Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland

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Summary

Information about soil VOC inventories and exchange rates in different soils is very scarce. Seasonality of soil VOC exchange rates is also largely unknown, despite the increasing interest in some soil volatile compounds, such as monoterpenes, because of their important role in soil ecology. We aimed to explore and quantify soil VOC exchange rates in a Mediterranean shrubland and their seasonality. Measurements of soil VOC exchange were taken using GC-MS and PTR-MS techniques, together with soil temperature, soil moisture and soil CO₂ efflux measurements, during two annual campaigns with contrasting precipitation. Methanol, acetic acid, ethyl acetate, acetaldehyde, acetone, C₃ and C₄ carbonyls (such as methyl ethyl ketone), α-pinene and limonene, showed the highest emission rates. Maximum soil monoterpene emission rates were very low (0.003 nmol m⁻² s⁻¹) compared with foliar monoterpene emission rates. The emission rates of the other VOCs were also low (maximum 0.8 nmol m⁻² s⁻¹) except for methanol (1.2 nmol m⁻² s⁻¹). Maximum soil uptake rates for some VOCs, such as methanol and acetonitrile (ranging from 0.1 to 0.5 nmol m⁻² s⁻¹) were, however, comparable with foliar uptake rates. Further studies are needed to corroborate these results and the possible importance of the soil VOC sink in regional chemistry-climate models. Long-term severe drought increased soil monoterpene emission rates in this Mediterranean shrubland. The increases seem to be linked to changes in the soil’s physical properties induced by low soil moisture. Unlike monoterpenes, other soil VOC emission rates decreased when soil moisture was low. The results suggest a seasonal control of soil temperature on the emission rates of monoterpenes and other VOCs. The emission rates increase with soil temperature. Positive correlations between the VOC exchange rates and the soil CO₂ fluxes suggest that phenology of roots and microorganisms also controls seasonal changes in soil VOCs in this Mediterranean shrubland.

Introduction

Volatile organic compounds (VOCs) are reactive trace substances present in gaseous form in the troposphere, which interact with other atmospheric trace compounds, affecting distributions of air pollutants such as NOₓ, PANs, and particles (Atkinson & Arey, 2003) and playing a central role in tropospheric ozone formation (Fuentes et al., 2000; Chen & Griffin, 2005). For this reason, considerable effort has been made to identify the sources and to quantify the amounts of VOCs (Lamb et al., 1987; Müller, 1992).

VOCs originate from three main sources: anthropogenic activities, biomass burning and the biosphere. The biosphere is the largest source of VOCs, its emissions surpassing several times those from anthropogenic and biomass burning sources (Guenther et al., 1995). Natural sources of VOC emissions to the atmosphere include marine and fresh water, soil and sediments, microbial decomposition of organic material, geological hydrocarbon reservoirs, plant foliage and woody material. Among terrestrial ecosystems, foliar emissions (mainly isoprene and terpenes) from woodlands are considered the largest source (Guenther et al., 1994; Fuentes et al., 1996). Isoprene and monoterpenes, and some partly oxygenated VOCs such as formaldehyde, acetaldehyde, acetone, methanol, ethanol, formic and acetic acids, are synthesized in plants and emitted in large amounts into the atmosphere (Guenther et al., 1994; Fall, 1999; Fall et al., 1999; Seco et al., 2007). Most studies have focused on these compounds, because they are highly reactive and have a greater ozone formation potential. These volatile compounds are also involved in numerous

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physiological and ecological interactions in forest ecosystems (Gershenzon, 1994; Fall et al., 1999; Peñuelas & Llusia, 2003).

In contrast to foliar VOC exchanges, soil exchanges of non-methane VOCs with the atmosphere have received little attention, because they probably represent less than a few per cent of total global VOC exchange (Lamb et al., 1987; Guenther et al., 1995), though further investigation is needed to verify this assumption. In fact, there is still a lack of data on soil VOC inventories in different soil and ecosystem types. There are also many uncertainties about the soil VOC exchange process, because several factors are involved in the emission and uptake of VOCs by soils.

There is now evidence that soil may be both a VOC source (Guenther, 1999) and a sink (Van Ginkel et al., 1987; Bender & Conrad, 1993). Litter is the largest source of VOC emissions from natural soils (Hayward et al., 2001; Schade & Goldstein, 2001), followed by roots (Janson, 1993; Chen et al., 2004; Lin et al., 2007) and microorganisms (Scholler et al., 2002). Physical adsorption of VOCs to soil particles (Pignatello & Xing, 1996) and biodegradation by microorganisms (van Roon et al., 2005a) or adsorption and degradation by root tissue (Simonich & Hites, 1995; Newman et al., 1997; Cho et al., 2005) are known to produce soil VOC uptake.

The role of monoterpenes and other VOCs in soil ecology is far from well understood. However, important roles such as the VOC-mediated interactions among bacteria/fungi and plants have been described in recent years. For example, Ryu et al. (2003) reported that 2,3-butanediol and acetoin are synthesized and emitted by plant growth-promoting Bacillus strains that enhance growth and induce systemic resistance of Arabidopsis thaliana leaves, and/or inhibition of root and leaf development. Volatiles in soil also mediate plant-plant interactions (Nishida et al., 2005) and plant-insect interactions (Nordenhem & Nordlander, 1994; Chamberlain et al., 2001). Other authors have reported the potential for monoterpenes to alter rates of nutrient cycling because these compounds inhibit nitrification in soil (White, 1994; Smolander et al., 2006) but serve as a carbon and energy source for soil microbes (Misra et al., 1996; Owen et al., 2007).

