

Comparison of methane, nitrous oxide fluxes and CO₂ respiration rates from a Mediterranean cork oak ecosystem and improved pasture

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Abstract

Background and aims During the recent decades, cork oak (*Q. suber*) mortality has been increasing in Mediterranean oak woodland endangering the economical and environmental sustainability of the “montado” ecosystem. This fact in combination with climate change and conversion of forestland to pasture may significantly affect the soil-atmosphere greenhouse gases (GHGs) exchange. Our study evaluates the impact of oak trees as compared to pasture on net ecosystem GHG (CH₄, N₂O, and CO₂) exchange as well as the main environmental factors influencing this exchange.

Methods We used field chamber measurements for the collection of GHGs under three different conditions: 1)

open area (OA), 2) under tree canopy area (UC) and 3) improved pasture (IP). Experiments were done under typical Mediterranean climate at central Portugal in 2010 and 2011.

Results The UC had higher nitrification potential, soil C/N ratio, electrical conductivity, litter input and soil organic matter (SOM) than OA and IP. SOM positively correlated with soil CH₄ and N₂O fluxes but not with soil CO₂ respiration rates. Soil water content (SWC) drives both CH₄ and N₂O fluxes. Under certain conditions, when SWC reached a threshold (7 % for CH₄ and 3 % for N₂O) the result was net uptake and that net uptake increased with SWC. This was the case for the UC and OA. Conversely, for the IP soil water content above 4 % promoted net CH₄ release.

Conclusions Our results show that cork oak influences soil properties and consequently GHGs fluxes. In the UC the input of litter for SOM together with soil moisture, favoured microbiological activity and related GHGs fluxes. Soil temperature is a secondary factor in the studied conditions. Our results also emphasized the potential impact posed by decreased cork oak tree density in the functioning of the “montado” ecosystem.

Keywords Evergreen oak · Greenhouse gases · Litter · Mediterranean · Organic matter · Root density

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Abbreviations

GHG Greenhouse gases
IP Improved pasture
OA Open area
Rs Soil CO₂ respiration rate

SOM Soil organic matter
UC Under tree canopy

Introduction

Climate change scenarios for Mediterranean regions can particularly affect the south-western Iberian Peninsula (IPCC 2007), which is dominated by evergreen oak species (*Quercus suber* and *Quercus ilex*) and which can be exploited in combination with silvopastoral systems known as ‘*montado*’ ecosystems. Worldwide, Portugal has the largest area of *Q. suber* (cork oak) of ca 730,000 ha, and 32 % of the global acreage. It provides about 50 % (ca 150,000 t) of the total world production of cork (Apcor 2010). In Portugal, cork oaks together with holm oak (*Q. ilex*) occupy about 1.1 million ha, and the largest area is centred in Alentejo.

In spite of the slight expansion (+12 %) of cork oak areas in Portugal over the last 40 years, mean tree density dropped by 23 % over the last decade (AFN 2010). During the years 1998–2011, which include the extreme drought of 2005, cork oak mortality showed a fourfold increase, and fire occurrence doubled, showing an increase in tree vulnerability and signs of decline of the cork species in Portugal (Garcia-Herrera et al. 2007). In parallel with drought and tree mortality, more land has been converted into pasture. Such change in soil use can affect the atmospheric levels of greenhouse gases (GHGs) and cause climatic change (Gundersen et al. 2012). According to (Copeland et al. 1996), land cover can affect climate because vegetation characteristics, such as albedo, roughness length, leaf area, and fractional coverage, influence temperature, humidity, wind speed, and precipitation.

Conservation of the *montado* ecosystem depends on its economical sustainability and on the market demand for cork products (Bugalho et al. 2011). The economic benefits of *montado* include other activities carried out under cork oak canopies such as crop growing, grazing and semi-intensive or extensive cattle production (Potes et al. 2011). Concerning the use of agricultural land in Portugal, the area of arable land decreased ca 50 % between 1989 and 2009 whereas the areas of permanent pastures increased by comparable amount (from 20.9 % in 1989 to 48.7 % in 2009) (INE 2011). The new improved pastures are composed of grasses in combination with legumes and are expected to increase long-

term carbon and nitrogen sequestration (WRR 2001; WWF 2007).

Deforestation, agriculture and intensive grazing may affect water resources, ecosystems and regional climate to a similar or greater extent than would climate change driven by global changes in atmospheric chemistry alone (Livesley et al. 2009; Hiltbrunner et al. 2012). Land use can modify the biogeochemical equilibrium of an ecosystem and related net GHGs fluxes, such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) (Smith and Conen 2004; Castaldi et al. 2006; Gundersen et al. 2012). Anthropogenic activity in the form of land use management can affect naturally occurring soil bacteria in ways that will increase/decrease the rate of decomposition of soil organic matter (SOM) and hence affect C and N cycles and the production of GHGs (Kirschbaum 2006). Land-use change can alter the production and consumption of GHGs by changing key soil hydrological, chemical and physical properties that influence soil nutrients, carbon cycling and microbial activity (Butterbach-Bahl and Kiese 2005; Dalal et al. 2008).

An important priority of terrestrial GHG flux studies is to create a database of gas fluxes for different ecosystems in order to define parameters and validate biogeochemical models (Parton et al. 2001). However, much of the available data was collected in temperate regions, boreal forests, prairies, agricultural areas or tropical forests (Smith et al. 2000; Maljanen et al. 2003; Siljanen et al. 2012). There is a lack of information regarding dry Mediterranean ecosystems (Castaldi et al. 2006; Correia et al. 2012). Such ecosystems occupy one-third of the land surface area of the planet and are particularly vulnerable to climate change (Emanuel et al. 1985).

Climate change is expected to increase temperature and spring/summer moisture deficits in the Mediterranean region, reduce soil nutrient availability and increase exposure to carbon dioxide (CO₂) and ozone (O₃) which may threaten the forest ecosystems (De Vries and Posch 2010). It is expected that rising temperatures will increase soil CO₂ fluxes (R_s) (De Dato et al. 2010), although this effect may not always be detected (Giardina and Seiler 2004) due to its modulation by rainfall patterns (Talmon et al. 2011).

Soil plays a key role in net global GHG fluxes, including fluxes of N₂O and CH₄ because they are biologically produced and then subsequently consumed by soil microorganisms (Chapuis-Lardy et al. 2007). The main sources of N₂O in soils are microbial nitrification and

denitrification and the latter can also consume N_2O (Schmidt et al. 2004). The production of N_2O in forest soils depends on soil characteristics (e.g. moisture, temperature, aeration, pH, SOM) and tree species composition (Butterbach-Bahl et al. 2002; Skiba et al. 2004, 2009; Weslien et al. 2009). The diffusivity of CH_4 through the soil profile is the primary limiting factor upon CH_4 oxidation and this is influenced by soil moisture, texture and bulk density. Furthermore, soil N status can limit CH_4 oxidation directly by inhibiting/competing with the monooxygenase enzyme of methanotrophs (Castro et al. 1995). Forest soils compared to agricultural soils, generally function as a net sink for CH_4 and emit less of the stronger GHG N_2O , thus having a general net (total) GHG mitigation effect (Livesley et al. 2009). Annual mean soil exchange fluxes of CH_4 and N_2O , measured for Mediterranean cork oak forests ($38^\circ 31'\text{N}$ $8^\circ 00'\text{W}$, Herdade da Mitra, Portugal) was reported as $-0.19 \text{ gC m}^{-2} \text{ yr}^{-1}$ and $0.0004 \text{ gNm}^{-2} \text{ yr}^{-1}$, respectively (Gundersen et al. 2012).

