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 (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.

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

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^{-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 100000 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 >80% 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 (gs) 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.

194 Emissions from sunlit and healthy *E. multiflora* needle clusters and *Q. ilex* leaves were
195 measured from three random plants in each plot. Air temperature was measured by an
196 automatic meteorological station, and soil moisture was measured by time-domain
197 reflectometry (Delta-T Devices Ltd, Cambridge, England), both about every 30 min on
198 the day of sampling.

199

200 2.3. Gas-exchange measurements and sampling of isoprenoid emissions

201 A and g_s were measured and isoprenoid emissions were collected simultaneously using
202 a Licor-6400XT gas-exchange system (LI-COR, Lincoln, Nebraska USA). A and g_s were
203 measured at a photosynthetic quantum flux density (PPFD) of 1000 ± 20 μmol m^{-2} s^{-1}
204 under a controlled CO_2 concentration of 400 ± 2 ppm. One *E. multiflora* needle cluster or
205 one *Q. ilex* leaf was enclosed in a clip-on gas-exchange cuvette with a surface area of 2
206 cm^2. The emitted isoprenoids were pumped from the cuvette through a stainless-steel tube
207 (89 mm in length and 6.4 mm external diameter) manually filled with adsorbents (115 mg
208 of Tenax® TA and 230 mg of SulfiCarb®) separated by sorbent-retaining springs, fixed
209 using gauze-retaining springs and closed with air-tight caps (Markes International Inc.
210 Wilmington, USA). The flow across the tubes was measured with a Bios Defender 510
211 flow meter (Bios International Corporation, Butler, USA) and controlled at about 300 mL
212 min^{-1} with a metallic valve. The flow was generated using a Q-MAX air-sampling pump
213 (Supelco, Bellefonte, USA), and the sampling time was 10 min. The hydrophobic
214 properties of the activated adsorbents minimized any sample displacement by water,
215 without chemical transformation in the tube. Isoprenoid concentrations were determined
216 by reference to trapped standards (α-pinene, β-pinene, 3-carene, limonene, sabinene and
217 dodecane). The tubes were conditioned before isoprenoid sampling with a stream of 100
218 mL min^{-1} of purified helium at 350 °C for 35 min. The trapping and desorption
219 efficiencies of liquid and volatilized standards such as α-pinene, β-pinene or limonene
220 (the main terpenes accounting for about 65–90% of total emissions) were near 100%.
221 Blank samples of air without leaves in the cuvette were collected for 10 min immediately
222 before each measurement. The sampled parts of the leaves were cut to the perimeter
223 enclosed in the Licor-6400XT cuvette and stored in a portable cooler at 4 °C, taken to the
224 laboratory and oven-dried at 60 °C to constant weights. The metallic tubes (with trapped
225 BVOCs) were stored at 4 °C until analysis.

226

227 2.4. Isoprenoid analyses

228 The isoprenoids were analyzed using a GC-MS system (HP59822B, Hewlett Packard,
229 Palo Alto, USA) with an automatic sample processor (Combi PAL, FOCUS-ATAS GL
230 International BV 5500 AA Veldhoven, The Netherlands). The desorber was an OPTIC3
231 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands), and
232 samples were applied to a 30 m × 0.25 mm × 0.25 μm film capillary column (HP-5,
233 Crosslinked 5% pH Me Silicone; Supelco, Bellefonte, USA). A detailed description of
234 the chromatographic method is provided by Mu et al. (2018).

235 The terpenes were identified by comparing their retention times with those of standards
236 from Fluka (Buchs, Switzerland), published spectra, GCD ChemStation G1074A HP and
237 the wiley7n mass-spectra library. The concentrations of common terpenes such as α-
238 pinene, 3-carene, β-pinene, myrcene, limonene, and sabinene were determined from
239 calibration curves every five analyses using four terpene concentrations ($r^2 > 0.99$ for the
240 relationships between the signal and terpene concentrations). The most abundant terpenes
241 had very similar sensitivities, with differences <5% among the calibration factors.

242

243 2.5. Statistical analyses

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

254

255 3. Results

256 3.1. Seasonal and daytime variation of air temperature and soil moisture

257 Annual air temperature and soil moisture were similar between the control and N
258 treatments, with variation <10% for both sites. Mean air temperature on the sampling
259 dates in Garraf ranged between 10.1 ± 0.84 °C in winter mornings in 2015 and 34.4 ±
260 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.

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.

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).

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).

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 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.

Declare on interest

All authors declare that there are no conflicts of interest relevant to this work.

Acknowledgements

This work was financially supported by the Spanish Government project CGL2016-79835-P, the Catalan government project SGR2017-1005 and the ERC Synergy project SyG-2013-610028 IMBALANCE-P. We are also grateful for the financial support from the China Scholarship Council.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

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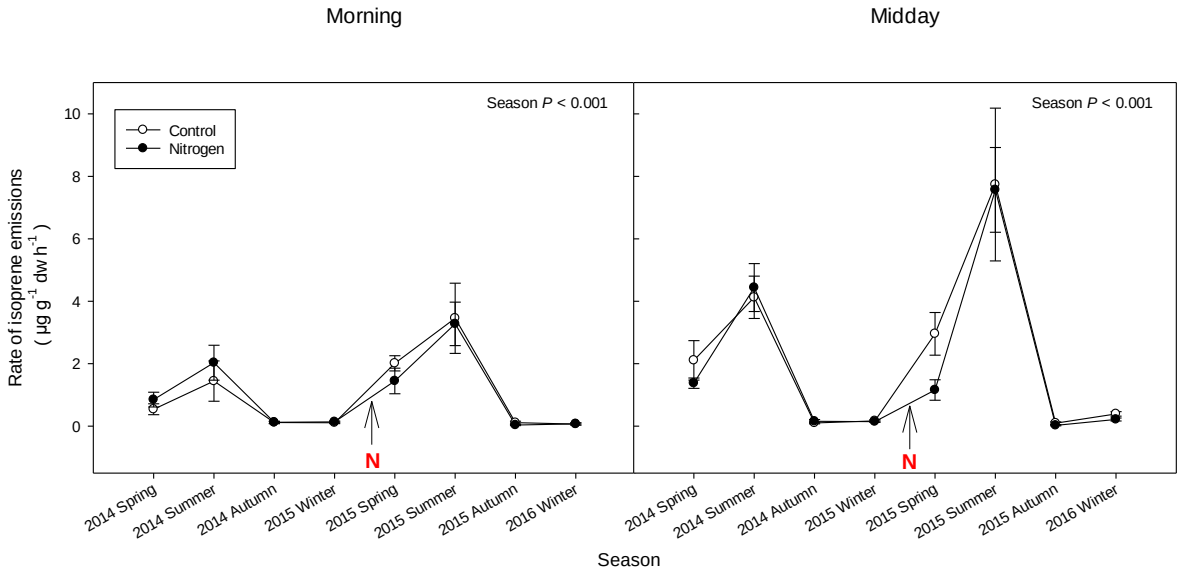
A)



B)