Climatic (IPCC, 2007) and ecophysiological models such as GOTILWA (Sabate et al., 2002; Peñuelas et al., 2005) predict increased drought in the near future in the Mediterranean Basin, which may affect soil VOC emission and uptake directly (Asensio et al., 2007), or indirectly through its effects on plants and soil micro-organism activities (Ogayà & Peñuelas, 2003; Emmett et al., 2004; Sardans & Peñuelas, 2005).

Given the lack of information on soil-atmosphere VOC fluxes, the importance of soil VOCs on soil ecology, and the predictions of increased drought in Mediterranean ecosystems, we conducted this study of soil VOC exchange rates in a Mediterranean shrubland at the Garraf Natural Park, in Catalonia. Our aims were (i) to explore and quantify the soil VOC exchange rates, with special attention to monoterpenes, in a typical calcareous Mediterranean shrubland, (ii) to investigate interannual and seasonal variations in soil VOC exchange rates, and (iii) to study the links between soil VOC exchange rates and soil temperature, soil moisture and soil CO₂ efflux.

Material and methods

The study site and species description

The study was carried out in a dry shrubland (Rosmarino-Ericion) at the Garraf Natural Park, in Catalonia, northeast Spain (41°18’N, 1°49’E), at 210 m above sea level, on a south-south-east slope (13°). The climate is typically Mediterranean (annual average temperature 15.1°C and annual average precipitation 455 mm). The site, which is located on terraces of abandoned vineyards, suffered severe fires in the summers of 1982 and 1994. The soil is a Petrocalcic Calcixerept (Soil Survey Staff, 1998), thin (12–37 cm), with a loamy texture and abundant calcareous nodules, and soil pH is 7.7. Physico-chemical soil properties measured within 0–15 cm soil depth are shown in Table 1. Currently the vegetation covers 60–70%, with a maximum height of 70 cm. The dominant species at the study site are

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Petrocalcic Calcixerept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil texture</td>
<td>% Sand / % Silt / % Clay</td>
</tr>
<tr>
<td></td>
<td>42.9 / 38.7 / 18.4</td>
</tr>
<tr>
<td>Mineral horizon (mean 0–15 cm)</td>
<td>1.28</td>
</tr>
<tr>
<td>Mineral horizon bulk density (Mg m⁻³)</td>
<td></td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>2.04</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Carbon store (kg C m⁻²)</td>
<td>1.46</td>
</tr>
<tr>
<td>Nitrogen store (kg m⁻²)</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean annual soil water content (% vol/vol)</td>
<td>18</td>
</tr>
<tr>
<td>Min. (% wilting point)</td>
<td>5</td>
</tr>
<tr>
<td>Max. (% field capacity)</td>
<td>26</td>
</tr>
<tr>
<td>Soluble ammonium NH₄⁺ (mg kg⁻¹ soil)</td>
<td></td>
</tr>
<tr>
<td>Summer 04</td>
<td>1.45 ± 0.13</td>
</tr>
<tr>
<td>Winter 04–05</td>
<td>1.45 ± 0.09</td>
</tr>
<tr>
<td>Spring 05</td>
<td>1.18 ± 0.37</td>
</tr>
<tr>
<td>Soluble nitrate NO₃⁻ (mg kg⁻¹ soil)</td>
<td></td>
</tr>
<tr>
<td>Summer 04</td>
<td>13.57 ± 4.39</td>
</tr>
<tr>
<td>Winter 04–05</td>
<td>22.83 ± 2.54</td>
</tr>
<tr>
<td>Spring 05</td>
<td>9.10 ± 0.87</td>
</tr>
<tr>
<td>Olsen-P (mg g⁻¹ soil)</td>
<td></td>
</tr>
<tr>
<td>Summer 04</td>
<td>2.81 ± 0.25</td>
</tr>
<tr>
<td>Winter 04–05</td>
<td>1.90 ± 0.50</td>
</tr>
<tr>
<td>Spring 05</td>
<td>4.17 ± 0.64</td>
</tr>
</tbody>
</table>
site, Erica multiflora L., Globularia alypum L., Pinus halepensis L. and Rosmarinus officinalis L., are evergreen species that typically occur on basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland.

**Sampling**

Measurement campaigns were carried out on two consecutive sunny days in each season of two annual sampling periods (one with a normal climate, between spring 2003 and winter 2003/2004, and a dry one, between autumn 2004 and summer 2005): spring 2003 (9 and 10 May), summer 2003 (19 and 20 August), autumn 2003 (19 and 20 November), winter 2003/2004 (27 and 28 January); autumn 2004 (9 and 10 November), winter 2004/2005 (1 and 2 February), spring 2005 (25 and 26 May) and summer 2005 (4 and 5 August). Soil CO2 fluxes and soil VOC exchange rates were measured during the morning (from 07.00 to 12.00 solar time).