Soil-atmosphere exchange of the GHG carbon dioxide (CO_2) from oak woodlands has been assessed (Pereira et al. 2007; Jongen et al. 2011; Correia et al. 2012) but little is known about non- CO_2 fluxes, e.g. CH_4 and N_2O (Carneiro et al. 2010; Shvaleva et al. 2011). In addition, the contribution (source or sink) of *montado* to regional soil-atmosphere net CH_4 and N_2O exchange, the impact of land-use change, and oak trees on soil GHG fluxes remains poorly understood.

The present work aims to contribute to filling this knowledge gap. We measured net soil CH_4 , N_2O and CO_2 effluxes in evergreen oak woodlands for 20 months during 2010–2011, considering three different conditions: open area (OA) and under tree canopy (UC) of the natural understory (NU), and improved pasture (IP). We hypothesize that in nutrient poor soils, trees create patches of higher organic matter concentration and microbial activity, which influence soil properties and drive net soil GHG fluxes. Therefore, big differences are to be expected between net soil GHG fluxes under the canopy or in open areas or pastures. Based on this conceptual frame, the objectives of this study were: 1) to assess the effect of abiotic factors (e.g. soil characteristics) on soil-atmosphere net GHG exchange in a Mediterranean evergreen oak woodland; 2) to evaluate the impact of increasing the area of the IP on net GHG fluxes compared to the NU; 3) to determine whether plant cover (cork oak trees) can affect GHG fluxes, and 4) to clarify the main factors influencing soil GHG fluxes and how they interact.

Materials and methods

Site description

The experimental site was located in Herdade da Machoqueira do Grou ($39^\circ 08'18.29'' \text{N}$, $8^\circ 19'57.68'' \text{W}$), 30 km northeast of Coruche, Portugal. The region has a typical Mediterranean climate with hot and dry summers and mild wet winters. Long-term average meteorological data for this area show that more than 80 % of annual precipitation ($ca\ 680 \pm 210 \text{ mm}$) occurs between October and May and the mean annual temperature is $\sim 15.9^\circ \text{C}$ (Inst. de Meteorologia, Lisbon). The study site is a typical evergreen cork oak open woodland with 50 year old trees and a density of 177 trees/ha, and is a certified *montado* which assures sustainable management. The natural understory consists of Mediterranean shrub species such as *Cistus salvifolius* L., *Cistus crispus* L., *Lavandula stoechas*, and *Ulex* spp. and grasses. The understory vegetation actively grows in winter and early spring, with senescence starting generally at the end of May. From the transect analysis of the site (six transects of 200 m each), we determined that the herbaceous layer covers 42 %, the litter 31 %, the shrubs 18 %, and bare soil 9 % of the area. The soil is a Cambisol (FAO) with a soil water-holding capacity of 20 % and from the surface to 1 m depth consists of 81 % sand, 14 % silt and 5 % clay. In the fall of 2009, part of the site ($ca. 18 \text{ ha}$) was ploughed and reseeded with a legume-rich mixture (e.g. *Trifolium* spp., *Ornithopus* spp., *Biserrula pelecinum*) resulting in the IP (Jongen et al. 2011). This agricultural practice is intended to improve productivity and soil fertility (Crespo 2010) and common for agro-silvo-pastoral systems (such as the cork oak forests) in Portugal (Jongen et al. 2013).

Three different areas of approximately 25 m^2 each were used to characterize soil-atmosphere net GHG fluxes. Two areas were established in the natural understory: one under tree canopies (UC), another in large open areas ($ca\ 100 \text{ m}^2$) between trees (OA), and the third area was set in the improved pasture (IP). The distance between study areas were $ca\ 300\text{--}400 \text{ m}$. Soil-atmosphere net GHG fluxes were sampled from three chambers installed randomly in each of the UC and OA areas, and from four chambers installed in the IP area. Chambers (polypropylene cylinders, Technical University of Lisbon, Portugal) of 30 cm diameter were pushed to a depth of $ca. 10 \text{ cm}$ into the soil, giving a headspace volume of $10.43 (\pm 0.08) \text{ dm}^3$. These were

closed with a stainless-steel lid (41.5 cm in diameter) fitted with sample ports (0.6 cm diameter), which could be closed and opened by lock valves. The distance between chambers in each study areas (UC, OA and IP) was *ca* 5 m.

Standard meteorological data for rainfall (ARG100, Environmental Measurements Ltd., Gateshead, UK), air humidity and temperature (CS215, Campbell, Inc., Logan, USA) were collected continuously at 30 min intervals by a CR10X, datalogger (Campbell Scientific, Inc., Logan, USA) that was installed on the study site (natural understory). Rainfall data are relevant to all three study areas, i.e. UC, OA and IP.

Measurements: soil GHG effluxes and temperature

Fluxes of CH₄ and N₂O between the soil and the atmosphere were measured with a closed chamber technique as described in (Shvaleyeva et al. 2011). Measurements were taken monthly for 20 months (from May 2010 to December 2011) in the UC and OA sites, and for 13 months (from May 2010 to May 2011) in the IP. Additionally, soil net CH₄, N₂O and CO₂ fluxes were collected before (20th October) and after (26th and 27th October) the first autumn rain event since 1st September, 2011.

Measurements of CH₄ and N₂O gas fluxes between the soil and the atmosphere involved the collection of three gas aliquots per chamber. The chamber was closed at time 0, and samples were taken immediately beforehand and at 30 and 60 min later. Each time a 100 ml sample was withdrawn using a plastic syringe and flushed through 20 ml gas vials. These were sent to CEH, UK for analysis on a gas chromatograph (GC, HP5890 Series II, Hewlett Packard, Agilent Technologies UK Ltd., Stockport, UK) fitted with an electron capture detector (ECD) and a flame ionization detector (FID) for N₂O and CH₄ analysis, respectively. GC accuracy was 30 ppb for N₂O and 70 ppb for CH₄. Samples were analysed within 30 days of collection. A storage test vial, enclosing standard concentrations of 0.3 mg l⁻¹ N₂O and 1.8 mg l⁻¹ CH₄ and 1 mg l⁻¹ N₂O and 100 mg l⁻¹ CH₄ in N₂ for several months showed that leakage in and out of the sample vials was negligible.

Soil CO₂ net fluxes (i.e. soil respiration R_s) were measured using a closed loop dynamic chamber (EGM-3, PP-System, Amesbury, USA). For measurement the flux chamber was placed on permanent collars (10 cm tall and 12 cm diameter and inserted 2 cm into the soil) installed close to each of the static

chambers for N₂O and CH₄ measurements. Simultaneously, soil temperature at 5 cm depth was measured near to the collars using a hand held digital thermometer. The GHG flux measurements were always performed between 09:00 h and 13:00 h.

