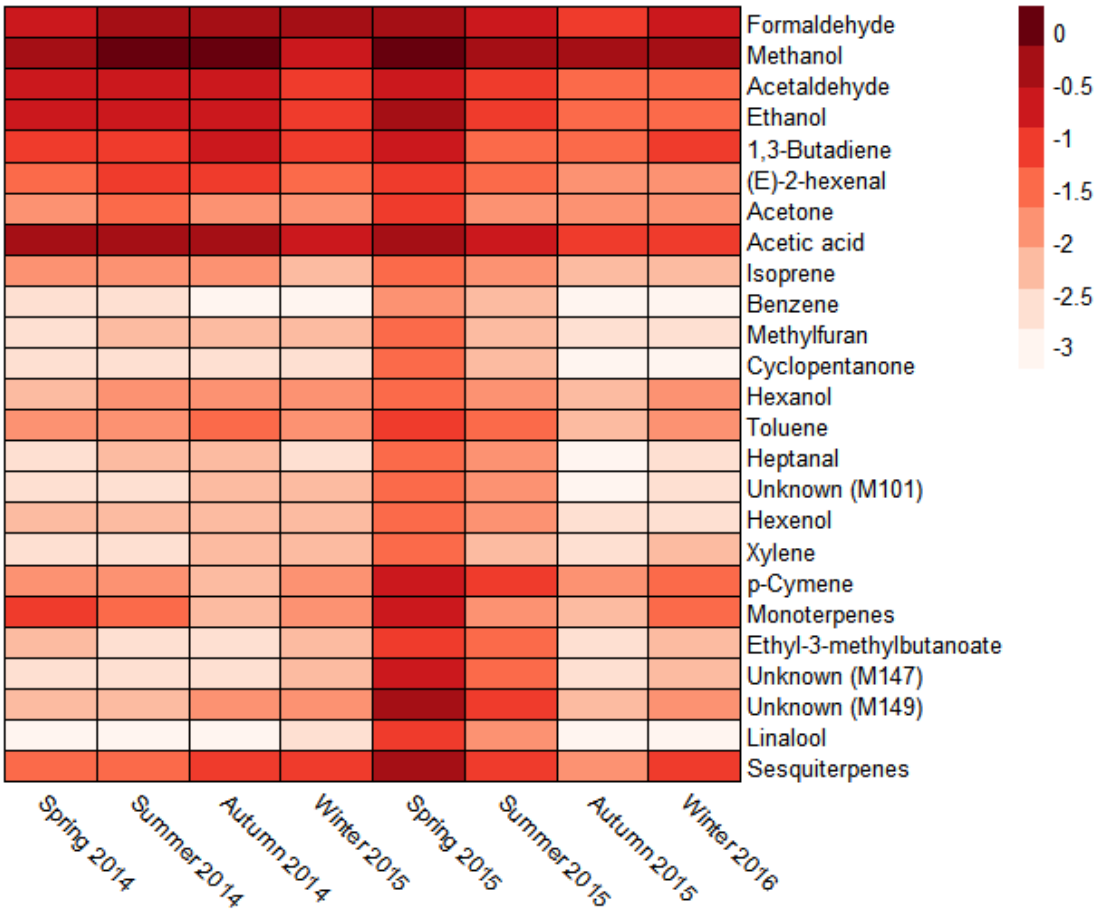


Fig. S1. Log10-transformed values of seasonal concentrations of detected volatile organic compounds (nmol mol^{-1}, ppb) in (A) shrubland (2014: n = 10–12; 2015 and 2016: n = 5–6) and (B) holm oak forest soils (2014: n = 5–6; 2015 and 2016: n = 4).

Chapter 4. Ground level isoprenoid exchanges associated with *Pinus pinea* trees in a Mediterranean turf

Table S1. Randomized sampling plan for the four studied trees during the morning. T1-T4 are first to fourth study trees, respectively.

Distance(m)	9h-10h	10h-11h	11h-12h	12h-13h
0	T4	T3	T2	T1
1	T1	T4	T3	T2
2	T2	T1	T4	T3
3	T3	T2	T1	T4
4	T4	T3	T2	T1
5	T3	T4	T1	T2
6	T2	T3	T4	T1
7	T1	T2	T3	T4
8	T4	T1	T2	T3
9	T1	T4	T3	T2
10	T2	T3	T4	T1

Table S2. The component of terpene emissions of *P. pinea* for green needle (a) from the study of Staudt et al. (2000) and dense litter zone (≤1 m) (b) from this study in summer mornings.