**Measurements of soil CO2 flux, temperature and moisture**

Soil respiration was measured in situ using a flow-through chamber method and an infrared gas analyser system (EGM-4, PP Systems, Hitchin, Hertfordshire, England). A vented soil chamber system was used with PVC collars (12.5 cm in diameter and 8 cm in height) installed permanently 3.4 cm into the soil. The collars were covered by a PVC lid with two outlets. One outlet was connected to the IRGA analyser by a teflon tube. The other outlet was open to external air entry. Air inside the chamber was pumped (constant flux 0.4 litre minute$^{-1}$) to the CO2 analyser by the EGM-4 integral DC pump. The flow was measured with a bubbler flowmeter. Equilibration of CO2 concentration in the effluent stream occurred after 20 minutes. Before the collar was covered, we measured external air CO2 concentrations. Net soil CO2 fluxes were calculated from the stable difference in CO2 concentration between the outlet air and the inlet air. Measurements were automatically corrected for temperature and pressure by the EGM-4 analyser. The accuracy of CO2 measurements was estimated to be 1%. Stability of the measurements was provided by the periodic ‘Auto-Zero’, resulting in automatic correction for sample cell contamination, source aging, detector sensitivity variations and pre-amplifier gain changes.

Twenty-seven collars were distributed randomly in a 1 ha field site. The collars were installed in winter 2002 and were permanently placed into the soil, in order to minimize possible effects of mechanical disturbance during measurements. Litter recently fallen inside the PVC collars was removed before sampling to obtain CO2 emissions only from mineral soil, roots and micro-organisms.

Together with each gas exchange measurement, soil temperature was measured with a digital soil thermometer (TO 15, Jules Richard instruments, Argenteuil, France) and soil moisture with a HH2 soil moisture meter connected to an ML2x soil moisture sensor (Delta-T Devices Ltd, Cambridge, England). These measurements were taken at 10 cm depth, just beside each PVC collar, to avoid mechanical disturbances to the enclosed soil. Temperature above the soil surface was also measured.

**Measurements of soil VOC exchange with the atmosphere**

Measurements of soil VOC exchange were conducted immediately after those of soil CO2 fluxes. Air from the cuvette was pumped through a T system to a glass tube (11.5 cm long and 0.4 internal diameter) filled with VOC adsorbents Carbotrap C (300 mg), Carbotrap B (200 mg) and Carbosieve S-III (125 mg) (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool and treated as described by Llusia & Peñuelas (2000).

The VOC flow from the soil chamber to the glass tube varied between 0.2 and 0.3 litre minute$^{-1}$, depending on the adsorbent and quartz wool packing and model of pump used. Soil VOCs were sampled for 5 minutes and the flow was regulated with a peristaltic pump (Portable Escort Elf Pump, P/N 497701 S/N A2-31854; Mine Safety Appliances Co., Pittsburgh, Pennsylvania, USA). The flow adjustment was determined with a bubble flowmeter. Glass tubes were stored in a portable fridge at 4°C and taken to the laboratory, where they were stored at −30°C until analysis (within 1 week).

VOC analyses were conducted in a GC-MS (Hewlett Packard HP59822B, Palo Alto, California). Trapped VOCs were desorbed (Thermal Desorption Unit, Model 890/891; Supelco, Bellefonte, Pennsylvania) at 250°C for 3 minutes and injected into a 30 m × 0.25 mm × 0.25 μm film thickness capillary column (Supelco HP-5, Crosslinked 5% Ph Me Silicone). After sample injection, the initial temperature (40°C) was increased at 30°C minute$^{-1}$ up to 60°C, and thereafter at 10°C minute$^{-1}$ up to 150°C, maintained for 3 minutes, and thereafter at 70°C minute$^{-1}$ up to 250°C, which was maintained for another 5 minutes. Helium flow was 1 ml minute$^{-1}$. The identification of VOCs was conducted by GC-MS and comparison with standards from Fluka (Chemie AG, Buchs, Switzerland) and the GCD Chemstation G1074A HP reference library. The reference compounds used for the identification were: 3-hexen-1-ol, α-pinene, β-pinene, limonene and dodecane. Calibration with a range of solutions of these standards every seven analyses was used for quantification. VOC calibration curves ($n = 5$ different VOC concentrations) were always significant ($r^2 > 0.97$) for the relationship between signal and VOC concentration. The most abundant terpenes had very similar sensitivities (differences were less than 5%). In order to detect possible column contamination during the analyses, a blank sample of pure pentane was injected and analysed twice, prior to starting the GC-MS analyses and at the end of the analyses.

We measured the background values of the VOCs in the atmosphere near the soil surface, using a blank PVC soil chamber in order to detect possible contaminations from the system. We used the background values to calculate the exchange rates on
a mass balance basis. This way we measured either a positive soil VOC ‘exchange rate’, i. e. a flux from the soil to the atmosphere (‘emission rate’), or a negative soil VOC ‘exchange rate’, i. e. flux from the atmosphere to the soil (‘uptake rate’).