Soil analyses

To determine soil mineral nitrogen concentration, four samples close to each static chamber were collected on the same days as gas sampling. Soil samples consisted of a 3 cm diameter core from the top 20 cm layer. In the laboratory all samples were sieved (1 mm mesh) and separated into three parts. One part was used for the determination of gravimetric soil water content (SWC, %). The second part was used for the determination of nitrate (NO₃⁻) and ammonium (NH₄⁺) concentrations by the spectrophotometric method as described in Fangueiro et al. (2008). Finally, the third part of the soil samples was air-dried and analyzed for: 1) organic matter content according to (Nelson and Sommers 1996) using a CO₂ Infrared Detection Promacs TOC Analyser (Skalar, Netherlands). Organic matter content was determined using the conventional Van Bemmelen factor of 1.72, i.e.: organic matter (%) = carbon (%) × 1.72, and 2) potential of nitrogen mineralization with the anaerobic incubation method as described by Lober and Reeder (1993) with the modification of Fangueiro et al. (2008). Air dried soil (10 g) was weighed in a 60 ml syringe and mixed with 25 ml of distilled water. Ten replicates were prepared for each soil sample. Five replicates were incubated anaerobically for 8 days at 40 °C in the dark and the remaining five replicates were immediately treated with KCl (2M). The potential of net N mineralization (PM) was calculated as the difference between post- and pre-incubation NH₄⁺-N concentrations (Fangueiro et al. 2008). Total nitrogen in the soil was quantified by the Kjeldahl method (Horneck and Miller 1998).

Soil pH in the upper 20 cm was determined in a water extract (1:10, w/v) with a selective electrode (Micro pH 2001, Criston). Soil electrical conductivity (EC) was measured in a soil-water suspension (1:5, w/v), as described in Fangueiro et al. (2008).

Tree litter fall and root density

Tree litter fall was determined with 16 litter baskets placed in two transects across the site with periodic sampling throughout 2011. Root density expressed in dry mass per

m² of soil was determined in soil samples of 20×20×20 cm, collected in October, 2011 (three for each area). In the laboratory, roots were separated from the soil, washed, and dried at 65 °C for 48 h.

Statistical analysis

To examine the differences between plots we used a One-way Analysis of Variance (One-way Anova). Differences between group means were identified by *post-hoc* Tukey HSD test when statistically significant differences were found. The Pearson Product Moment Correlation coefficient was used to display the strength of the association between pairs of variables. All statistical relationships were considered significant at $P<0.05$, and statistical analyses were carried out using STATISTICA (Version 7, StatSoft, Inc. 2004).

Results

Seasonal changes and soil properties

Climate conditions during the years 2010 and 2011 were similar (Fig. 1). Total annual precipitation was 893 and 883 mm in 2010 and 2011, respectively, with comparable monthly distribution, although 2010 had a slightly drier summer. The mean average air temperature was 16 °C in 2010 and 15.7 °C in 2011. The average summer temperature in 2010 as compared with 2011, was 5 °C higher.

Soil water content (SWC) at 10 cm depth ranged from 2 % to 17.3 % in the UC, from 1 % to 15.5 % in the OA, and from 0.5 % to 12.7 % in the IP (Fig. 2a). Soil from the UC was significantly wetter ($P<0.001$) than in the IP and OA during the study period. Interestingly, SWC was 3-fold higher in the UC than in the IP during the summer months. Soil water content in the OA was, in most cases, an intermediate values, between the SWC of the IP and UC with values closer to those of the IP during dry months (Fig. 2a). SWC was negatively correlated with soil temperature during the whole period of observation ($R^2=0.50$, $P<0.001$).

Soil temperature recorded in the upper 5 cm ranged from 11 °C to 28 °C in the UC, from 8 °C to 39 °C in the OA, and from 9 °C to 31 °C in the IC (Fig. 2b). The UC had lower soil temperatures than the OA or IP during most of the study period, especially in warmer summer months. This difference was particularly striking in 2010 when the OA and IP, with much less tree

shadowing, had 10 °C higher soil temperatures than the UC. In 2011 the difference in soil temperature was only 4 °C in summer.

Net soil CH₄ fluxes

Results showed that the soil was mainly a net sink for CH₄; low emission rates were only observed on four out of the 18 dates (Fig. 2c). For the periods when measurements were made for two consecutive years (May to December), fluxes, both uptake and release rates, were larger in the period May to December 2010 than 2011 and ranged from -39 to $19 \mu\text{g C m}^{-2} \text{ h}^{-1}$ in 2010 as compared to the same period in 2011 (-13 to $3 \mu\text{g C m}^{-2} \text{ h}^{-1}$). This could be related to a big variability in soil temperature between sites (ca 10 °C), which was more pronounced in May–August of 2010 (Fig. 2b). Soil CH₄ uptake tended to be less negative in May–August, 2010 with increasing soil temperature but no statistical significant correlation between CH₄ fluxes and soil temperature was found. The only differences between sites were registered in February of 2011, when CH₄ uptake in the UC and OA was larger than in the IP, and in March of 2011, when CH₄ uptake in the UC was more negative than in the OA and IP. Although soil moisture increased significantly after the first autumn rains in October 2011 (Fig. 2b), our results showed no differences in soil CH₄ uptake or release after soil rewetting by rainfall. However, a significant negative correlation was observed between CH₄ fluxes and SWC for the UC (38 %, $P<0.05$ $n=11$) and OA (67 %, $P<0.05$, $n=11$) sites when SWC was higher than 7 % (Fig. 3a, b), i.e. above this limit soil CH₄ uptake had more negative values with increasing SWC. In contrast, for the IP soil water content above 4 % was positively correlated ($P<0.009$) with CH₄ release (Fig. 3c).

Net soil N₂O fluxes

Both, net uptake and net release of N₂O occurred and fluxes varied from -8.8 to $11 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ (Fig. 2d). As for CH₄, also N₂O uptake and release rates were larger in 2010 compared to 2011 (Fig. 2b), but similarly to CH₄, no correlation between N₂O fluxes and soil temperature in 2010 was found. The most pronounced differences between the studied sites were observed in July, August and October 2010, with higher N₂O release in the UC compared to the IP and OA. The first rainfall in October 2011 did not affect soil N₂O effluxes in either the UC, or OA.

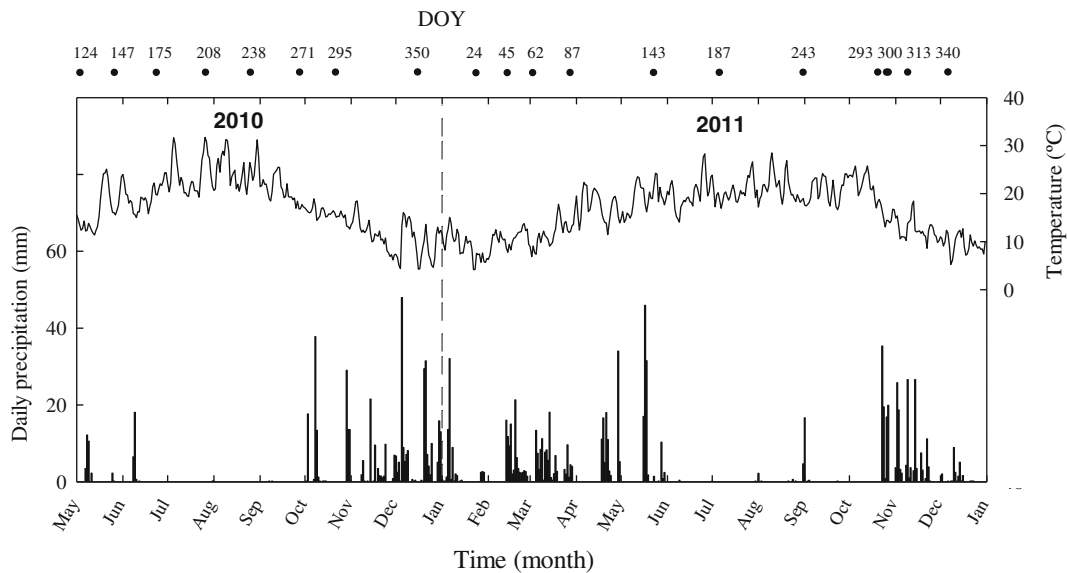


Fig. 1 Precipitation (mm) and air temperature (°C) measured during the study period from May, 2010 to December, 2011. Dots at the top of the figure indicate days of sampling during the trial. Data is relevant for all three study sites, i.e. UC, OA and IP

Our data showed that N_2O fluxes correlated with SWC only in the UC (45 %, $P < 0.008$, $n = 8$). Whenever SWC was above 3 %, soil N_2O fluxes had more negative values (Fig. 3d).