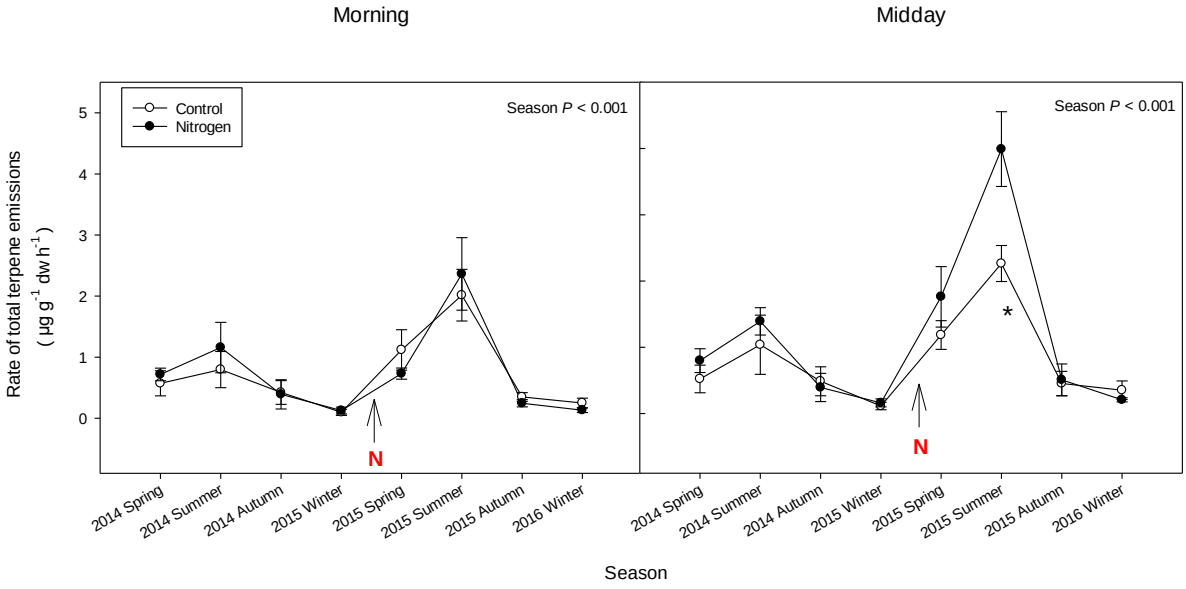
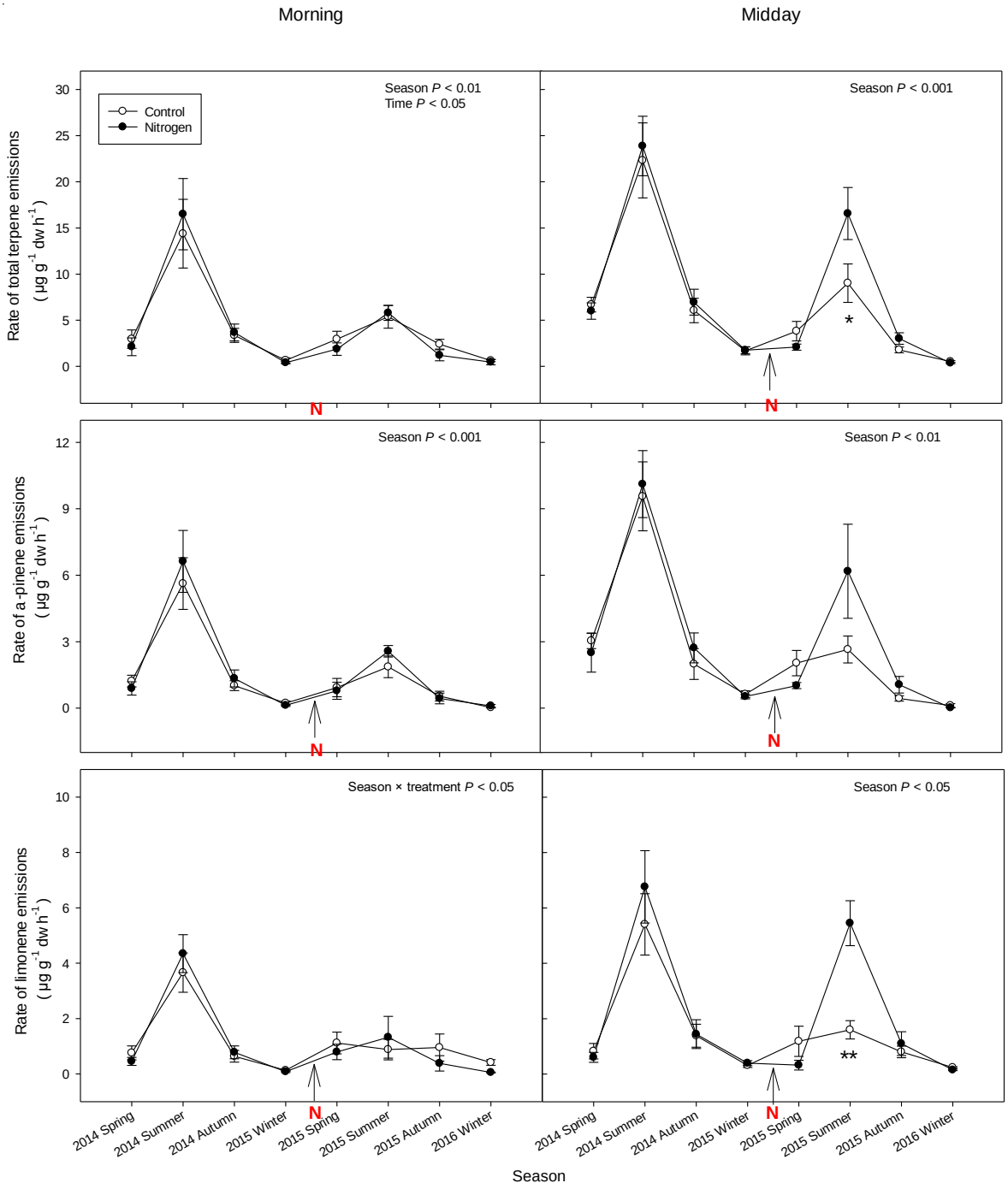


Fig. 1. Seasonal variation of the rates of emission of isoprene (A) and total terpenes (B) for *Erica multiflora*. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means ($n = 6$). Significant differences between treatments identified by Student's *t*-tests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



745

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

750

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752

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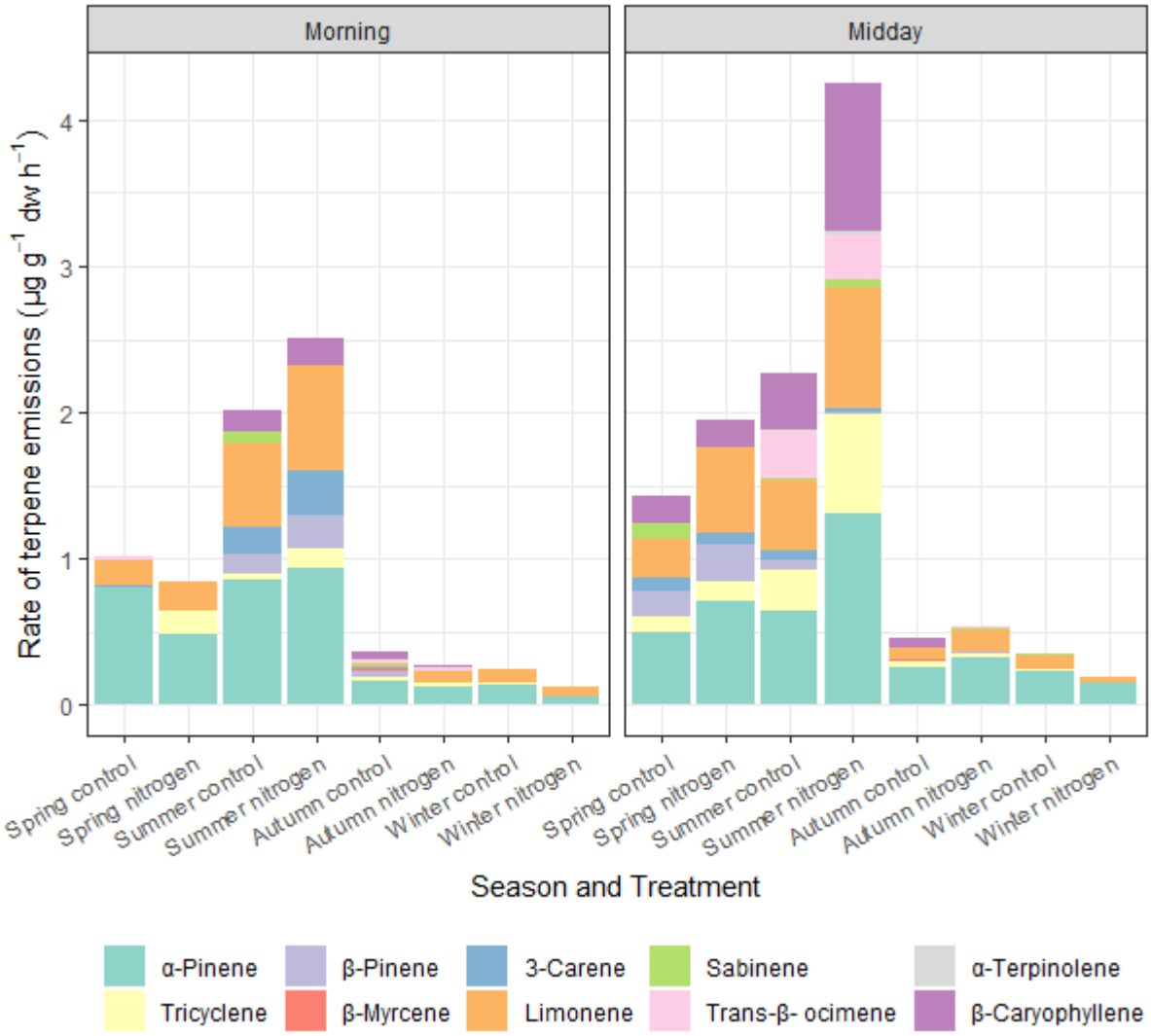
A)