From November 2004 to August 2005 soil VOCs were additionally sampled in Tedlar bags in nine PVC collars and analysed in the laboratory with the proton transfer reaction mass spectrometry (PTR-MS) technique. We scanned all masses between 22 and 205 to study seasonal variations. After equilibration of the CO₂ concentration in the effluent stream, air from the cuvette was impelled by a pump towards a 3 litre Tedlar bag. To minimize degradation of VOCs by solar radiation in the field, Tedlar bags were enclosed inside a dark bag and put into the shadow. In the laboratory, bags were stored in a dark cool chamber at 4°C until analysis by PTR-MS (within a week). Preliminary control measurements have shown no significant changes of several VOC standards (oxVOCs and terpenoids) during this storage period under these conditions. The air from the Teflon bags was pumped to the PTR-MS inlet. The PTR-MS system (PTR-MS-FTD hs; Ionicon Analitik, Innsbruck, Austria) consists of three parts: the ion source where ions are produced by a hollow cathode discharge using water vapour as the molecular source of ions; the drift tube where proton transfer reactions to the trace constituents in the air occur (VOCs with a higher proton affinity than that of water 166.5 kcal mol⁻¹, including most unsaturated and almost all oxygenated hydrocarbons, undergo a proton-transfer reaction with H₃O⁺); and finally the ion detector, a quadrupole mass spectrometer, which provides sensitive detection of the mass-selected ions that are characteristic of the molecules of interest. PTR-MS and its use in VOC analysis have been described in detail in Lindinger et al. (1998) and Fall et al. (1999). Here, the PTR-MS drift tube was operated at 210 Pa and 40°C, with a drift field of 600 V cm⁻¹; the parent ion signal was maintained at ca. 3 × 10⁸ counts per second during the measurements. We also sampled VOCs in the atmosphere near the soil surface, using a blank PVC soil chamber and Tedlar bags, in order to detect possible contaminations from the system. We used the background values of all masses between 22 and 205 to calculate the exchange balance for each compound. Mass identification was based on the literature and the use of calibration standards for common compounds: methanol, acetaldehyde, acetone, isoprene and α-pinene (Sigma-Aldrich and Abelló-Linde, Barcelona, Spain). However, due to the impossibility of using standards for all the compounds, there are still many unidentified masses. As different VOCs with the same mass cannot be separately measured, we combined PTR-MS with GC-MS techniques to determine the different monoterpenes with the same mass.

Statistical analyses

Repeated measures analyses of variance (ANOVA) were conducted for the exchange rate of every mass measured with the PTR-MS as dependent variable. Simple and multiple regression analyses were conducted to explore the relationships of the exchange rates of monoterpenes and of every mass measured with the PTR-MS with soil CO₂ fluxes, soil moisture and temperature. All analyses were performed with the STATVIEW 5.01 software package (Abacus Concepts Inc.).

Results

Soil temperature, moisture and soil CO₂ flux. Interannual and seasonal variations

Total rainfall in the first annual sampling period (from May 2003 to January 2004) was 495.4 mm, while total rainfall in the second annual sampling period (from November 2004 to August 2005) was 248.2 mm (Figure 1). While the first annual sampling period was significantly wetter than the second (soil moisture 19.01 ± 1.53 vs. 12.78 ± 1.01%; P < 0.005; Figure 1), the mean soil temperatures in each period were not significantly different (soil temperature 18.9 ± 1.3 vs. 17.9 ± 1.3°C; Figure 1). Soil CO₂ flux mean value did not differ significantly between the first and the second sampling periods (0.98 ± 0.01 vs. 0.95 ± 0.07 μmol m⁻² s⁻¹, Figure 1).

Soil temperature, moisture and soil CO₂ flux showed seasonal changes throughout the years (overall season effect P < 0.0001, Figure 1). Soil respiration increased in the wet seasons, spring and autumn, for both sampling periods (Figure 1).

During the first sampling period the highest soil temperature and the lowest soil moisture coincided in summer (August 2003, 30°C and 8%, Figure 1). During the second sampling period the highest soil temperature values were measured in spring and summer (May and August 2005, 26°C and 24°C, respectively; Figure 1). The lowest soil moisture values for this period were measured in winter (anomalously dry) and summer (February and August 2005, 9% and 10%, respectively; Figure 1).

Soil monoterpane exchange rates. Interannual and seasonal variations

Total monoterpane exchange rates measured during the study years were very small and ranged from uptake to net emissions (from −0.003 ± 0.001 to 0.003 ± 0.002 nmol m⁻² s⁻¹; Figure 2). α-pinene and limonene were the most common monoterpenes detected with the highest exchange rates (Figure 2) followed by camphene (Table 2). β-pinene, β-myrcene and Δ⁴-carene were also detected in some seasons although with very low exchange rates (Table 2).

In the first wet period, generally there was soil uptake of monoterpenes, except for small emissions measured in spring 2003 (Figure 2). In the second dry sampling period there were net emissions (Figure 2).
Seasonal changes in soil monoterpenes were small in most cases and no significant overall seasonal effect on total monoterpene or individual monoterpene exchange rates was found (Figure 2). However, the uptake rates of total monoterpenes and limonene increased from spring to winter, during the first sampling period (2003–2004, Figure 2). During the second sampling period 2004–2005, maximum total monoterpene emission rates were recorded in autumn and summer (November 2004 and August 2005, Figure 2).

**Figure 1** Seasonal course of rainfall, soil temperature, soil moisture and soil CO₂ flux measured in the two annual sampling periods, 2003–2004 and 2004–2005. Upper panel: bar diagram represents total monthly rainfall. Middle panel: soil temperature and moisture respectively. Lower panel: seasonal course of soil CO₂ efflux. Error bars indicate standard error of the mean (n = 27). Significances for the overall season effect, the sampling period effect (year) and the interaction between seasons and year effect (repeated measurements ANOVA) are indicated inside the panels.

**Figure 2** Seasonal course of soil exchange rates of total monoterpenes, α-pinene and limonene. Error bars indicate standard error of the mean (n = 27). Significance for the overall global effect of the season or the year (repeated measurements ANOVA) is indicated inside the panels. Significance for the season effect in one sampling period (repeated measurements ANOVA) is also indicated inside the panels.

Seasonal changes in soil monoterpenes were small in most cases and no significant overall seasonal effect on total monoterpene or individual monoterpene exchange rates was found (Figure 2). However, the uptake rates of total monoterpenes and limonene increased from spring to winter, during the first sampling period (2003–2004, Figure 2). During the second sampling period 2004–2005, maximum total monoterpene emission rates were recorded in autumn and summer (November 2004 and August 2005, Figure 2).
Table 2 Seasonal course of soil exchange rates (nmol m\(^{-2}\) s\(^{-1}\)) of other detected monoterpenes during the sampling annual periods 2003–2004 and 2004–2005. Values are means ± standard error (n = 27). Nd, not detected.