Soil CO_2 respiration rate (R_s)

Our results showed that during the study period CO_2 respiration rate ranged between 33 and 241 $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ (Fig. 2e). Largest R_s values were recorded in late June 2010 from the UC and OA when soil temperature was 25 ± 0.7 °C and 35 ± 1.1 °C, and SWC was 4.9 ± 0.5 % and 2.9 ± 0.5 %, respectively. In 2011 R_s was measured throughout the summer drought, when SWC exhibited the lowest value in the year (between 0.6 % and 2.9 %) and soil temperature the highest values (between 22 °C and 27 °C) (Fig. 2a, b). During the summer R_s maintained relatively constant values (Fig. 2e). Over the study period R_s in the IP was, on average, significantly lower ($P < 0.001$) than in the OA and UC (Fig. 2e). Soil rewetting in 2011 stimulated ecosystem respiration, i.e. after the dry summer rainfalls in 26th October (4 days after rainfall, Julian day DOY 299) and 27th October (5 days after rainfall, Julian day DOY 300) led to an increase ($P < 0.01$) in R_s by 4.8-fold, 2.4-fold and 1.2-fold on the IP, UC and OA sites, respectively (Figs. 1 and 2e). Despite this positive response of R_s to the higher SWC, we found no correlation

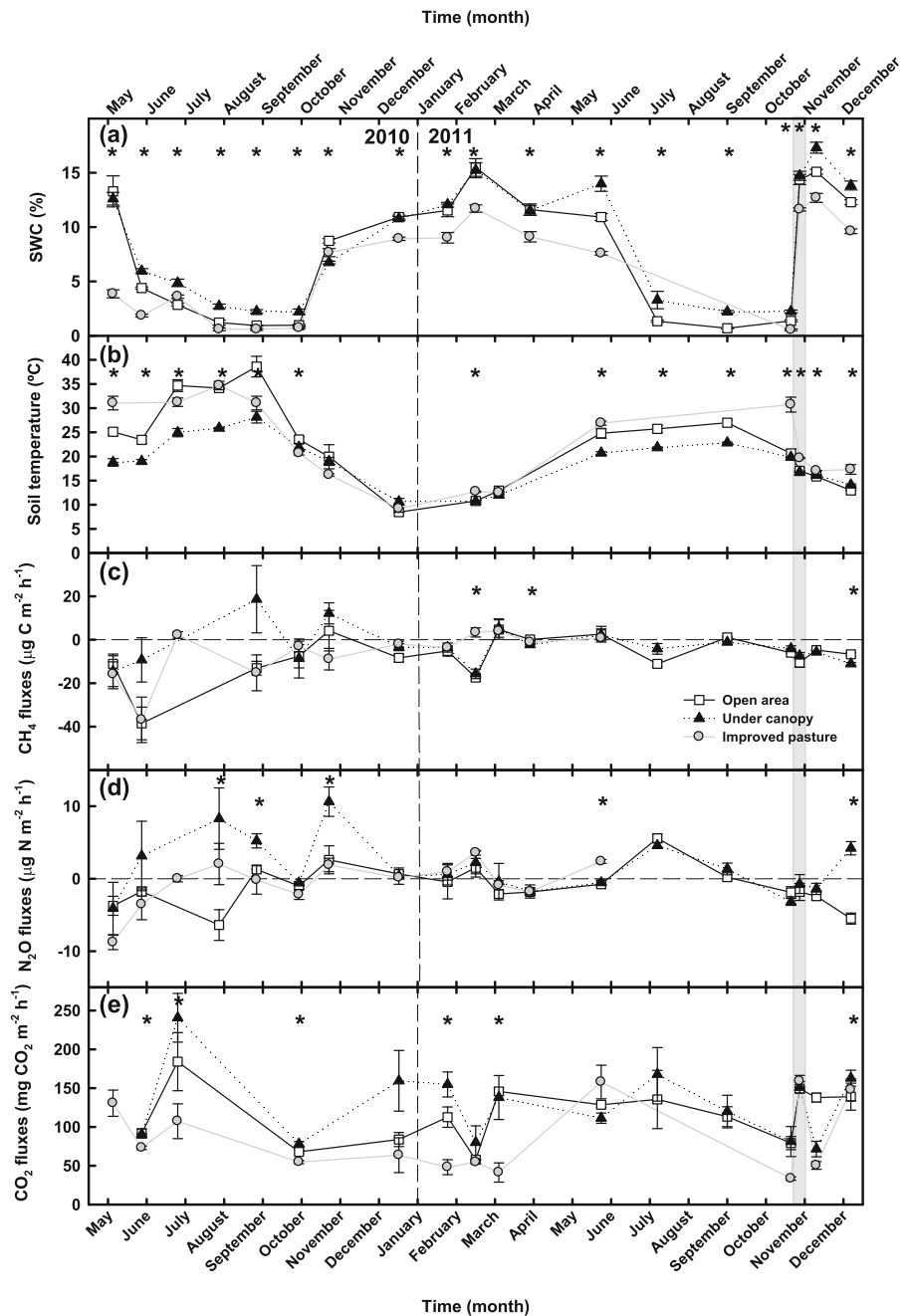
between R_s and either SWC, or soil temperature during the entire period of study for UC and OA. However, a significant correlation was observed between R_s and SWC (91 %, $P < 0.05$, $n = 6$) in IP, whenever SWC was below 10 %. Whenever soil water content was above 10 %, soil temperature was the driving factor of R_s ($R^2 = 0.49$, $P < 0.05$, $n = 6$). Our results also showed a positive correlation ($P < 0.05$) between cumulative rainfall for 5 and 7 days and R_s in the IP.

Soil total N, NH_4^+ -N and NO_3^- -N content

Soil total N in the UC was 1.4-fold higher ($P < 0.01$) as compared to the OA and had a significantly higher nitrification potential (mineralization capacity) relative to the OA and IP (Table 1).