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)

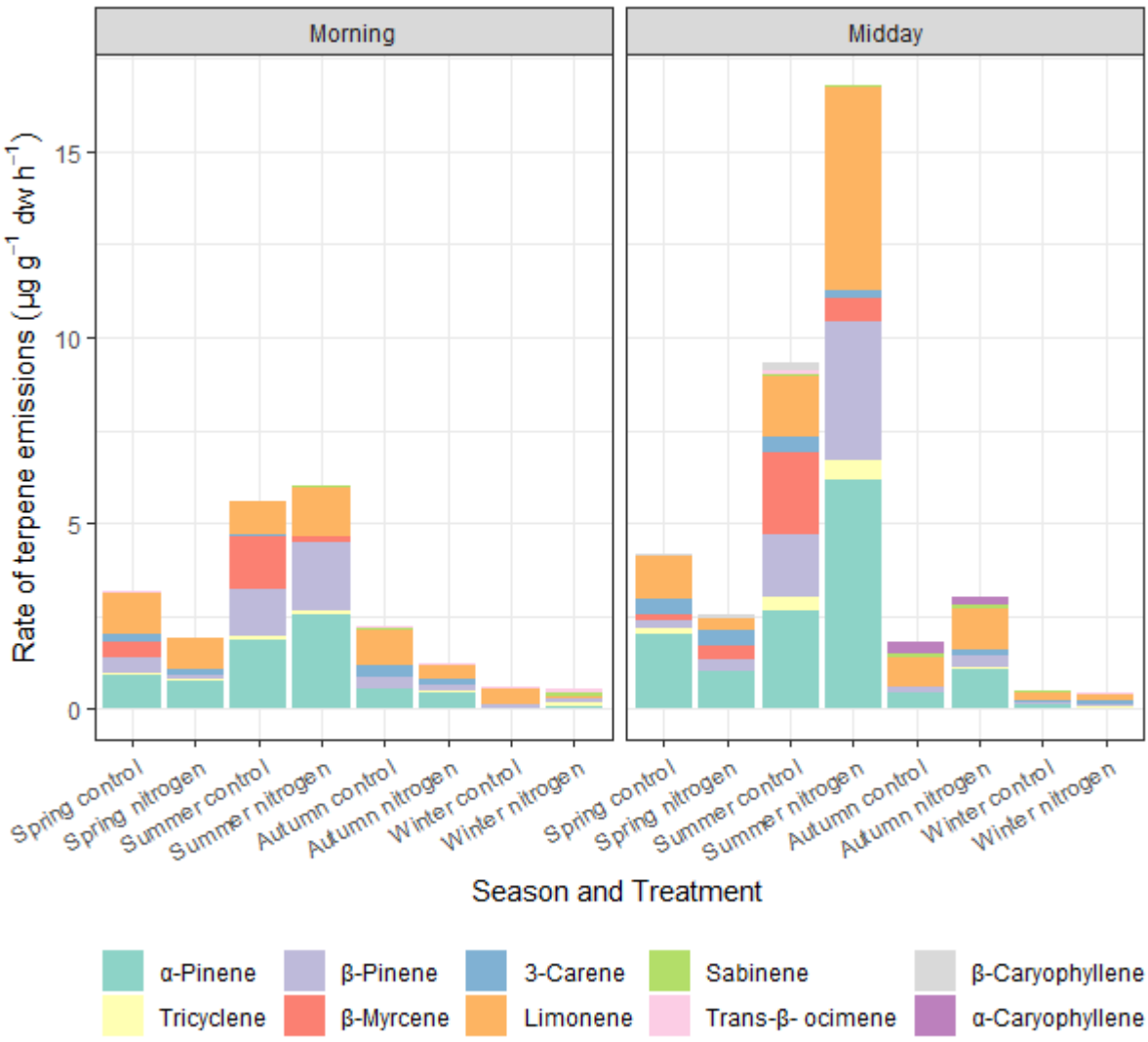
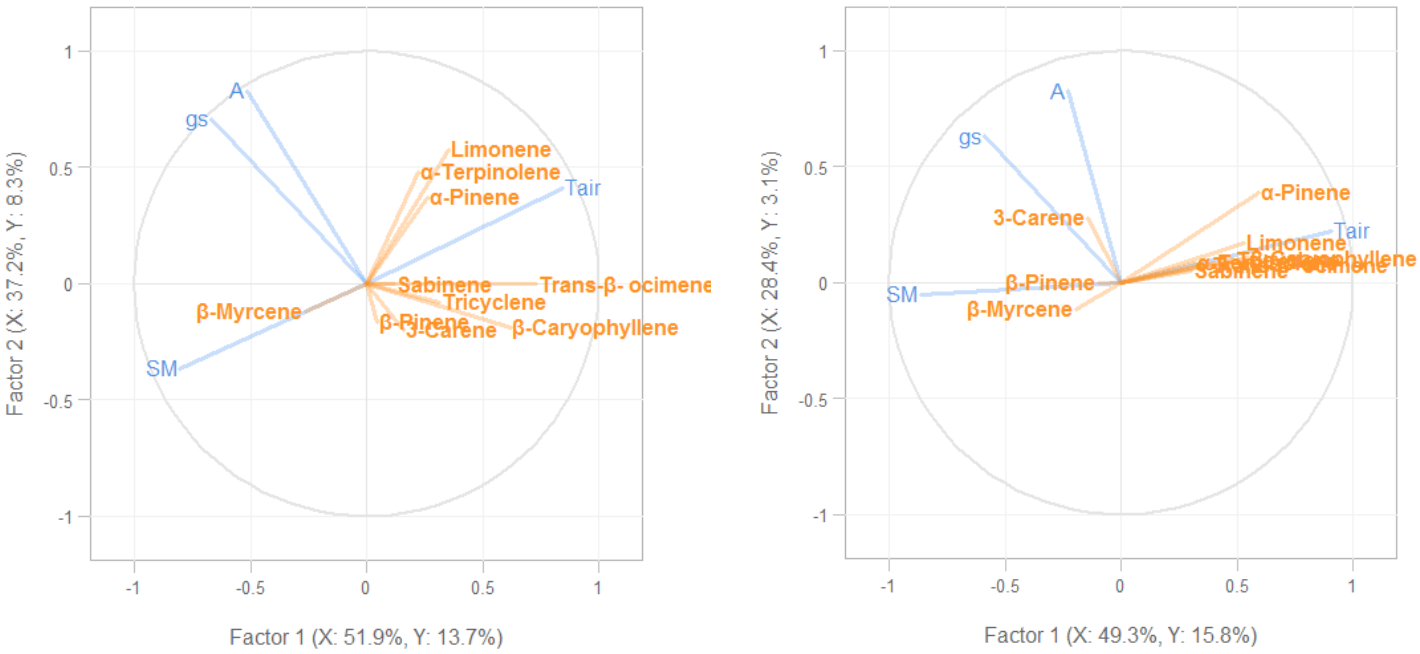


Fig. 3. Distribution of seasonal terpene emissions for *Erica multiflora* (A) and *Quercus ilex* (B) in the morning and at midday for the fertilized year.