<table>
<thead>
<tr>
<th>Period</th>
<th>camphene</th>
<th>β-pinene</th>
<th>β-myrcene</th>
<th>Δ^2-carene</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2003</td>
<td>0.0000079 ± 0.000036</td>
<td>0.000171 ± 0.000159</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Aug 2003</td>
<td>Nd</td>
<td>0.0000040 ± 0.000004</td>
<td>Nd</td>
<td>0.000040 ± 0.000034</td>
</tr>
<tr>
<td>Nov 2003</td>
<td>−0.000377 ± 0.000164</td>
<td>−0.000092 ± 0.000098</td>
<td>−0.000022 ± 0.000150</td>
<td>0.000024 ± 0.000060</td>
</tr>
<tr>
<td>Jan 2004</td>
<td>−0.000178 ± 0.000125</td>
<td>−0.000030 ± 0.000057</td>
<td>0.000119 ± 0.000089</td>
<td>0.000015 ± 0.000115</td>
</tr>
<tr>
<td>Nov 2004</td>
<td>0.000552 ± 0.000386</td>
<td>0.000162 ± 0.000179</td>
<td>0.0003 ± 0.000409</td>
<td>0.000291 ± 0.000226</td>
</tr>
<tr>
<td>Feb 2005</td>
<td>0.000129 ± 0.000078</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>May 2005</td>
<td>0.000132 ± 0.000132</td>
<td>0.000018 ± 0.000018</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Aug 2005</td>
<td>0.001127 ± 0.000595</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

**Table 3** Air concentrations of masses of volatile organic compounds in nmol mol\(^{-1}\) (ppbv), detected by PTR-MS during the 2004–2005 sampling period in Garraf. Only those masses that were identified and that were significantly affected by season or treatment are shown. Data are means ± standard error; n = 27. The most likely VOC corresponding to each mass based on standard calculations of the PTR-MS system and literature is indicated in brackets.

<table>
<thead>
<tr>
<th>Mass number (compound)</th>
<th>VOC concentration/nmol mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>November 2004</td>
</tr>
<tr>
<td>M31 (formaldehyde)</td>
<td>5.24 ± 0.40</td>
</tr>
<tr>
<td>M33 (methylal)</td>
<td>109.05 ± 2.13</td>
</tr>
<tr>
<td>M43 (acetic acid, ethyl acetate, 2-3 butanedione)</td>
<td>12.24 ± 1.18</td>
</tr>
<tr>
<td>M45 (acetalddehyde)</td>
<td>9.11 ± 1.14</td>
</tr>
<tr>
<td>M57 (E)-2-hexenal</td>
<td>2.98 ± 0.16</td>
</tr>
<tr>
<td>M59 (acetone)</td>
<td>7.92 ± 0.21</td>
</tr>
<tr>
<td>M61 (acetic acid)</td>
<td>4.59 ± 0.30</td>
</tr>
<tr>
<td>M73 (C(_3) and C(_4) carbonyls, methyl ethyl ketone)</td>
<td>0.99 ± 0.16</td>
</tr>
<tr>
<td>M97 (heptanal, 2e-4e-hexadienal)</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>M99 (C(_6) oxygenated compounds)</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>M101 (hexanal)</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>M114</td>
<td>0.01 ± 0.09</td>
</tr>
<tr>
<td>M123 (sesquiterpenes)</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>M127 (C(_6) aldehydes)</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>M137 (monoterpenes, sesquiterpenes)</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>M139 (C(_10) aldehydes fragment)</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>M143 (hexenyl acetates)</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>M155 (linalool, 1, 8-cineole)</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>M199 (C(_13) unsaturated alcohols)</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

**Exchange rates of other VOCs**

Background air concentrations for all identified masses (analysed with the PTR-MS technique during the 2004–2005 sampling period) with a significant seasonal change in exchange rate are shown in Table 3. Among all the identified masses, maximum soil emission rates ranged from 0.23 to 2.5 nmol m\(^{-2}\) s\(^{-1}\). These maximum soil emission rates were measured for compounds such as M33 (methanol), M45 (acetalddehyde) and M43 (acetic acid) shown in Figure 3 and M73 (C\(_3\) and C\(_4\) carbonyls), M59 (acetone) and M61 (acetic acid) shown in Figure 4. M137 (monoterpenes and sesquiterpenes), M57 [(E)-2-hexenal], M31 (formaldehyde), M97 (heptanal) and M99 (C\(_6\) oxygenated compounds) emission rates were lower, ranging from 0.01 to 0.04 nmol m\(^{-2}\) s\(^{-1}\) (Figures 3, 4). Even lower emission rates were measured for other compounds such as M123 (sesquiterpenes), M139 (C\(_{10}\) aldehydes fragment) and M155 (linalool) (Figure 3) or M127 (6-methyl-5-hepten-2-one), M199 (C\(_{13}\) unsaturated alcohols) and M143 (hexenyl acetates) (Figure 4). Their maximum emission rates ranged up to 0.005 nmol m\(^{-2}\) s\(^{-1}\). Maximum soil VOC uptake rates measured, among the identified masses, ranged from −0.002 to −0.1 nmol m\(^{-2}\) s\(^{-1}\) in VOCs such as M33 (methanol), M45 (acetalddehyde) and M43 (acetic acid) (Figure 3) and M59 (acetone) (Figure 4).
Figure 3 Soil VOC exchange rates detected during the 2004–2005 sampling period using PTR-MS technique that presented maximum exchange rates in spring. Error bars indicate ± standard error of the mean (n = 9). Significances for the overall season effect (repeated measurements ANOVA) are indicated inside the panels. The most likely VOC corresponding to each mass based on standard calibrations of the PTR-MS system and on the literature is indicated in brackets.
The analysis of the soil VOCs by PTR-MS during the sampling period 2004–2005 showed that most VOC exchange rates changed significantly through the seasons. All the identified compounds showed their maximum emission rates in spring (May 2005, Figure 3) or summer (August 2005, Figure 4) whereas the minimum emission rates (sometimes uptake rates) were more frequent in autumn or winter (November 2004 and February 2005, Figures 3, 4).