Soil NH_4^+ -N content was low during the whole study period, ranging between 0 and 5.6 $\text{mg NH}_4^+\text{-N kg}^{-1}$ dry soil (Fig. 4a). As well as soil GHG fluxes, NH_4^+ -N and NO_3^- -N contents at all sites were more variable and higher from May 2010 until August 2010 when compared to equivalent period in 2011 (Fig. 4a, b). Nitrate contents varied between sites, i.e. NO_3^- -N content ranged between 1.2 and 4.4 $\text{mg N-NO}_3^-\text{ kg}^{-1}$ dry soil for the IP, from 0 to 12.3 $\text{mg N-NO}_3^-\text{ kg}^{-1}$ dry soil for the OA and between 0 and 9.7 $\text{mg N-NO}_3^-\text{ kg}^{-1}$ dry soil for the UC (Fig. 4b). In most measurements, NH_4^+ -N and NO_3^- -N content in the IP were higher ($P < 0.05$) as to compare with NH_4^+ -N and

Fig. 2 **a** Soil water content (%), **b** soil temperature recorded in the upper 5 cm, **c** soil CH_4 fluxes ($\mu\text{gCm}^{-2}\text{h}^{-1}$), **d** soil N_2O ($\mu\text{gNm}^{-2}\text{h}^{-1}$) fluxes, and **e** CO_2 respiration rate ($\text{mgCO}_2\text{m}^{-2}\text{h}^{-1}$) measured at the study site from May, 2010 to December, 2011. Data was collected once per month. Values are means \pm SE ($n=6$). Significant differences between plots ($P<0.01$) are indicated by stars directly above data points. In (e), values for CO_2 respiration rates in July, August, October and November, 2010 and April, 2011 were not registered due to lack of sensitivity of the equipment



NO_3^- -N content in the OA and UC, except in June and August 2010 and March, 2011.

Soil characteristics, litter fall and root density

The influence of trees on soil properties can be perceived from Table 1. Soil organic matter and C/N ratio were significantly higher in the UC compared to

the IP and OA. The presence of trees leads to a double input of dry mass per m^2 and per year in the UC as compared to the OA that is due to the litter fall (i.e. leaves, branches, flowers and fruits). In addition, the UC area has more than 2-fold and 4-fold higher root density when compared to the OA and IP, respectively. This higher SOM and C/N ratio of the UC reflect the long term effect (*ca* 50 years) that tree

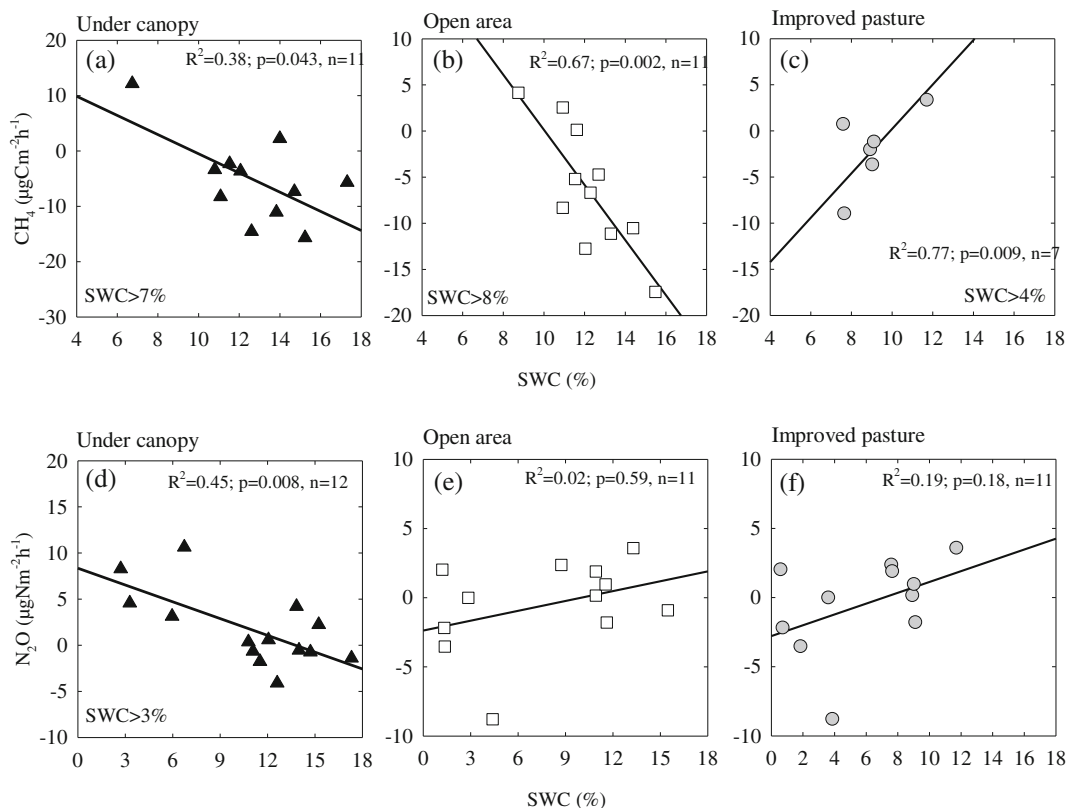


Fig. 3 Annual variation in soil CH₄ and N₂O fluxes correlated with SWC for the three study sites (OA, UC and IP). Negative correlations between CH₄ fluxes and SWC were observed for OA and UC above 7 %, and also for N₂O fluxes for UC above 3 %

presence and root regeneration and growth has had on the soil.

Considering the overall data, we found a significant correlation between SOM and soil CH₄ and N₂O fluxes (Fig. 5a, b). For individual treatments the correlation coefficient was significant for the UC ($R^2=0.64$, $P<0.001$ for CH₄ and $R^2=0.48$, $P<0.001$ for N₂O) and for the IP ($R^2=0.68$, $P<0.05$ for CH₄ and $R^2=0.86$, $P<0.01$ for N₂O). No correlation was observed between SOM and soil CO₂ fluxes.

Discussion

Net soil CH₄ fluxes

In general, our ecosystems appeared to act as methane sinks. The magnitude of the observed soil-atmosphere net CH₄ fluxes are within the range of values obtained for other Mediterranean and temperate, nitrogen-limited forest ecosystems (Rosenkranz et al. 2006;

Castaldi et al. 2007). Our data also confirmed the hypothesis that the soil in evergreen oak woodlands in Portugal maintained a consistent potential for methane oxidation (Shvaleva et al. 2011) during the course of the years 2010–2011. The measured uptake rates, in most cases of study (Table 2), stress that in dry ecosystems, methanotrophs are present and active (Gundersen et al. 2012).

Based on dimethyl sulphoxide (DMSO) studies, CH₄ oxidation in poor-N forest soils is carried out by methanotrophs (Saari and Martikainen 2001). Methanotrophs living at high CH₄ concentrations are well known (Siljanen et al. 2012), while the microbes capable of oxidising atmospheric CH₄ at low concentrations (i.e. with a high affinity for CH₄) are largely unknown (Menyailo and Hungate 2003).

Our data showed that the soil mineral N content is compatible with its use for growth by methanotrophic bacteria, whereas high rates of nitrification can produce toxic effects on CH₄-consuming bacteria through the production of N₂O and NH₂OH (Bowden et al.

