



Environmental and biological controls on CH₄ exchange over an evergreen Mediterranean forest



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ABSTRACT

Methane (CH₄) fluxes in a Mediterranean holm oak (*Quercus ilex* L.) forest were measured over 19 months, using eddy covariance technique and soil chambers measurements, complemented by microbiological characterization of CH₄-cycling microorganisms along a vertical soil profile.

CH₄ budget at the site was close to the neutrality, although periodical switching of the ecosystem from sink to source was observed at daily and seasonal scale. Forest soil represented a net sink for atmospheric CH₄ throughout the whole study period (mean uptake rate of $1.64 \times 10^{-3} \mu\text{mol m}^{-2} \text{s}^{-1}$).

Different environmental parameters influenced CH₄ fluxes at different time scales.

At half-hour timescale, CH₄ fluxes followed a half-sinusoid curve with high emission recorded during the central hour of day (mean emission rate of $1.05 \times 10^{-2} \mu\text{mol m}^{-2} \text{s}^{-1}$). Stomatal conductance, solar radiation (global and UV) and latent heat flux were the main drivers controlling ecosystem CH₄ emissions, suggesting the occurrence of a plant-mediated transport through xylem as a way of CH₄ emissions from canopy and supporting the hypothesis of UV-induced production of CH₄ from leaves at the site.

At daily scale, CH₄ fluxes were strongly connected to temperature and precipitation which promote CH₄-oxidizing microorganisms activity during the cold season and methanogens activity during the dry season.

This study is the first long-term CH₄ eddy covariance measurement above a Mediterranean forest and demonstrates that, at present, the ecosystem is a small sink of CH₄ when considering both the soil and vegetation processes together, and this sink capacity is strictly connected to the water availability. Future changes in temperature and precipitation patterns may increase CH₄ emissions, turning the ecosystem to a source of CH₄.

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1. Introduction

Methane (CH₄) is the most abundant hydrocarbon and the third greenhouse gas in the atmosphere after water vapour (H₂O) and carbon dioxide (CO₂). In the frame of climate change, this gas is of particular concern for its global warming potential (over 100 years), 28–36 times higher compared to CO₂ (IPCC, 2013) and for its indirect effect on aerosols and other chemical compounds through modification of the atmosphere oxidative capacity (Shindell et al.,

2009). Compared to preindustrial values, CH₄ concentration has increased by 2.5 times (Etheridge et al., 1992). The total global CH₄ source strength has been estimated at 600 T CH₄ y⁻¹ (Lelieveld et al., 1998) due to both natural sources, mainly wetlands, and anthropogenic activities, such as biomass burning, fossil fuel production, livestock and rice paddies (Denman et al., 2007).

In terrestrial ecosystems, the largest biological sink for CH₄ is represented by microorganisms in aerobic soils (Stuedler et al., 1989) which consume 1–10% equivalent of the total global emissions of CH₄ (Watson et al., 1992). The global terrestrial sink of CH₄ has been estimated to be 29 Tg CH₄ y⁻¹, with a wide uncertainty range (Smith et al., 2000). Soil CH₄ can be oxidised by both methanotrophic and nitrifying bacteria (Castro et al., 1995). CH₄ production by methanogenic bacteria is strictly limited to anaerobic environments, and plays a minor role in well-drained soils.

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In forest ecosystems, soil is the primary compartment where CH_4 exchange takes place: uptake of atmospheric CH_4 is driven by methanotrophic bacteria, responsible for CH_4 oxidation in aerobic soils, whereas emission is regulated by methanogenic archaea, under anoxic conditions (Trotsenko and Khmelenina, 2002). Recently, several studies have suggested a contribution of forest vegetation to CH_4 emission (Keppler et al., 2006) although the significance of this emission is still under debate (McLeod and Keppler, 2010; Bruhn et al., 2012). The main mechanisms of CH_4 emission identified so far are: contribution via the transpirational stream through the xylem (Zeikus and Ward, 1974), anaerobic source in the trunk (Mukhin and Voronin, 2011; Covey et al., 2012), induction by UV radiation from plant tissues (McLeod et al., 2008; Vigano et al., 2008), wounding (Wang et al., 2009), heat (McLeod et al., 2008; Vigano et al., 2008) or reactive oxygen species (Sharpaty, 2007; Vigano et al., 2008).

CH_4 exchange between ecosystems and atmosphere is commonly measured using soil enclosures with different configurations (Pihlatie et al., 2013). In the last decade, the eddy covariance (EC) technique was successfully employed to study CH_4 fluxes at ecosystem level (Rinne et al., 2007; Detto et al., 2011). Although EC method have been widely applied over CH_4 emitting ecosystems, such as wetland and peatland (see Petrescu et al., 2015 for a review), few studies were carried over forests. Most of them report net emission of CH_4 , as Hommeltenberg et al. (2014), who measured a CH_4 emission of $0.33 \pm 0.02 \times 10^6 \mu\text{mol m}^{-2} \text{y}^{-1}$ over a bog pine forest in Germany; or Shoemaker et al. (2015), who indicates a net CH_4 emission up to $94.34 \pm 12.81 \times 10^6 \mu\text{mol m}^{-2} \text{y}^{-1}$ from forested subtropical wetlands in Florida. Some studies observed a net CH_4 sink, as Smeets et al. (2009), who measured a CH_4 daily uptake ($-0.37 \times 10^3 \mu\text{mol m}^{-2} \text{d}^{-1}$) over a Ponderosa pine forest in California, and Wang et al. (2013), who recorded a small CH_4 sink (average flux: $-2.7 \pm 0.13 \times 10^{-3} \mu\text{mol m}^{-2} \text{s}^{-1}$) over a temperate forest in Ontario. Few studies show a periodical switch of forests from source to sink over diurnal or seasonal cycle, as Sakabe et al. (2011), who measured CH_4 fluxes over a Japanese evergreen coniferous, which was a net sink during Spring and Summer, and a net source during Autumn and Winter.