Relationships between soil VOC exchange rates and soil temperature, moisture and soil CO₂ flux

The relationships between total and individual monoterpene exchange rates as dependent variables and soil moisture, temperature and soil CO₂ flux as independent variables are plotted in Figure 5. Total monoterpene and α-pinene emission rates decreased significantly in response to increases in soil moisture, although the coefficients of determination were low.


\[ R^2 \approx 0.08, \ P < 0.05 \text{ and } R^2 \approx 0.13, \ P < 0.005, \ \text{respectively,} \]

(Figure 5). However, no significant correlations or clear trends were found in the relationships between monoterpane exchange rates and either soil temperature or soil CO2 flux (Figure 5). Neither were there significant relationships when the data were separated into the two different sampling periods, 2003–2004 and 2004–2005.

Significant and positive correlations were found between exchange rates of other soil VOCs (the compounds shown in Figure 3) and soil moisture (correlations not shown). For all these VOCs, emission rates increased considerably in spring, when soil moisture was high (May 2005, Figure 1). However, they did not increase in the other rainy season, autumn 2004. Thus the coefficients of determination found with soil moisture were quite low (\( R^2 \) up to 0.24). Exchange rates of other VOCs showed higher positive correlations with soil temperature than with soil moisture, with a common trend to increase their emission rates when soil temperature increased in summer (Figure 4). We found a better fit between VOC exchange rates and soil temperature than for soil moisture (\( R^2 \) up to 0.40). The majority of the masses that showed a significant correlation with soil CO2 fluxes tended to increase their emission rates when soil CO2 flux increased, e.g. M45 (acetaldehyde), M137 (monoterpenes and sesquiterpenes) and other VOCs in Figure 6. Yet the relationships found, when significant, again showed low \( R^2 \) values (up to 0.30).

Given the relatively poor relationships with the single variables, multiple regression analyses were performed between total and individual monoterpane exchange rates (dependent variables) and soil moisture, temperature and soil CO2 flux (predictor variables). The results showed no relationship between limonene exchange rates and predictor variables. However, results showed that the combination of soil moisture, temperature and CO2 flux explained a higher percentage of the variability in total monoterpenes and \( \alpha \)-pinene exchange rates (\( R^2 \approx 0.19, \ P < 0.005, \ R^2 \approx 0.18, \ P = 0.005, \ \text{respectively} \)) than the single variables (Figure 5). The beta coefficient for soil moisture variable was negative and significant in both total monoterpenes and \( \alpha \)-pinene multiple regressions (\( P < 0.005 \) and \( P < 0.001, \ \text{respectively} \)). The beta coefficients for CO2 flux variable were always positive although only significant in the total monoterpenes multiple regression (\( P < 0.05 \)). In all cases, beta coefficients of soil temperature were not significant and lower than soil moisture and CO2 beta coefficients.

**Discussion**

**Soil VOC exchange rates in a Mediterranean shrubland**

The monoterpenes \( \alpha \)-pinene and limonene, along with the other monoterpenes shown in Table 2, have also been detected in other studies of soil VOC exchange in a Mediterranean holm oak forest soil (Asensio et al., 2007) and in a non-Mediterranean Sitka spruce forest soil (Hayward et al., 2001). As we had removed the litter layer, the principal source of these terpenoids emitted by soil is likely to have been the root systems of...
plants. We were not able directly to identify the origin of the detected compounds because our VOC sampling method includes all living organisms in soil. However, *Pinus halepensis* is abundant at this site and the finding of large amounts of α-pinene, limonene, β-pinene and camphene in *Pinus* root emissions and content (Lin *et al.*, 2007) suggests that roots are the likely source of these monoterpenes. As our sampling method did not damage the roots, below-ground parts of the plants were probably affecting the total soil terpene emission rates through the production and release of terpenes to the rhizosphere by living roots or decomposing plant material.

In accordance with Asensio *et al.* (2007), our results show that soil monoterpane emission rates in general are very low compared with monoterpane emissions from leaves. For example, Peñuelas & Llusia (1999) reported values of about 18 nmol m\(^{-2}\) s\(^{-1}\) from *Pinus halepensis* leaves in summer. So soil monoterpane emissions may represent only a small part of the total monoterpane fluxes emitted to the atmosphere by a vegetated land surface. Maximum soil monoterpane emission rates reported in this study were similar to those maximum emission rates reported by Asensio *et al.* (2007) in a Mediterranean holm oak soil forest (0.003 nmol m\(^{-2}\) s\(^{-1}\) and 0.004 nmol m\(^{-2}\) s\(^{-1}\), respectively). Therefore these results indicate that emissions to the atmosphere are not one of the most important fates for soil-derived monoterpenes, which might be stored in the soil mineral layers rather than emitted to the atmosphere. However, so far there are few studies on the monoterpane content of soils (White, 1994; Smolander *et al.*, 2006; Lin *et al.*, 2007), so research into terpene content in different soil layers is still needed.