Table 1 Soil properties, litter fall and roots density in each experimental site: Improved pasture (IP), Open area (OA) and Under canopy (UC). Letters show significantly differences ($P<0.05$) for each parameters. Values are mean (\pm SE)

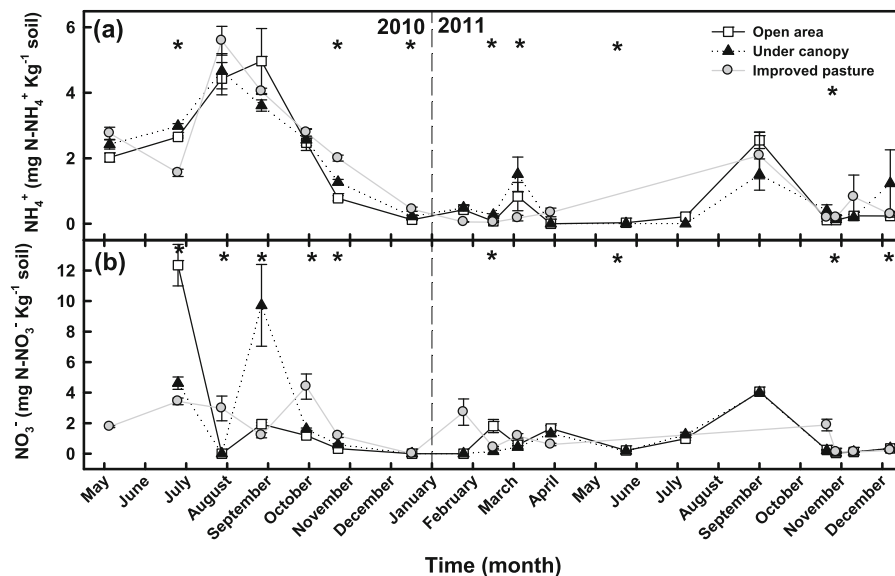
	Organic matter, %	N, mg kg ⁻¹ DW	C/N	Nitrification potential, $\mu\text{gN kg}^{-1}$ DW	pH	Electrical conductivity, mS cm ⁻¹	Roots density, gDW m ⁻²	Litter fall, gDWm ⁻² y ⁻¹
IP	2.5 \pm 0.1 ^a	1 \pm 0.7 ^{ab}	13.5 ^a	30 ^a	5.0 \pm 0.1 ^a	15 \pm 2 ^a	119 \pm 33 ^a	–
OA	2.4 \pm 0.1 ^a	0.8 \pm 0.03 ^a	16.6 ^{ab}	40 ^a	5.2 \pm 0.1 ^b	14 \pm 1 ^a	314 \pm 58 ^a	140
UC	4.3 \pm 0.3 ^b	1.1 \pm 0.1 ^b	22.1 ^b	240 ^b	5.1 \pm 0.1 ^{ab}	30 \pm 3 ^b	693 \pm 70 ^b	290

2000). As it was shown in Cruz et al. (2008), soil nitrate concentrations of around 40–50 ppm, inhibit methane oxidation by soil microorganisms by 10–20 %. Since in our study sites the nitrate concentrations were three times lower, we may assume that there would be little or no inhibitory effect on soil methane oxidation by methanotrophic bacteria. Our results also confirm previously obtained data for oak woodlands in Portugal, showing that low soil inorganic nitrogen content ($\text{NH}_4\text{-N}$ ranged between 0 and 13.5 mg $\text{NH}_4\text{-N kg}^{-1}$ dry soil and $\text{NO}_3\text{-N}$ ranged between 0.9 and 15.1 mg $\text{NO}_3\text{-N kg}^{-1}$ dry soil) is an important factor in determining that soils act mainly as a methane sink (Shvaleyeva et al. 2011).

Correlations between net CH_4 oxidation, soil water content in forest soils and grasslands have been reported in (Bowden et al. 2000; Bradford et al. 2001; Castaldi and Fierro 2005) but findings are not consistent and no

general rule seems to apply. For example, Ambus and Christensen (1995) observed no correlation between soil moisture and CH_4 uptake, whereas Dobbie and Smith (1996) observed a weak negative correlation and Castro et al. (1995) a strong negative correlation. Which of the two process (CH_4 consumption vs CH_4 production) will be predominant following the increase SWC will depend on the soil type (texture, porosity, profile depth) and other ecosystem characteristics (forests, grasslands). This might favor the formation of special microsites for CH_4 fluxes.

Our results showed that SWC drives both CH_4 and N_2O fluxes under certain conditions. That is, whenever SWC reached a certain threshold (7 % for CH_4 and 3 % for N_2O) net uptakes were observed to increase with SWC in natural understory (UC and OA). On the contrary, in the IP area, we observed the opposite trend, i.e. net CH_4 release increased with increasing SWC above

**Fig. 4** **a** Soil $\text{NH}_4^+\text{-N}$ (mg kg⁻¹ N DW) and **b** $\text{NO}_3^-\text{-N}$ (mg kg⁻¹ N DW) contents measured during the study period for UC, OA and IP on the same days as gas sampling was performed. Values are means \pm

SE ($n=6$). Significant differences between sites ($P<0.01$) are indicated by stars directly above data points

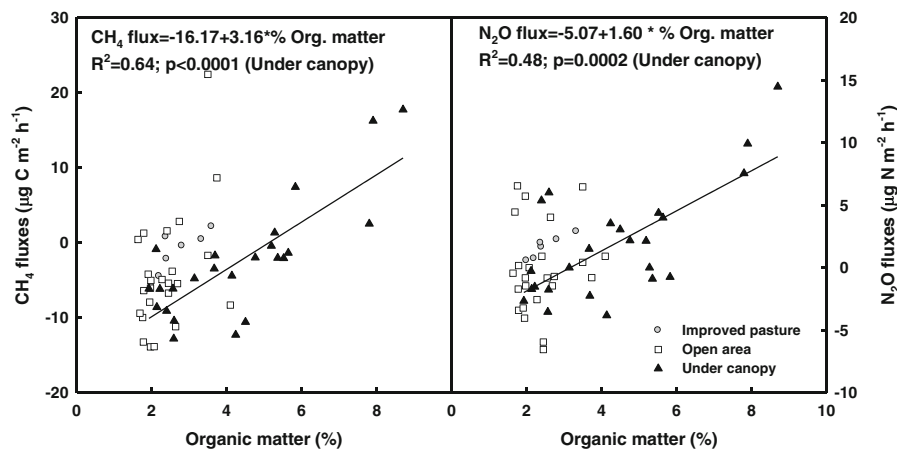


Fig. 5 Correlation between SOM content and soil CH₄ and N₂O fluxes in the UC, OA and IP. A *trend line* is shown only for the UC

4 %. We suggest that the different relationship that we observed between CH₄ and N₂O fluxes and SWC in the NU and the IP can be attributed to differences in soil microenvironment (soil bacterial communities) that we were not able to identify in this study.

Net soil N₂O fluxes

The low N₂O fluxes found in this work have been reported in previous studies for Mediterranean forest soils (Ryden 1981; Rosenkranz et al. 2006; Castaldi et al. 2006; Gundersen et al. 2012). Although both nitrification and denitrification can produce N₂O effluxes, our study does not allow us to distinguish their respective contribution.

In general, dry soils have larger aeration, which would be unfavourable for N₂O production by denitrification (Castaldi et al. 2006) or by nitrifier denitrification. Furthermore, drought limits the overall microbiological activity, reducing the amount of N cycled in the ecosystems. Therefore, lack of available N together with low water content would limit N₂O production in summer. In Mediterranean ecosystems, total N tends to be low (Rovira and Vallejo 1997). Mineralization rates are also generally low due to allelopathic compounds leached from plants and the quality of the sclerophyllous leaf, typical of such ecosystems (Gallardo and Merino 1992). This would result in low availability of mineral N in the soil, which could limit the rate of both nitrification and denitrification.

Higher N₂O release was observed in July, August and October in 2010 in the UC when compared with the IP and OA. There are various parameters that might have contributed to the N₂O peak and could partly explain these emissions. For example, higher soil moisture in the UC as compared with the OA and IP may have favoured microbial activity. On the other hand, the level of N-deposition and the NO₃⁻ in the soil might have promoted the sudden release of N₂O (Davidson et al. 2000).