Erica multiflora

A) Control B) Nitrogen



Quercus ilex

C) Control D) Nitrogen

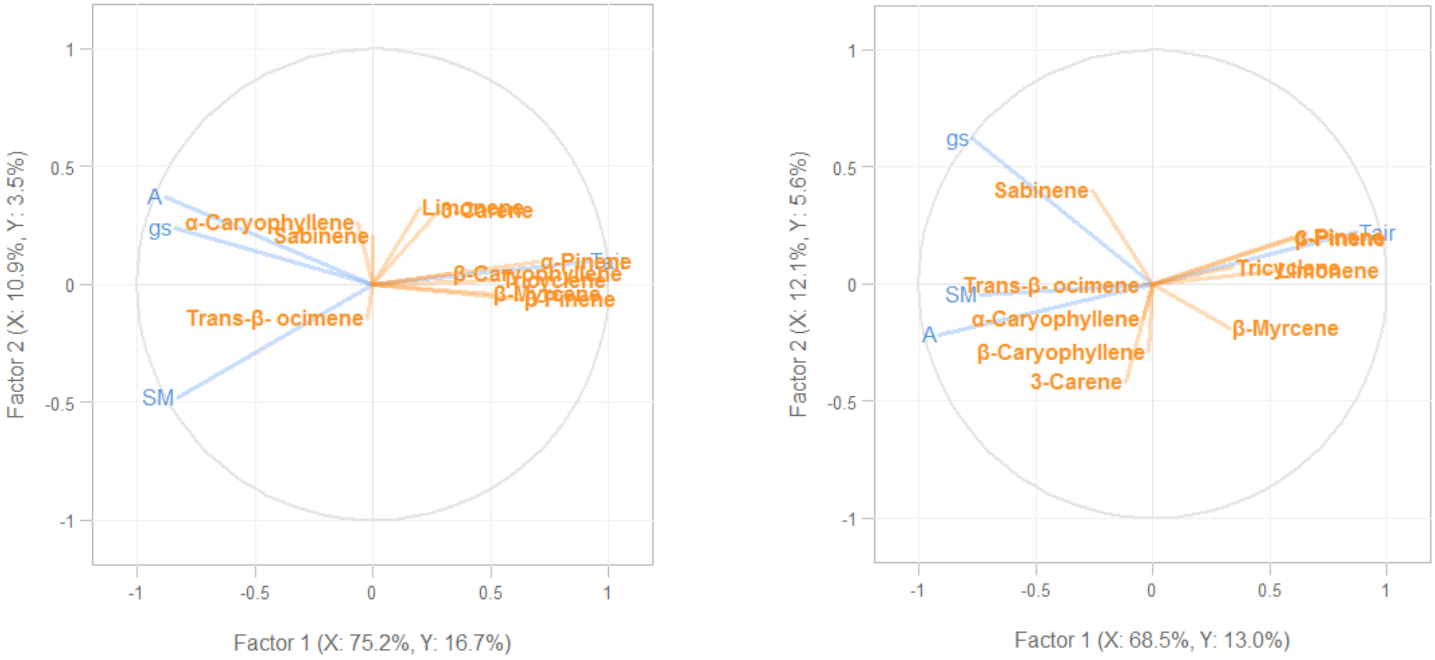


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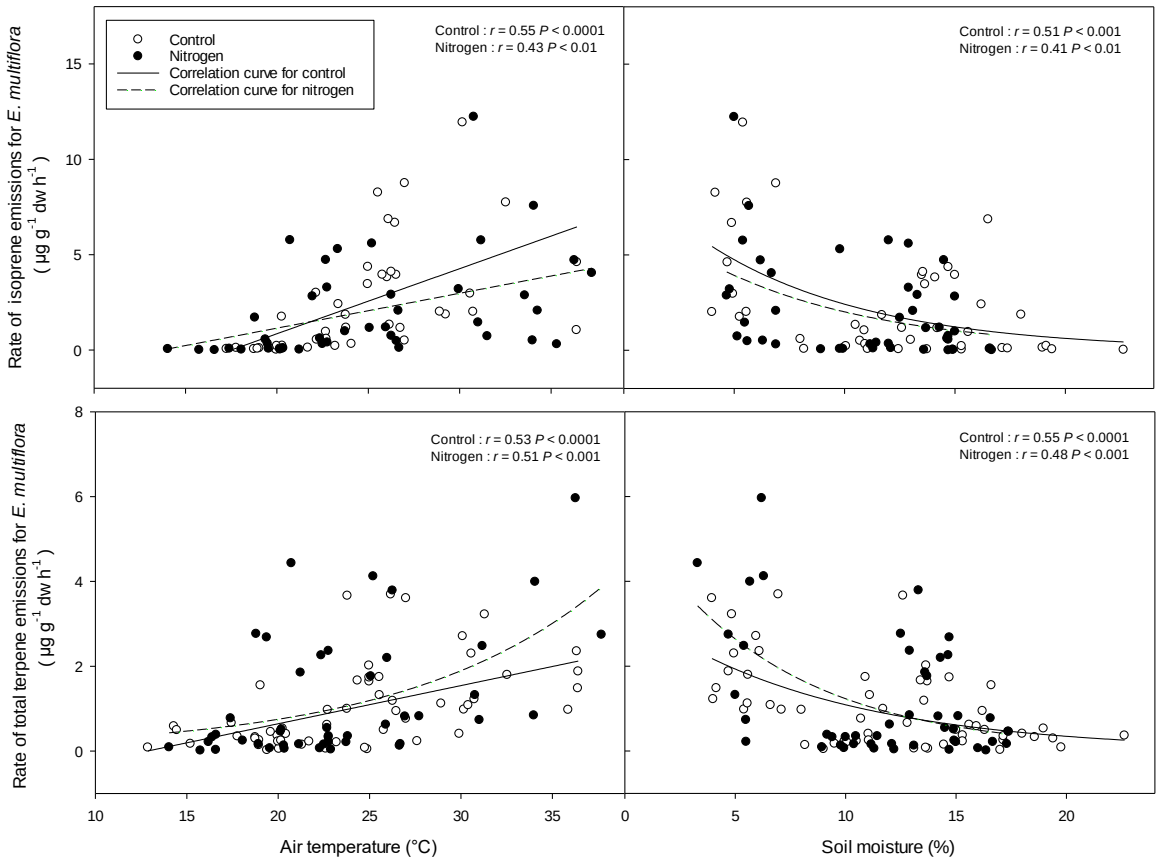
Fig. 4. Partial least squares (PLS) regression between main environmental or physiological parameters and terpene emissions for *Erica multiflora* (A for control treatments, B for nitrogen treatments) and *Quercus ilex* (C for control treatments, D for nitrogen treatments) in the fertilized year. Blue 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.