Soil monoterpane uptake in this shrubland was lower than monoterpane uptake measured in the holm oak forest soil (−0.003 nmol m\(^{-2}\) s\(^{-1}\) and −0.01 nmol m\(^{-2}\) s\(^{-1}\), respectively). This difference is possibly due to differences in the physical soil features or in the soil microflora, because soil VOC uptake activity occurs both by physical adsorption of VOCs by soil components (Pignatello & Xing, 1996) and by biodegradation by soil microorganisms (van Roon *et al.*, 2005a). In fact, soil enzyme activity and soil CO\(_2\) flux, which are good indicators of microorganisms’ activity in soils (Hanson *et al.*, 2000; Baum *et al.*, 2003), were also lower in this shrubland soil than in the holm oak forest soil (Sardans & Peñuelas, 2005; Asensio *et al.*, 2007).

The air concentration of the protonated compound of mass 33 (methanol, Table 3) was higher than other values reported in the literature for other natural sites [from 1 to 41 nmol mol\(^{-1}\) (ppbv), reviewed by our group in Seco *et al.*, 2007]. It is not

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**Figure 6** Examples of the relationships found between some identified VOC exchange rates and soil CO\(_2\) flux during the 2004–2005 sampling period. The most likely VOC corresponding to each mass based on standard calibrations of the PTR-MS system and on the literature is indicated in brackets.
clear what may have caused such high background concentrations of methanol. In fact, Schade & Custer (2004) have recently also reported high methanol mixing ratios up to 25 nmol mol\(^{-1}\) near an agricultural soil surface. Maximum methanol soil emission rates (1.2 nmol m\(^{-2}\) s\(^{-1}\), Figure 3) are similar to or lower than other methanol soil emissions (maximum about 1.7 nmol m\(^{-2}\) s\(^{-1}\)) reported above a bare agricultural field by Schade & Custer (2004). These latter authors suggest a methanol source at the soil surface and that its production is probably linked to a physico-chemical degradation process of soil organic matter. As we removed the litter layer, the source of soil methanol emissions in this study should be in the soil.

Except for methanol (Figure 3), the emission rates of the other volatile compounds measured with the PTR-MS technique were in the range or slightly higher than those reported in a holm oak forest soil by Asensio et al. (2007) (maximum emission rates up to 0.6 nmol m\(^{-2}\) s\(^{-1}\) in this shrubland and up to 0.3 nmol m\(^{-2}\) s\(^{-1}\) in the holm oak forest). These results show again that soil VOC emission rates in general are very low, except for methanol.

Monoterpene and sesquiterpene (M137) emission rates measured in spring (May 2005; Figure 3), using the PTR-MS technique, were approximately 10 times higher than the monoterpene emission rates measured using the GC-MS technique (Figures 2, 3). The higher abundance of mass 137 might indicate the presence of high sesquiterpene amounts released by roots. In fact, sesquiterpenes are one of the most abundant compounds in Pinus roots (Lin et al., 2007). The sum of all masses documented in the literature as sesquiterpenes or sesquiterpene fragments (M109, M123, M149 and M205) clearly reflected the same emission pattern as mass 137, but the sum of these masses only doubled the value for mass 137 in spring 2005, indicating that there must be other sources contributing to the high mass 137 values of that spring sampling.

Our results showed maximum soil uptake rates ranging from −0.1 to −0.5 nmol m\(^{-2}\) s\(^{-1}\) in some compounds like M33 (methanol, Figure 3), M42 (acetonitrile), M46, M59 (acetone), M88 and M89 (ethyl acetate) (not shown). These values are comparable with foliar uptake rates reported by other authors (ranging from −0.4 to −3 nmol m\(^{-2}\) s\(^{-1}\)) for some compounds like MIBK (methyl isobutyl ketone) (Tani et al., 2007), monoterpenes (Copolovici et al., 2005) or M31 (formaldehyde) (Filella et al., 2006). However, there is still insufficient knowledge about the soil VOC sink strength to evaluate whether it could be small but non-negligible on a global scale. Soil VOC and other soil gas uptake fluxes have been described (Pegoraro et al., 2006; Chapuis-Lardy et al., 2007) but they have received little attention due to the relatively low uptake rates and the quite high uncertainties around soil VOC exchange processes. Soil VOC uptake rate depends on the air concentration and on the soil concentration resulting from the balance between soil VOC production and consumption (Pignatello & Xing, 1996), which may vary with the soil type and plant species.

**Interannual variation and seasonality of soil VOC exchange rates and relationships with soil temperature, moisture and CO\(_2\) flux**

Results indicate that a severe drought period increases soil monoterpene emission rates in this Mediterranean shrubland (Figure 2). Even though monoterpene exchange rates were very low, there were highly significant differences between the exchange rates measured in the 2 years of contrasting precipitation. This may be the result of lower uptake rates due to lower physiological activity during the drier sampling period 2004–2005. However, the fact that monoterpene exchange rates and soil CO\(_2\) fluxes were not significantly correlated (Figure 5) and that soil CO\(_2\) fluxes did not decrease significantly during the dry year 2004–2005 (Figure 1) suggest that the increase in soil monoterpene emission rates in response to lower soil water availability could be due to changes in soil physical properties induced by low soil moisture, rather than to a lower physiological activity of roots and microorganisms.