Net negative N₂O fluxes in forests that are reported in the literature vary widely, from -0.0014 to -484 µg N₂O-N m⁻² h⁻¹ (Chapuis-Lardy et al. 2007). The net N₂O uptake measured in our study falls within this range. A few reports in the literature suggest that the soil may function as a N₂O sink under dry conditions (Rosenkranz et al. 2006; Goldberg and Gebauer 2009) but underlying reasons have not yet been identified. Rosenkranz et al. (2006) linked negative fluxes in Mediterranean forest soils to very low N availability and high soil C content, and considered aerobic denitrification by heterotrophic nitrifiers as a possible pathway. This should be true for our studied soil.

Our data show that in most cases, both soil N₂O and CH₄ fluxes of natural understory were small, as compared with soil-atmosphere exchange of N₂O and CH₄ of other forest ecosystems in Europe (Gundersen et al. 2012). Besides, soil N₂O and CH₄ fluxes of natural understory (the UC and OA), in general, did not differ from N₂O and CH₄ fluxes of the improved pasture. Consequently, it is possible that soil gas exchange in forest-to-agriculture land-use scenarios in Mediterranean-type ecosystems, has

Table 2 Reported soil CH₄ and N₂O fluxes and soil CO₂ respiration rates in the Mediterranean type ecosystems

Country/ Location ^a	Vegetation type and species	MAT (°C)	MAP (mm)	Reported CH ₄ (μg C m ⁻² h ⁻¹)	Reported N ₂ O (μg N ₂ O-N m ⁻² h ⁻¹)	Reported CO ₂ (μmol CO ₂ m ⁻² s ⁻¹)	Method ^b	Reference
Central Portugal (39°08'18.29" N, 8°19'57.68"W)	Holm and cork oak forest: <i>Quercus ilex</i> L., <i>Quercus suber</i> L. Shrubs and grasses: <i>Cistus spp.</i> , <i>Lavandula stoechas</i> , <i>Rosmarinus officinalis</i> , <i>Ulex spp.</i> , etc. Shrubs and grasses: <i>Cistus spp.</i> , <i>Lavandula stoechas</i> , <i>Rosmarinus officinalis</i> , <i>Ulex spp.</i> , etc. Grasses: <i>Trifolium spp.</i> , <i>Ornithopus spp.</i> , <i>Vulpia spp.</i> , <i>Ornithopus spp.</i> , etc.	15.9	888	-16 to 19 μg C m ⁻² h ⁻¹	-4 to 11	0.4 to 1.5	MC, IR	Own study
Southern Portugal (38°31'40" N, 8°00'0.1"W)	Cork oak forest: <i>Quercus suber</i> L. Shrubs and grasses: <i>Cistus spp.</i> , <i>Lavandula stoechas</i> , <i>Rosmarinus officinalis</i> , Coppiced eucalypt: <i>Eucalyptus globulus</i> L.	15.5	669	-39 to 4.7 μg C m ⁻² h ⁻¹ -37 to 4 μg C m ⁻² h ⁻¹ -40 to -0.5 μg C m ⁻² h ⁻¹	-6 to 6 -9 to 4 -7.4 to 47.3	0.3 to 1.0 0.3 to 1.6	MC, IR	Shvaleva et al. (2011)
Southern Portugal (38°38' N, 8°36'W)	Shrubs and grasses: <i>Cistus spp.</i> and C3 grasses	15.9	608	-	-	1 to 3.6	IR	Correia et al. (2012)
Southern Portugal (38°47' N, 7°25'W)	Shrubs and grasses: <i>Cistus spp.</i> and C3 grasses	15.9	608	-	-	0.8 to 4.5	IR	Correia et al. (2012)
Spain, Southeast Andalusia (36°56'26.0"N, 2°01'58.8"W)	Steppe alpha grass: <i>Stenacissima</i> , <i>Chamaerops humilis</i> , <i>Rhamnus lycioides</i> , <i>Asparagus horridus</i> , <i>Rubia peregrina</i> , etc.	18	220	-	-	0.8 to 1.1 (annual average)	IR	Rey et al. (2011)
Central Spain (41°13'N, 0°55'E)	Holm oak forest: <i>Quercus ilex</i> L., <i>Phillyrea latifolia</i> L., <i>Arbutus unedo</i> L. Shrubs: <i>Erica arborea</i> L., <i>Juniperus oxycedrus</i> L., <i>Cistus albidus</i> L. Deciduous spp.	12	658	-	-	2.3±0.3 (annual average)	IR	Asensio et al. (2007)
Central Spain (40°35' 39.3"N, 3°55'30.5"W)	Holm oak forest: <i>Quercus ilex</i> L. Shrubs and grasses: <i>Cistus ladanifer</i> , <i>Lavandula stoechas</i> , <i>Rosmarinus officinalis</i> , <i>Lygeum spartum</i> , etc.	14	655	-8.1 to 4.1 μg CH ₄ -C m ⁻² h ⁻¹	-11.4 to 8.3	-	MC	Inclan et al. (2012)
Central Spain (40°43' 21.2"N, 4°07'20.1"W)	Scots pine forest: <i>Pinus sylvestris</i> L. Shrubs and grasses: <i>Genista cinerea</i> , <i>Adenocarpus hispanicus</i> , <i>Rosa canina</i> , <i>Pteridium aquilinum</i> , <i>Rumex scutellatus</i>	8	895	-24.5 to 6.1 μg CH ₄ -C m ⁻² h ⁻¹	-28.2 to 21.9	-	MC	Inclan et al. (2012)
Central Spain (40°34' 07.8"N, 4°08'54.8"W)	Pyrenean oak forest: <i>Quercus pyrenaica</i> L. Shrubs and grasses: <i>Fraxinus angustifolia</i> , <i>Cytisus scoparius</i> , <i>Genista florida</i> , <i>Rubus ulmifolius</i> , <i>Tamus communis</i>	13	788	-7.7 to 3.0 μg CH ₄ -C m ⁻² h ⁻¹	-7.7 to 13.5	-	MC	Inclan et al. (2012)
Central Italy (42°24'N, 11°55'E)	Coppiced oak: <i>Quercus cerris</i> L., <i>Q. suber</i> L., <i>Q. ilex</i> L. Shrubs: <i>Prunus spinosa</i> L., <i>Ruscus aculeatus</i> L., <i>Coletea arborescens</i> L.	14	755	-	-	1.7 to 7.8	IR	Tedeschi et al. (2006)
		15.8	760	-	-	-	MC	

Table 2 (continued)

Country/ Location ^a	Vegetation type and species	MAT (°C)	MAP (mm)	Reported CH ₄ (CH ₄ m ⁻² day ⁻¹)	Reported N ₂ O (μg N ₂ O- Nm ⁻² h ⁻¹)	Reported CO ₂ (μmol CO ₂ m ⁻² s ⁻¹)	Method ^b	Reference
Southern Italy (40°57'N, 1°33'E)	Shrubland: <i>Quercus ilex</i> L., <i>Phillyrea angustifolia</i> L., <i>Pistacia lentiscus</i> L., <i>Cistus</i> spp., <i>Myrtus communis</i> L., <i>Rosmarinus officinalis</i> L.			-0.39 to -16.1 mg CH ₄ m ⁻² day ⁻¹				Castaldi and Fierro (2005)
Italy, Tuscany (10°17'3"E, 43° 43'58"N)	Pine forest: <i>Pinus pinaster</i> L., some <i>Q. ilex</i> L., <i>Erica arborea</i> , <i>Phyllirea angustifolia</i> , <i>Rhamnus alaternus</i> , <i>Myrtus communis</i>	14.1	918	-73.3 (spring) -59.7 (autumn) μg C m ⁻² h ⁻¹	-4.3 (spring) and -7.9 (autumn)		AC	Rosenkranz et al. (2006)

MAT mean annual temperature, MAP mean annual precipitation, AC automated chamber technique, IR infrared gas analysis technique, MC manual chamber technique

^a Locations are reported from literature

^b Methodologies

no major potential contribution to atmospheric heating/warming.