A)


Control
Nitrogen
Correlation curve for control
Correlation curve for nitrogen
Control : r = 0.55 P < 0.0001
Nitrogen : r = 0.43 P < 0.01
Control : r = 0.51 P < 0.001
Nitrogen : r = 0.41 P < 0.01
Control : r = 0.53 P < 0.0001
Nitrogen : r = 0.51 P < 0.001
Control : r = 0.55 P < 0.0001
Nitrogen : r = 0.48 P < 0.001
Rate of isoprene emissions for E. multiflora (µg g-1 dw h-1)
Rate of total terpene emissions for E. multiflora (µg g-1 dw h-1)
Air temperature (°C)
Soil moisture (%)

B)

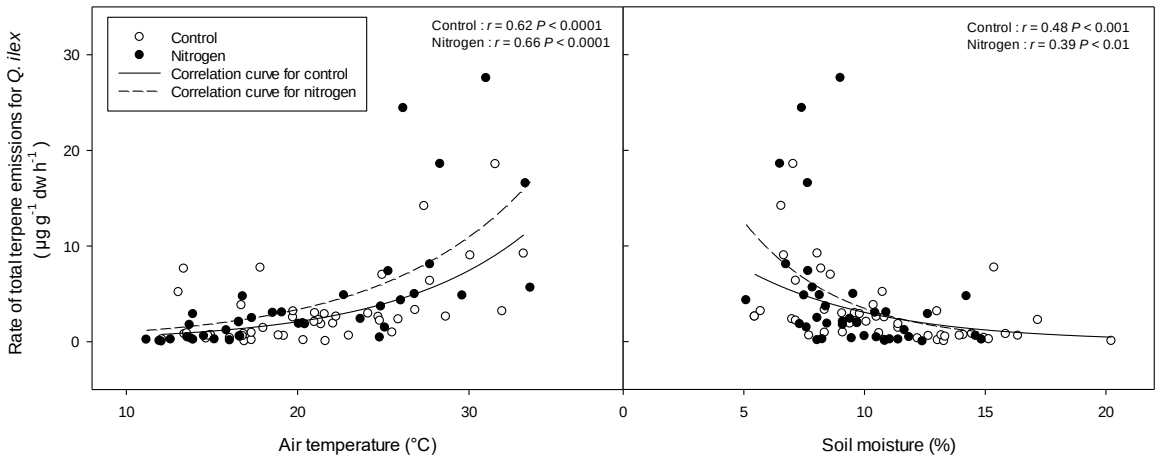


Fig. 5. Relationships for the rate of isoprenoid emissions with main environmental conditions (air temperature and soil moisture) for *Erica multiflora* (A) and *Quercus ilex* (B) in the fertilized year.

Supporting Information

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

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Tables

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**	

Figures

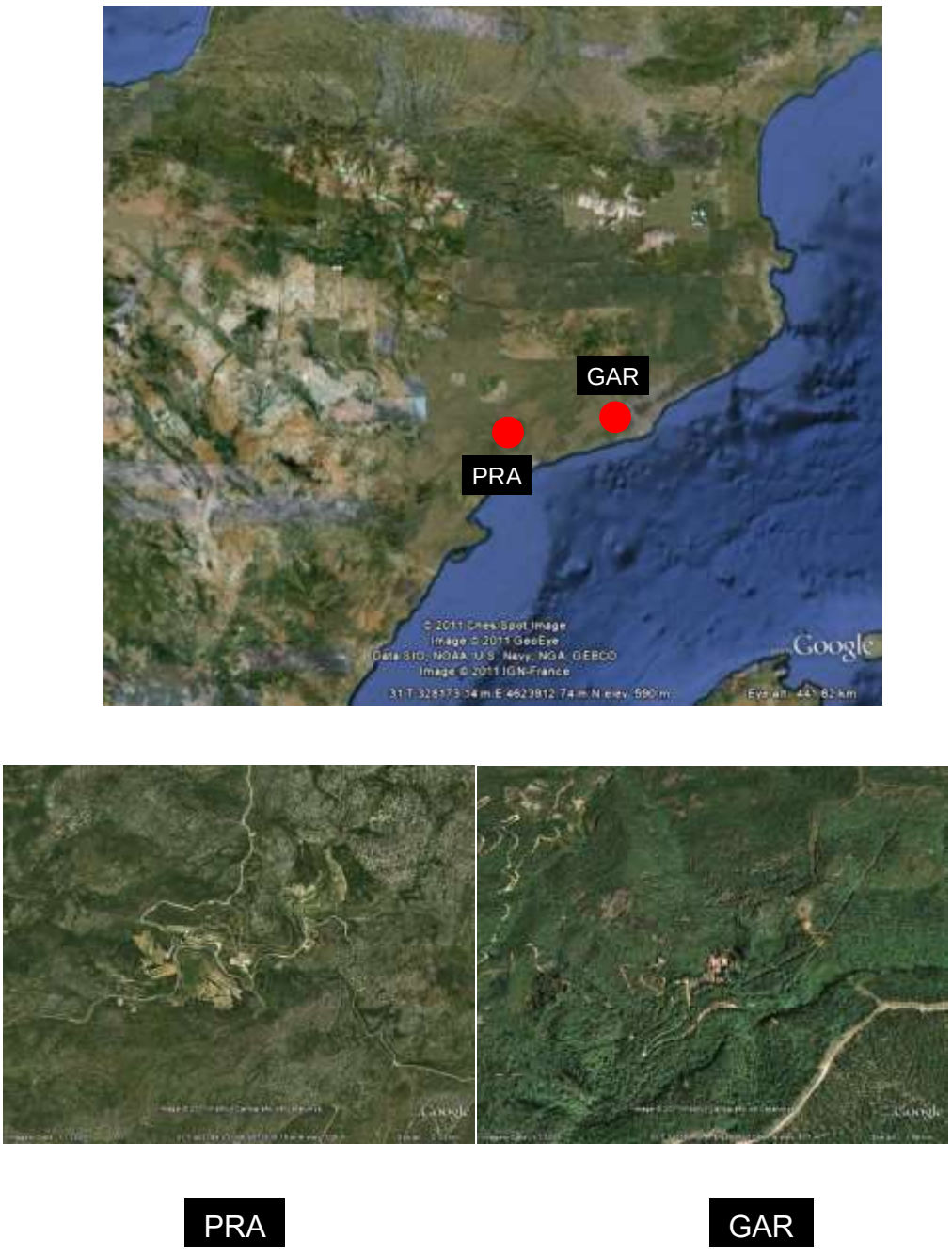


Fig. S1. Location and landform of experimental sites. GAR, Garraf; PRA, Prades.

Garraf (*Erica multiflora*) Prades (*Quercus ilex*)