Seasonal changes in total monoterpenes, α-pinene and limonene were smaller than interannual changes and the exchange rates measured were highly variable in some seasons (Figure 2). Total and individual monoterpene exchange rates did not show overall significant seasonal effects in the second, dry sampling period, although there were significant seasonal changes in soil moisture and temperature (Figure 1). These results, in addition to the very low, though significant, negative correlations found between monoterpene exchange rates and soil moisture (Figure 5), suggest that monoterpene exchange rates are less affected by seasonal soil moisture changes than by long-term severe changes.

Monoterpene emissions were recorded mainly during the warm seasons (spring and summer) and most monoterpene uptake was recorded during the cold seasons (autumn and winter), as shown in Figure 2. This is in agreement with the results of Asensio et al. (2007) and it is likely to be due to the higher volatilisation rates of volatile compounds in response to increases in temperature (van Roon et al., 2005b). However, general data correlation analyses showed no significant relationships between exchange rates of total and individual monoterpenes and soil temperature. Furthermore, multiple regression analyses showed that soil temperature is the least explanatory variable of soil monoterpene exchange rates measured in this study. Thus, results suggest that soil moisture and soil CO\(_2\) flux (indicating microbial and rhizosphere activity) are better predictors of soil monoterpene exchange rates (those of α-pinene, especially) than soil temperature in this Mediterranean shrubland.

There were significant seasonal variations in the exchange rates of other VOCs (Figures 3, 4), together with changes in soil temperature, moisture and soil CO\(_2\) flux (Figure 1). The effect of soil moisture on soil VOC exchange rates depends on the compound type, although results suggest that high soil moisture tended to increase soil VOC emissions, at least in those compounds that showed significant seasonal changes in their
exchange rates (Figures 3, 4). The positive correlations found between several soil VOC exchange rates and soil moisture provide evidence of this trend. However, the correlations were not strong because soil moisture effects on VOC exchange rates were constrained by low soil temperatures, for example, in autumn (November 2004, Figure 1). Similarly, we found positive correlations between soil VOC exchange rates and soil CO₂ flux (Figure 6), suggesting that phytodegradation of plants and soil microorganisms is also affecting VOC exchange rates. Soil CO₂ flux is considered to be a good indicator of the physiological activity of roots and microorganisms (Hanson et al., 2000). Thus, the increase of soil VOC emission rates in this Mediterranean shrubland in spring (Figures 3, 4) might be linked to the increase of root and microorganism activities in spring and autumn; for example, the root-surface and soil phosphatase activities (Sardans et al., 2006, 2007) in parallel with the increase of soil-available P in spring (Table 1). The decrease in available ammonium and nitrate in soil during spring and autumn (Table 1) corresponded to the plant growing seasons and it could be also related to the increase in soil VOC emission rates during spring (probably increases in root emissions).

Because maximum emission rates always occurred in spring or summer, when the soil temperature was high, we found significant positive correlations between several VOC exchange rates and soil temperature (not shown). Thus, results indicate again seasonal soil temperature controls on exchange of several soil VOCs, with emission rates increasing when soil temperature increases.

Conclusions and final remarks

Soil VOC exchange rates measured in this Mediterranean shrubland are the result of the synthesis and uptake of VOCs that are dependent on temperature, water availability, phenology of plants and soil microorganisms, soil physical traits and concentration of the compounds in the atmosphere near the soil. Alpha-pinene and limonene were the most common identified monoterpenes undergoing soil-atmosphere exchange. M33 (methanol), M43 (acetic acid, ethyl acetate), M45 (acetaldehyde), M59 (acetone) and M73 (C₃ and C₄ carbonyls) showed the highest emission rates among all the identified VOCs. Exudates from rhizosphere activities, production by roots and organic matter decomposition by microorganisms in the soil organic or mineral horizons may be the most important sources of these VOCs.

Soil monoterpenes and in general soil VOC emissions, except those of methanol, were very low compared with foliar VOC emission rates. However, some soil VOC uptake rates were comparable with those reported for leaves. These VOCs might be consumed by soil microorganisms or stored in the soil layers. Further studies are needed to corroborate these results and to investigate the possible importance of the soil VOC sink in chemistry-climate models.

Results suggest that severe long-term drought increases soil monoterpenes emission rates in this Mediterranean shrubland. The increase in soil monoterpenes emissions in response to lower soil water availability is probably linked to changes in soil physical properties induced by low soil moisture in this Mediterranean shrubland, because monoterpenes exchange rates and soil CO₂ fluxes were not significantly correlated. Conversely, other soil VOCs increased their emission rates when soil moisture increased, although the effects were constrained by soil temperature, for example in the cold seasons. Most of these compounds’ exchange rates showed significant correlations with CO₂ flux, suggesting that the increase in root and microbial activities during the growing seasons may increase these soil VOC exchange rates. High soil temperatures increased emission rates of monoterpenes and other VOCs, possibly due to higher volatilisation rates. The effect of soil moisture reduction on soil VOC exchange rates is still poorly understood because several factors are probably affecting soil VOC exchange rates. Climatic (IPCC, 2007) and ecophysiological models such as GOTILWA (Sabaté et al., 2002; Peñuelas et al., 2005) predict decreases in water availability in Mediterranean ecosystems for the next decades. Improved knowledge of the multiple interactions between rhizosphere components and soil emission and uptake processes will help to disentangle the effects of the projected drought on soil VOC exchange with the atmosphere.

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