Effect of first rain events on soil CH₄ and N₂O fluxes

Gundersen et al. (2012) considered the dry Mediterranean forests as resilient ecosystems concerning GHG emissions, since the rainfall exclusion/addition had no effect on CH₄ and N₂O exchange, although water was expected to be a limiting factor. In our study soil CH₄ and N₂O emissions were not significantly influenced by rain events in October 2011. It is possible that CH₄ and N₂O emissions were influenced by 1) a combination of higher SWC (lower soil O₂ concentration) after rains coupled with either gas transport limitation in soil saturated with water (Bradford et al. 2001), or substrate availability (amount of SOM and high molecular weight of soil organics that need more time for degradation) (Fang et al. 2009), 2) a summer moisture deficit that affects soil bacterial communities and resource competition, eliciting a strong selective pressure on the structure and functioning of communities (Lynch and Whipps 1990), 3) rapid gas flux pulses after rain events (during the first 24 h) that were not measured, or 4) a delay in soil gas responses for certain days that were not captured in the measurements.

Soil total N, NH₄⁺-N and NO₃⁻-N content

The higher soil mineral N content observed in the IP as compared to NU, might be related to the presence of leguminous plants that were reseeded in the IP before the study period. Besides, during spring-summer 2010, NH₄⁺-content was higher than in the same period of 2011, presumably because it could not be nitrified due to the lower precipitation in spring-summer 2010 (and the consequently lower soil moisture). Moreover, NH₄⁺-N and NO₃⁻-N content, as well as soil CH₄ and N₂O fluxes, were more variable and higher during May–August 2010 as compared to the same period in 2011, supporting the hypothesis that soil GHG fluxes were closely related to soil N content in these forms, microbial activity and N turnover (Gundersen et al. 2012).

Soil CO₂ respiration rate (Rs)

The effect of soil moisture on soil CO₂ respiration rates in the Mediterranean basin has been reported in a range of environments (forests, shrublands, grassland) (Joffre et al.

2003; Jarvis et al. 2007; Inglema et al. 2009; Correia et al. 2012; Jongen et al. 2013). Rewetting of dried soils is known to cause increased mineralization of C (Lundquist et al. 1999) and N (Cabrera 1993) and is coupled with a flush of CO₂ efflux (Jarvis et al. 2007). While these processes are not entirely a result of microbial activity, the underlying mechanisms may be related to changes in the diversity and activity of bacterial populations (Conrad 1996).

For Mediterranean ecosystems, maximum rates of net ecosystem carbon exchange coincide with the peak of photosynthesis in spring (Pereira et al. 2007), as well as with favourable conditions for soil microbial decomposition (Caritat et al. 2006). The decrease in *R_s* in summer is in line with the onset of the dry period followed by the decline of tree growth and senescence of the herbaceous vegetation (Baldocchi et al. 2010). However, we did not observe an increase of *R_s* in the peak of the growing season (spring) as was observed in other studies (Jarvis et al. 2007; Correia et al. 2012). This discrepancy can be partly attributed to the insufficient number of measurement days during this period (Fig. 2e). Besides, the largest *R_s* rates that were recorded in June, 2010 in the natural understory (UC and OA) are possibly related with a higher contribution of tree root respiration (Pinto et al. 2011), as herbaceous plants were senescent at this time of year. The correlation that was observed between *R_s* and SWC above the 10 % threshold in SWC for the IP is in agreement with previously published data for Mediterranean ecosystems (Asensio et al. 2007; Almagro et al. 2009; Correia et al. 2012) and reinforces that *R_s* is affected by other environmental factors besides purely climatic variability. Nonetheless, our results show that the first rain events in October 2011 (DOY 293) after a dry summer, increased soil CO₂ emissions (DOY 299 and 300). These data are in agreement with previously observed strong increases in *R_s* stimulated by autumn rains observed in the Mediterranean basin (Jarvis et al. 2007). Our results show that *R_s* in the NU tended to be higher than in the IP which can be due to a higher contribution of root respiration and to complex effects of trees on soil properties, namely ectomycorrhizas associated with *Quercus spp.*, and to diversity and structure of soil microorganisms (Haskl et al. 2004; Lejon et al. 2005).

Soil characteristics, litter fall and root density

Our results show that cork oak trees influence soil characteristics and thus affect GHGs (Table 1). In the UC, the

marked positive correlation between SOM (a key component, holding moisture, improving soil macropore structure and providing substrate for microbes) and soil CH₄ and N₂O, indicates that fluxes are highly dependent on soil conditions favourable to microbial activity. This implies that oak trees provide litter for decomposition, generating SOM and higher soil fertility (Caritat et al. 2006; Tate et al. 2007). Soil organic matter, nitrogen content, nitrification potential, and electrical conductivity were higher in the UC than in the IP and OA (Table 1). All of this, together with the highest root density and soil C/N ratio for the UC can affect the structure of the microbial community, species composition and diversity (Lejon et al. 2005).

Plants and microorganisms strongly compete for nitrogen and other nutrients released from SOM mineralization. Generally, the rhizosphere offers microbes an environment rich in nutrients, due to root exudates, hence rhizosphere CO₂ and N₂O emissions tend to be larger than in non-rhizosphere soil. This is largely in agreement with our data, which showed that over the study period, *R_s* for the IP with a lesser root density was, on average, significantly lower than for the UC and OA. However, we cannot rule out in our study that competition between plants and microorganisms can reduce nutrients availability for soil bacterial communities responsible for GHG effluxes.

Conclusions

Our results supported the hypothesis that in nutrient poor soils oak trees influence soil properties and affect net GHG exchange. That is, cork oak trees led to increased input of litter for SOM, which together with soil moisture plays an important role in soil-atmosphere GHGs exchange. The presence of trees results in higher root density in the natural understory compared to the improved pasture, leading to increased soil CO₂ respiration rates. Nonetheless, although we found no differences between NU and IP concerning CH₄ and N₂O fluxes, our results contribute to the understanding of the mechanisms underlying CH₄ and N₂O responses to changes in environmental conditions. We hypothesize that changing land-use from understory towards improved pasture (with increased mechanical disturbances of the top soil layer) can change the community structure of soil microorganisms that are sensitive to stress and, therefore, soil-atmosphere GHG exchange.

Further studies should focus on the spatial variability of evergreen oak woodlands in terms of soil characteristics, vegetation, and soil biodiversity over a longer period of time. Moreover, modelling approaches can improve our knowledge about the contribution of soil GHG fluxes from Mediterranean type ecosystems to the global GHG balance. This emphasized the need for better estimates of soil-atmosphere GHG fluxes, which can only be achieved with additional field data on microbiological communities.

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