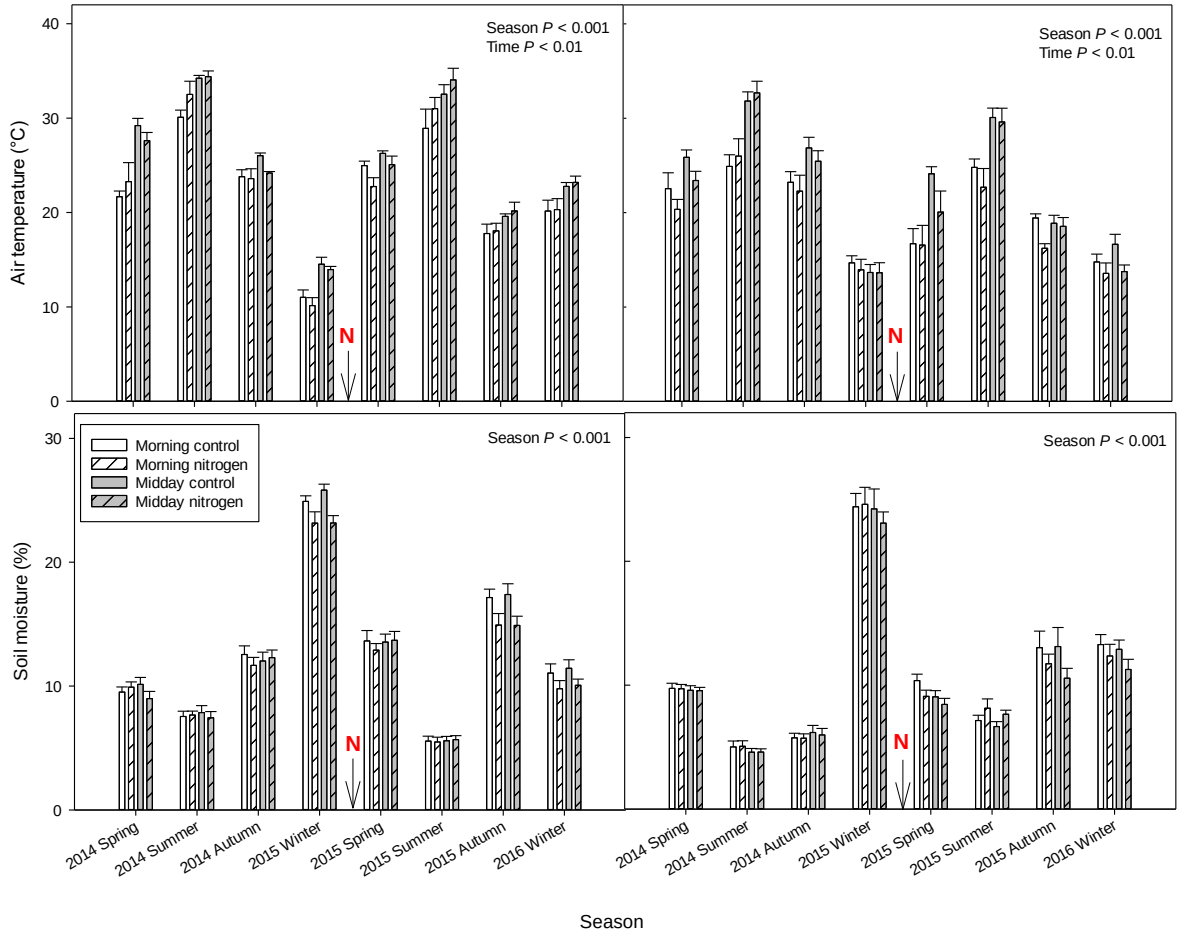
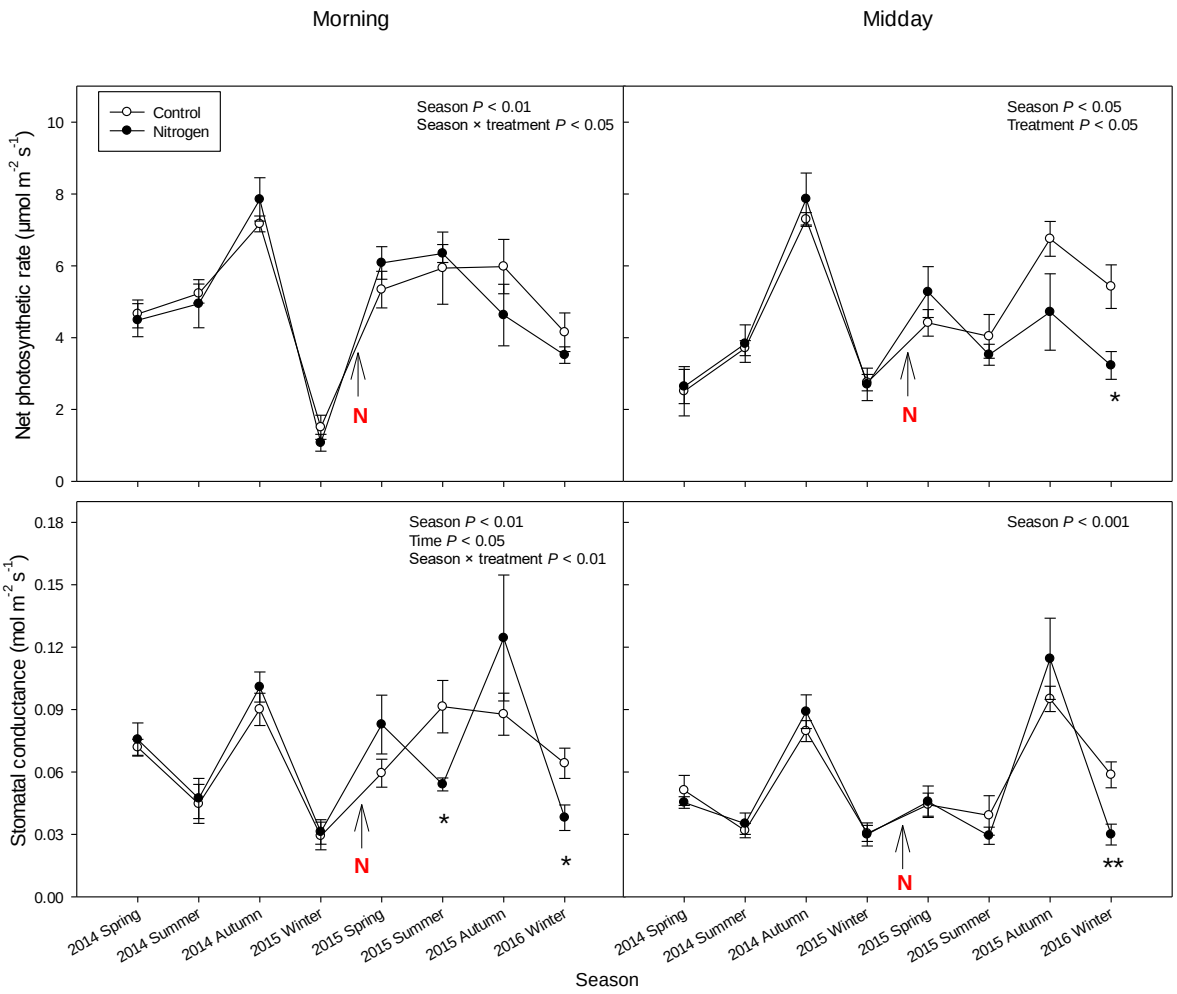


Fig. S2. Seasonal morning and midday variation of mean air temperature and soil moisture in Garraf and Prades. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means (n = 6). The significance of the effects of season and sampling time (repeated-measures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

