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\textsuperscript{−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 (gs) 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.

1. 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\textsuperscript{−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 (Guenter 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 > 80% of the total emission of BVOCs (Guenter 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 Llusia, 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 NOx 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), whereas 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 Gruulke, 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 Llusia, 2003). Low levels of N addition, however, can increase terrestrial plant productivity (Vitousek and Howarth, 1991; Pipovarov et al., 2016), and high levels exceeding critical loads may decrease productivity and render plants more susceptible to environmental stresses (Cardoso-Villena 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 Gruulke, 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 CO2 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 (Robbink et al., 2016; Alvarez-Clare et al., 2013) because of differences in resource availability, acquisition and strategies of use (Vourlitis and Pasquini, 2009; Pipovarov 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; Pipovarov 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; Llusia et al., 2011, 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 Llusia, 2001; IPCC, 2014). The important interaction between the emission of phylogenetic 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 (Llusia 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 (Valolahi 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 (Llusia et al., 2014; Yuan et al., 2017), and the results have been inconsistent (Blanch et al., 2007; Peñuelas and Staadt, 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), Llusia et al. (2014) reported that the emission of terpenes decreased in two Mediterranean leguminous species (Ornthopus 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 Staadt, 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 Staadt, 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 (gs) and rate of isoprenoid emissions in these two dominant species at two study sites, a Garraf shrubland and a Prades forest (Llusia et al., 2011, 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 (Fig. 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 (Bolós and Vigo, 1990; Llusíà 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 (Bolós and Vigo, 1990; Llusíà 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, 2 as N and two as control plots. The N treatments were fertilized with 60 kg N ha⁻¹ using NH₄NO₃ (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 gs were measured and isoprenoid emissions were collected simultaneously using a Licor-6400XT gas-exchange system (LI-COR, Lincoln, Nebraska, USA). A and gs were measured at a photosynthetic quantum flux density (PPFD) of 1000 ± 20 μmol m⁻² s⁻¹ under a controlled CO₂ 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 cm². 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, sabine 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% PhMe 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 sabine were determined from calibration curves every five analyses using four terpene concentrations (r² > 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 winter 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 gs

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. gs was always highest in autumn and lowest at winter. A and gs differed significantly between the nitrogen and control treatments in winter, lower by 49.0% (P < 0.05) in mornings for gs and by 40.5% for A (P < 0.05) and 40.8% for gs (P < 0.01) at midday. gs was 41.0% (P < 0.05) lower in the N than the control treatments in summer mornings. An
increased sharply in spring relative to the pre-treatments after the first addition of 15 kg N ha\(^{-1}\). A noticeably decreased and gs increased compared to autumn in 2014 after the third addition of 15 kg N ha\(^{-1}\).

For Q. ilex was always highest in spring and lowest in summer (Fig. S3B). gs was always highest in spring or winter and lowest in summer. An 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 gs 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...
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.
mostly at summer middays in 2015, at rates of about 1.3 and 0.8 μg g⁻¹ dw h⁻¹, respectively.

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-Pinen and limonene were emitted mostly at summer middays in 2014, at rates of about 10 and 7 μg g⁻¹ dw h⁻¹, respectively.

4. Discussion

4.1. Seasonal and daytime variations of A, gs 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 gs highest in spring or autumn when environmental conditions are favorable (Llusia et al., 2013; Liu et al., 2016), with A and gs 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 (Llusia 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 and S3). Plants could temporarily decrease photosynthetic activity under drought stress because of the increased resistance to CO₂ 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) (Llusia 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 CO₂ 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 gs, 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. An 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 (Ninemets et al., 2016; Llusia 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; Llusia et al., 2011; Allen et al., 2015). BVOC emissions, however, are also largely influenced by A and gs, 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.
multi
flo
ra were correlated positively with air temperature and nega-
tively with soil moisture, except for \( \beta \)-myrcene, which had the opposite trend. \( \beta \)-Pinene had the same relationship with \( \beta \)-myrcene, and 3-
carene emission became strongly correlated with physiological
activities in the N plots. Trans-\( \beta \)-ocimene, sabinene and \( \alpha \)-car-
yophyllene emissions by Q. ilex were not obviously correlated with any
parameter, and other species followed a common trend with environ-
mental conditions. \( \alpha \)-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 gs 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.

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.
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 gᵣ for both species (Fig. S6), which supports the idea that BVOC emissions are mostly influenced by environmental factors (Gershenzon et al., 1978; Loreto and Schnitzler, 2010; Holopainen and Gershenzon, 2010; Llusíà et al., 2011). Q. ilex emitted more isoprenoids and A was lowest in summer than in the other seasons (Figs. 2 and S3B), and higher percentages of fixed carbon were devoted to isopreneoid 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 gᵣ (Figs. S3A and S6).

5. Conclusions

This study investigated the seasonal and daytime variations in the response of isoprenoid emissions to experimental N deposition in dominant species at Mediterranean ecosystems. 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 synergic 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 Llusíà, 2001; Peñuelas 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.

Declaration of competing interest

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

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Appendix A. Supplementary data

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