(a)

	Emission rates (pmol m^{-2} s^{-1})				Average proportion
Time of day	9:00	10:35	11:00	12:35	
Needle temperature	20.9	26.4	26.4	34.7	
α-Pinene	1.2	2.9	3.4	8.4	0.021
Myrcene	1.3	3.8	4.4	9.6	0.025
Limonene	15.6	31.4	43.4	47.1	0.180
1,8-Cineole	2	7.7	8	17.3	0.046
Trans-β-ocimene	18.3	104.2	108.3	205.3	0.572
Linalool	0.4	14.2	14.8	54.8	0.110
Other terpenes	1.7	8.1	8.4	17.1	0.046

(b)

	Emission rates (μg m^{-2} h^{-1})		Average proportion
Time of day	9:00-13:00		
Soil temperature	29.3	28.4	
α-Pinene	15.3	30.5	0.090
β-Pinene	5.9	7.7	0.027
Myrcene	3.3	9.6	0.025
Limonene	66.2	175.7	0.477
β-, γ-Terpinene with sabinene	39.9	131	0.337
Other terpenes	3.6	11	0.044

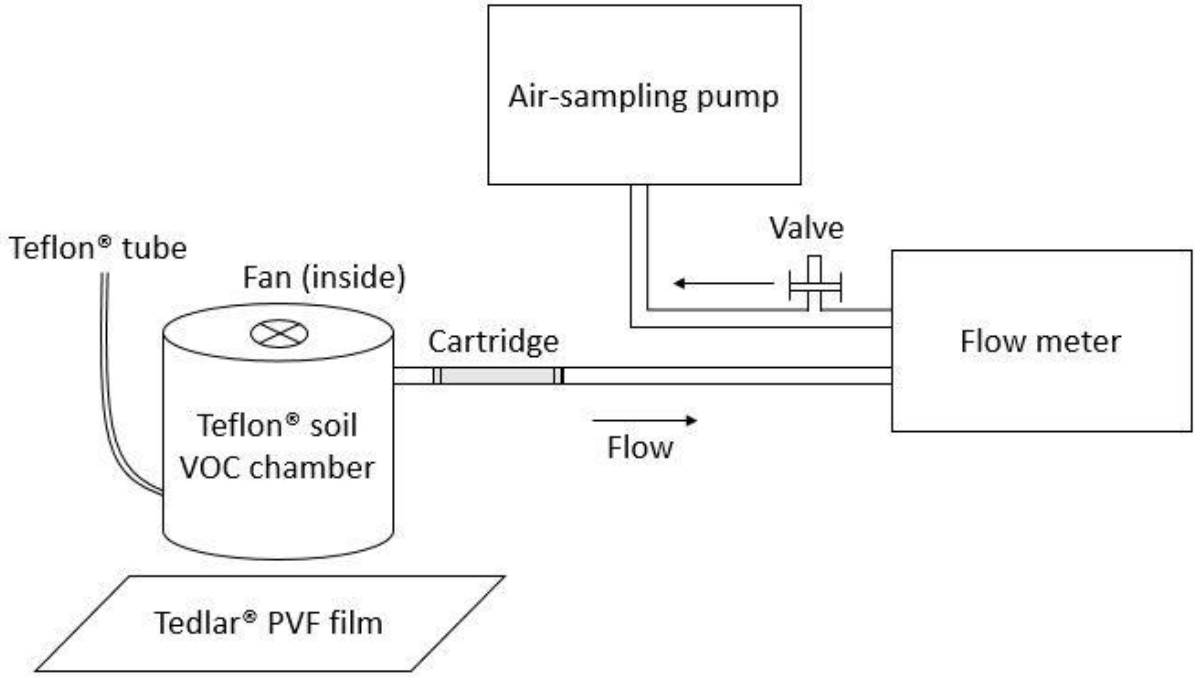


Figure S1. Schematic of the isoprenoid sampling.