A) *Erica multiflora*



B) *Quercus ilex*

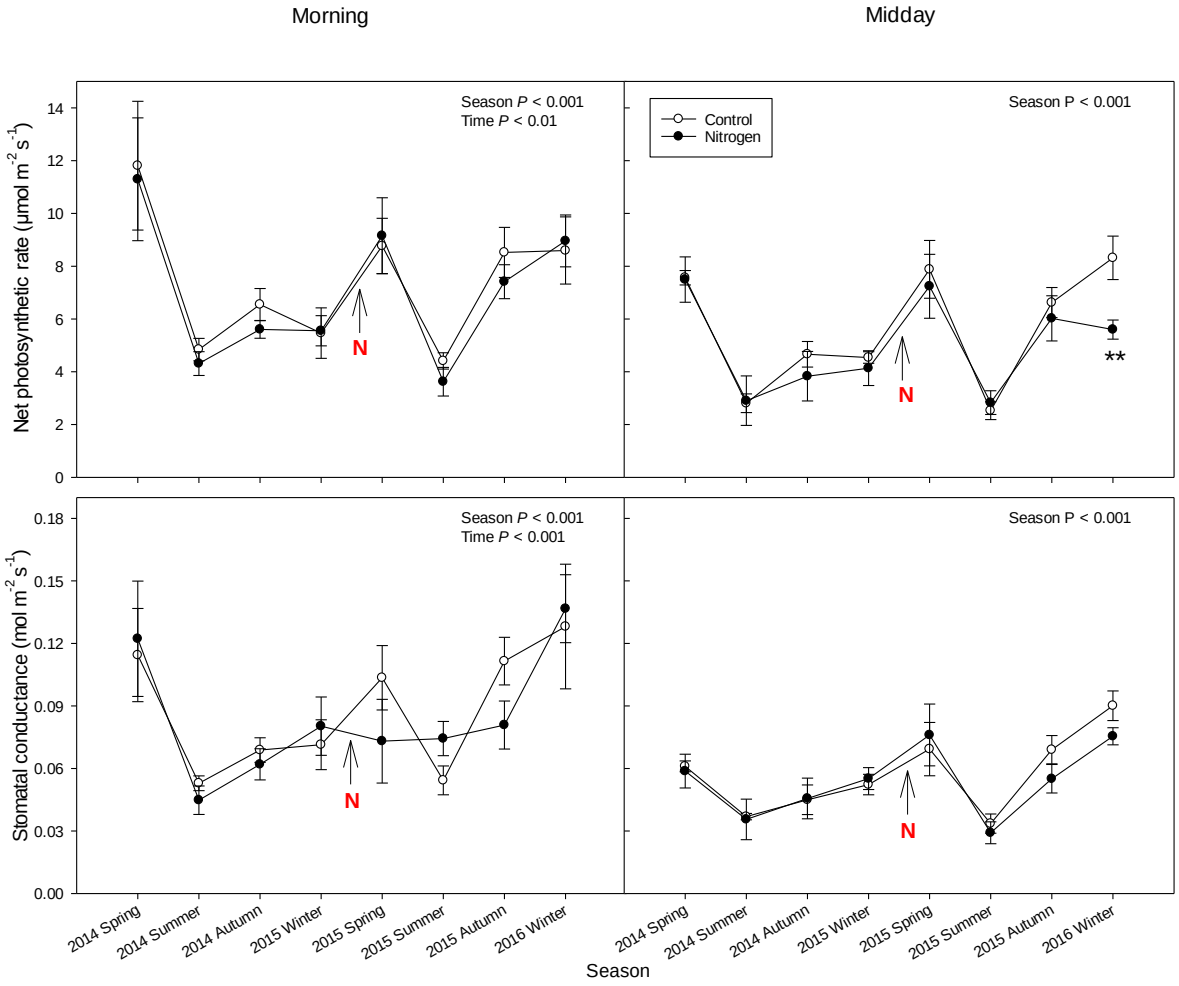


Fig. S3. Seasonal net photosynthetic rates and stomatal conductances for *Erica multiflora* (A) and *Quercus ilex* (B) in the morning and at midday. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means ($n = 6$). Significant differences between treatments 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.

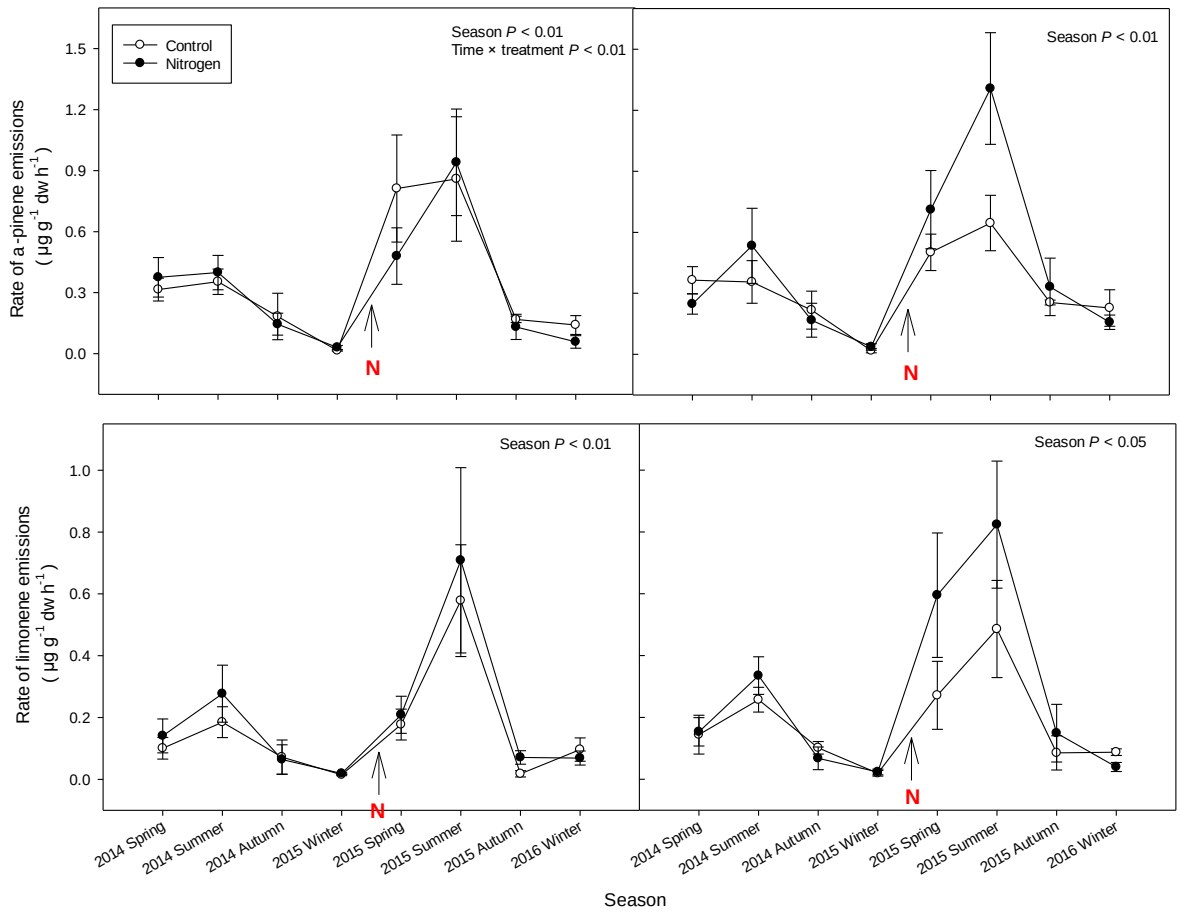


Fig. S4. Seasonal variation of the rates of emission of α-pinene and limonene for *Erica multiflora*. 'N' indicates the start of the fertilization treatment. Error bars indicate standard errors of the means (n = 6). The effects of season, treatment and sampling time are depicted in the panels when significant.