These studies demonstrate that CH_4 flux magnitude over different forest types is widely variable, depending on forest and soil types, and even the direction of fluxes can change, in response to different meteorological variables. This variability is mainly due to microbial responses to environmental factors influencing microbial activity, such meteorological conditions, soils texture, N availability and salinity (Dalal et al., 2008; Bissett et al., 2012; Zhang et al., 2014). Due to their role in controlling CH_4 behaviour, the understanding of soil microorganisms involved in the CH_4 cycle represents a cognitive platform for any CH_4 study aimed to explore the connection between ecosystems and CH_4 fluxes.

The Mediterranean region is characterized by pronounced seasonality where forest ecosystems are subjected to a wide range of climate conditions, making this area an ideal testing site for CH_4 fluxes dependence on meteorological parameters. Furthermore, this region is predicted to experience a pronounced decrease in precipitations and warming in the next years (Giorgi and Lionello, 2008).

This paper presents an integrated approach to study CH_4 dynamics in a Mediterranean holm oak forest in central Italy. 19-month long micrometeorological measurements above canopy were complemented by periodical measurements at localized sites, utilizing closed static soil chamber method, and one microbiological characterization of CH_4 -cycling microorganisms along a soil vertical profile, with specific focus on the methanogens and methanotrophic abundance.

Our study is the first long-term CH_4 EC measurements above a Mediterranean forest aims to explore environmental and biologi-

cal mechanisms controlling CH_4 exchange over a forest site where anaerobic soil production may not be predominant.

In particular, our aims were: 1) to assess the magnitude and the direction of CH_4 fluxes above the canopy in order to test whether the holm oak forest is a CH_4 sink or a source; 2) to evaluate the contribution of soil and vegetation to the total flux; 3) to test the effects of different environmental variables over CH_4 emission and uptake at different time-scales, assuming that the net CH_4 flux is correlated with the abundance of methane-cycling microbes in soil.

2. Materials and methods

2.1. Study site

The study was carried out in a holm oak (*Quercus ilex* L.) forest located within the presidential estate of Castelporziano, 25 km from Rome city centre ($41^\circ 70' 42'' \text{N}$, $12^\circ 35' 72'' \text{E}$), and is part of the Italian network for Long Term Ecological Research (LTER). The forest is an unmanaged coastal rear dune ecosystem, canopy mean height is 14 m and its structure is homogeneous with a leaf area index of $3.69 \text{ m}^2 \text{ leaf m}^{-2}$ ground. Soil has a flat topography, with a sandy texture, and low water-holding capacity. The main soil physic-chemical properties are as follows: 33 g kg^{-1} clay, 116 g kg^{-1} silt and, 851 g kg^{-1} sand; pH in H_2O 6.85; total organic C 8.73 g kg^{-1} ; total N, 0.56 g kg^{-1} ; C/N ratio 13.74. The understory vegetation is poorly developed and formed prevalently by small shrubs of *Phillyrea latifolia* L. Climate is typically Mediterranean with pronounced seasonality: Summer periods are hot and dry, Winters are moderately cold, whereas precipitation occurs prevalently during Spring and Autumn. Mean annual precipitation \pm standard deviation and mean annual temperature \pm standard deviation for the period 2012–2014 were $728 \pm 151 \text{ mm}$ and $16.1 \pm 6.8^\circ \text{C}$, respectively. For the same period, annual minimum and maximum temperatures \pm standard deviation were $-2.3 \pm 1.5^\circ \text{C}$ and $33.2 \pm 2.3^\circ \text{C}$. Mean of 3-month cumulative precipitation \pm standard deviation recorded during winters (January–March) and summers (July–September) of 2012–2014 were $256 \pm 115 \text{ mm}$ and $100 \pm 56 \text{ mm}$. More details about temperature and precipitation regime are reported in Fig. 6.

The wind pattern at the forest followed a sea-land breeze regime, the dominant wind direction is S-SW during the morning and N-NE during the afternoon as shown in Fares et al. (2014).

Measurements above canopy were carried out from the top of an experimental tower 22 m high during a 19-month long period (from August 2012 to March 2014). The contribution of the forest area around the tower to the eddy covariance (EC) measurements (fetch area) was evaluated according to Hsieh et al. (2000). Peak distance from measuring point to the maximum contributing source area outreached 80 m and 2 km up-wind from the tower during unstable and stable conditions, respectively.

2.2. Eddy covariance and meteorological measurements

CH_4 , CO_2 and H_2O vertical fluxes were calculated according to the EC technique (Aubinet et al., 2012). EC method integrates fluxes over a large area and is representative of the whole ecosystem. The flux calculation was made on an averaging period of half-hour, positive fluxes indicate gas release to the atmosphere while negative fluxes indicate uptake from the atmosphere.

CH_4 concentration was measured by an open path gas analyser (LI-7700, Li-Cor, Inc., Nebraska, USA), CO_2 and H_2O concentrations were measured by a closed path infrared analyser (LI-7200, Li-Cor, Inc., Nebraska, USA). Instantaneous three-dimensional wind velocity and direction were measured with a sonic anemometer (Windmaster 3d Anemometer, Gill Instruments Limited, UK). Raw

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