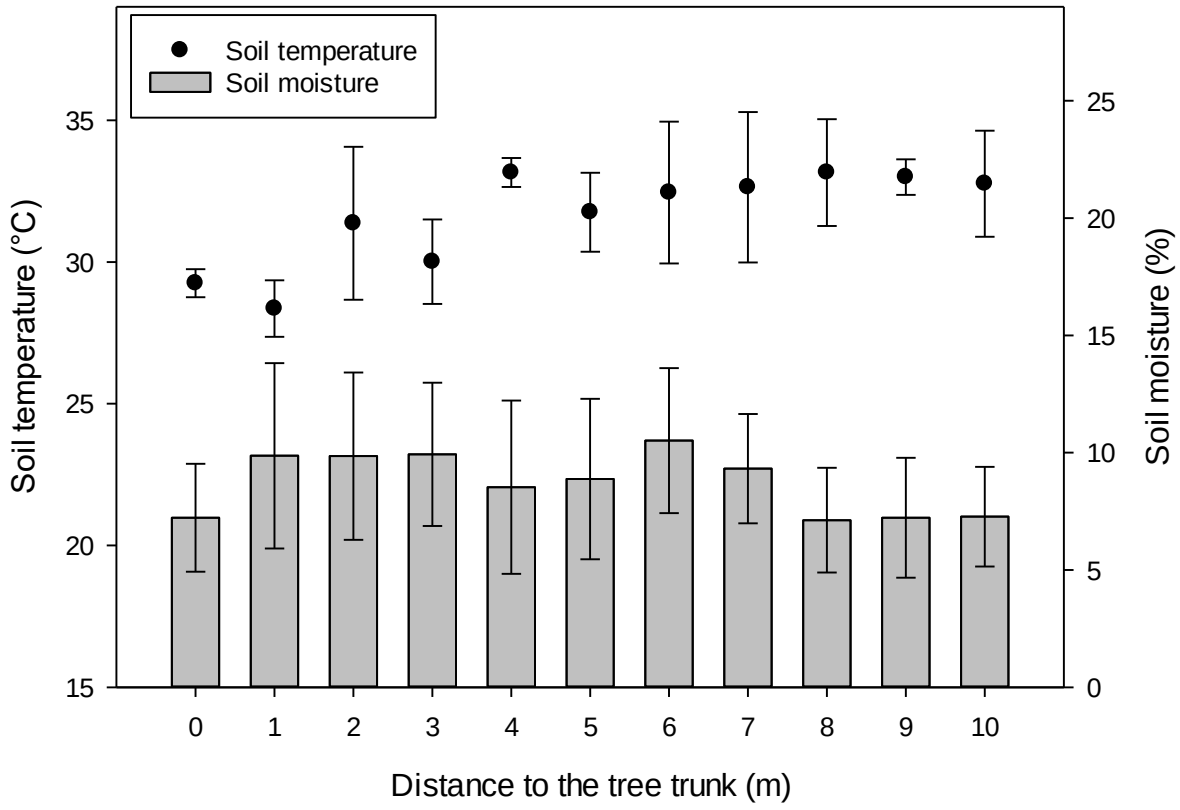


Figure S2. Mean soil temperature and moisture content along the transects. Data are means ±SE; n = 4.

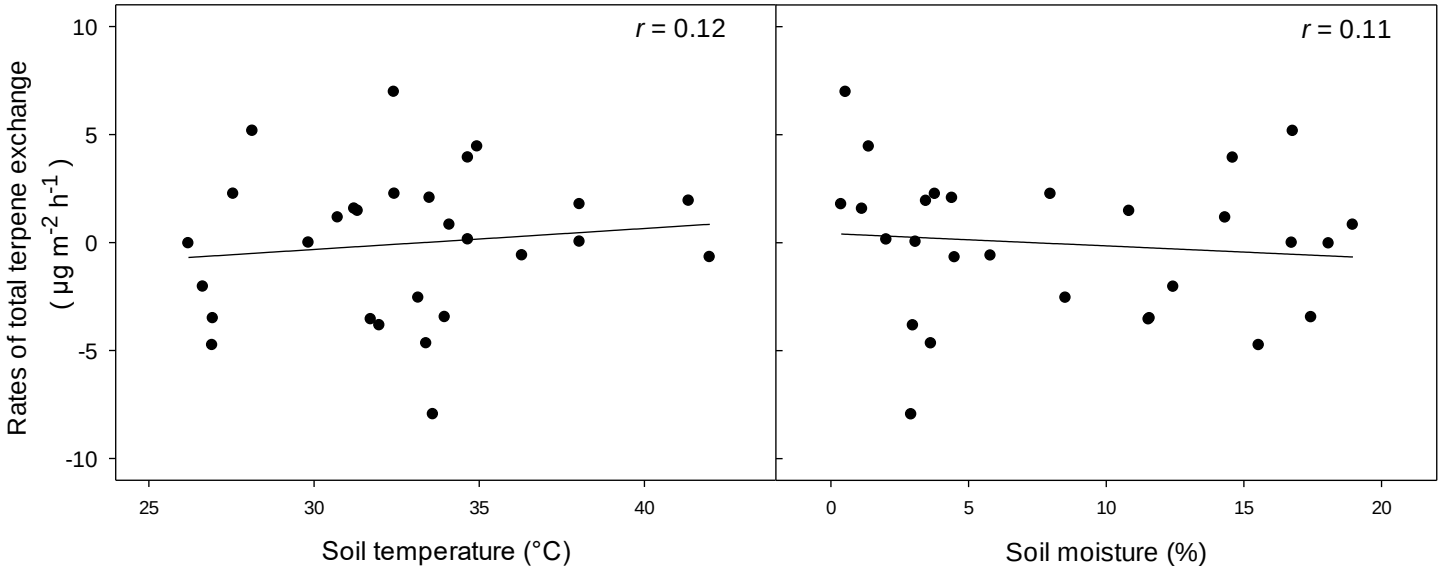


Figure S3. Relationships for the rates of total terpene exchange with soil temperature and soil moisture along the transects in turf zone (≥4 m).

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24th in February, 2021

Zhaobin Mu