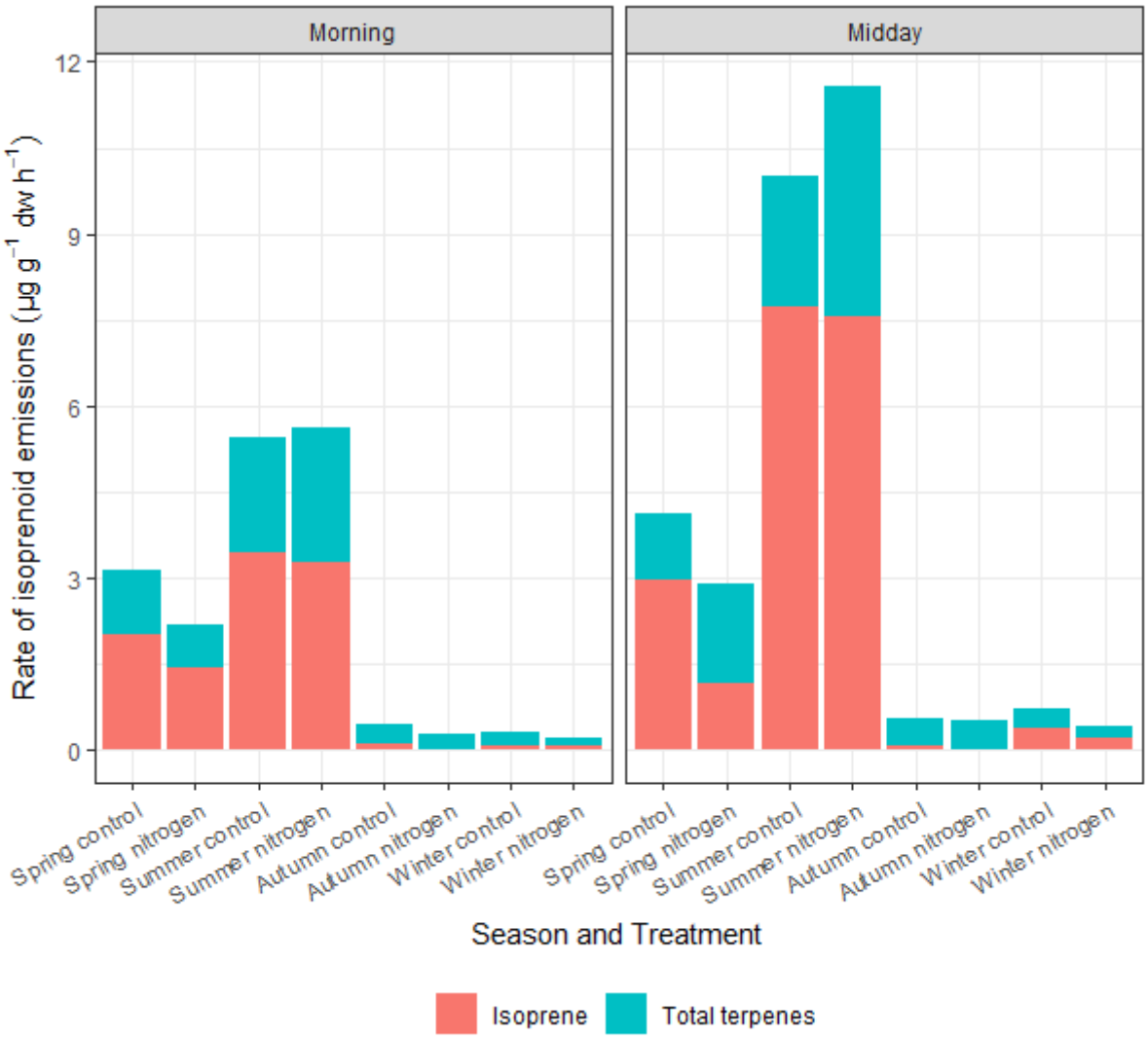
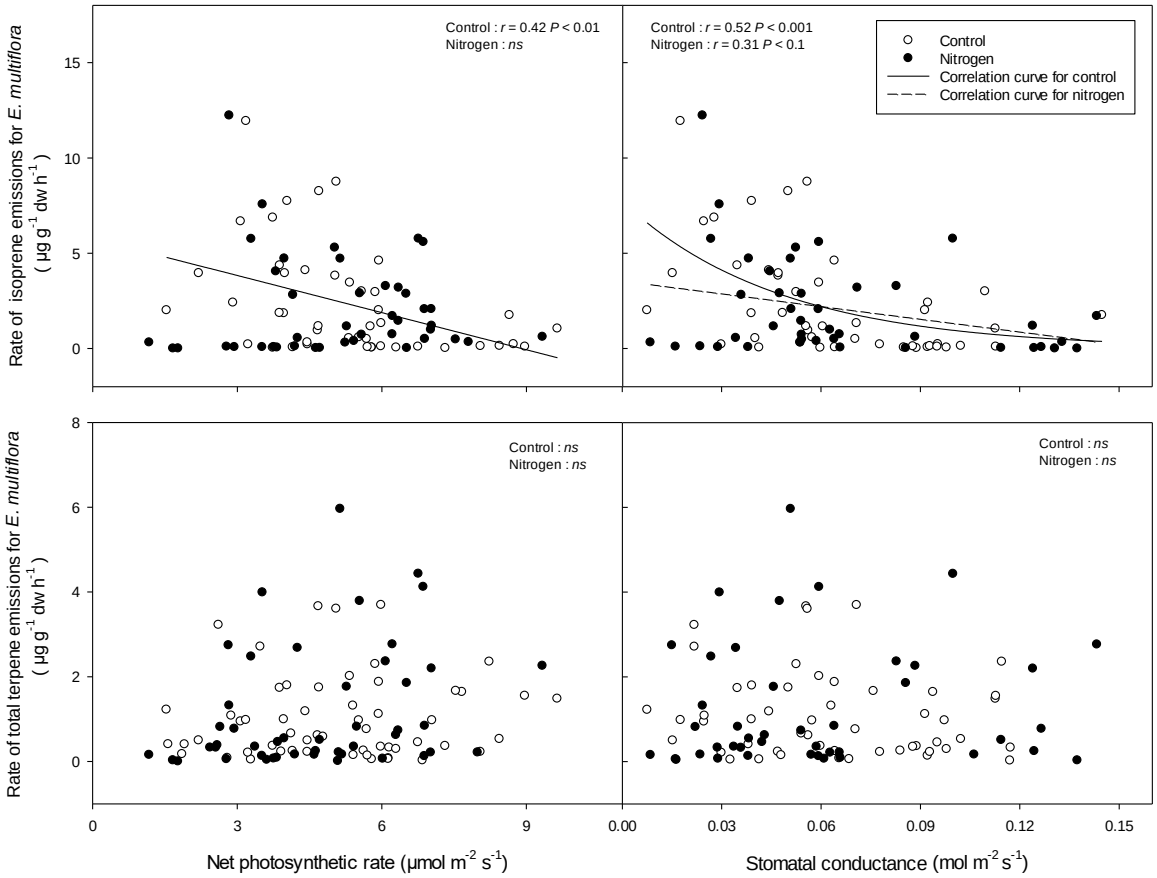


Fig. S5. Distribution of seasonal isoprenoid emissions for *Erica multiflora* in the morning and at midday for the fertilized year.

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)

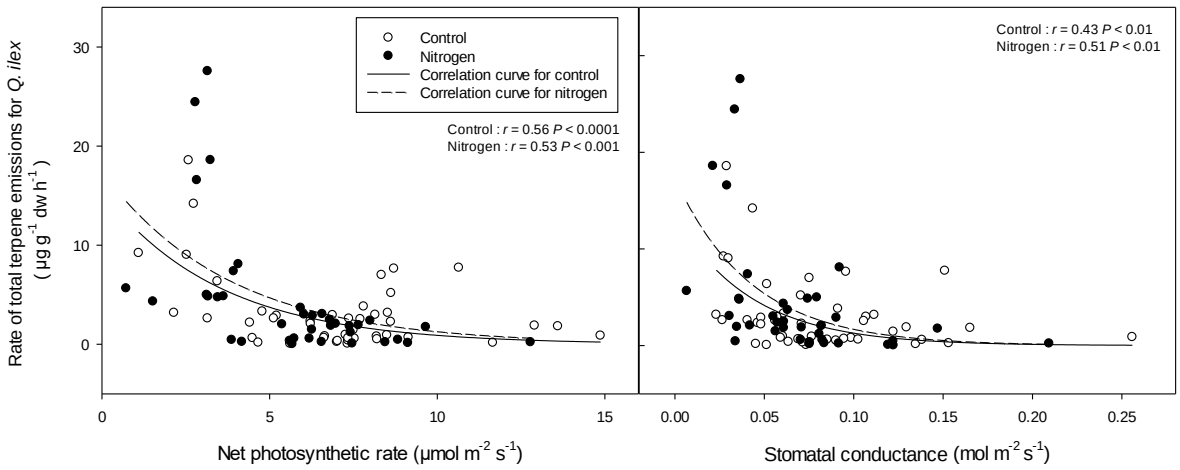


Fig. S6. Relationships for the rate of isoprenoid emissions with main physiological activities (net photosynthetic rate and stomatal conductance) for *Erica multiflora* (A) and *Quercus ilex* (B) in the fertilized year. ns, not significant.