Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil

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A B S T R A C T

Aerated soils represent an important sink for atmospheric methane (CH_4), due to the effect of methanotrophic bacteria, thus mitigating current atmospheric CH_4 increases. Whilst rates of CH_4 oxidation have been linked to types of vegetation cover, there has been no systematic investigation of the interaction between plants and soil in relation to the strength of the soil CH_4 sink. We used quasi-continuous automated chamber measurements of soil CH_4 and CO_2 flux from soil collar treatments that selectively include root and ectomycorrhizal (ECM) mycelium to investigate the role of rhizosphere activity as well as the effects of other environmental drivers on CH_4 uptake in a temperate coniferous forest soil. We also assessed the potential impact of measurement bias from sporadic chamber measurements in altering estimates of soil CO_2 efflux and CH_4 uptake. Results show a clear effect of the presence of live roots and ECM mycelium on soil CO_2 efflux and CH_4 uptake. The presence of ECM hyphae alone (without plant roots) showed intermediate fluxes of both CO_2 and CH_4 relative to soils that either contained roots and ECM mycelium, or soil lacking root- and ECM mycelium. Regression analysis confirmed a significant influence of soil moisture as well as temperature on flux dynamics of both CH_4 and CO_2 flux. We further found a surprising increase in soil CH_4 uptake during the night, and discuss diurnal fluctuations in atmospheric CH_4 (with higher concentrations during stable atmospheric conditions at night) as a potential driver of CH_4 oxidation rates. Using the high temporal resolution of our data set, we show that low-frequency sampling results in systematic bias of up-scaled flux estimates, resulting in under-estimates of up to 20% at our study site, due to fluctuations in flux dynamics on diurnal as well as longer time scales.

1. Introduction

Biogenic trace gases such as carbon dioxide (CO_2) and methane (CH_4) play a pivotal role in global climate change (Ciais et al., 2013; Tian et al., 2016). Anthropogenically driven increases in atmospheric CO_2 from fossil fuel combustion and land-use change are the main drivers of climate change. Increasing atmospheric CH_4 concentrations are now thought to contribute 20% of the total greenhouse gas warming (Ciais et al., 2013; Myhre et al., 2013). For anthropogenic CH_4 emission sources, rice cultivation, ruminants, landfills, and gas evasion during fossil fuel extraction dominate (Ciais et al., 2013; Myhre et al., 2013). Methane oxidation in upland soils represent an important sink for atmospheric CH_4, but poor constraints on the uptake of atmospheric CH_4 by soil microorganisms contributes to overall uncertainty in the global atmospheric CH_4 budget, and predictions of how soil-atmosphere feedbacks may modulate future changes in atmospheric CH_4 concentrations (Kirschke et al., 2013; Nisbet et al., 2014). Similarly, whilst the dynamics and drivers of CO_2 exchange from terrestrial ecosystems are reasonably well understood (Jung et al., 2011), there remain significant uncertainties around feedbacks between plants, soil microbes, and the potential role of rhizosphere priming effects (Talbot et al., 2013).

Trace gas fluxes between soil and atmosphere are directly influenced by the spatial and temporal variations in biotic and abiotic conditions and biogeochemistry. For CO_2 in particular, the role of temperature and soil water availability on heterotrophic decomposition

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of soil organic matter is well described (Barron-Gafford et al., 2011; Moyano et al., 2012), and also the role of autotrophic (root derived) substrate supply to the rhizosphere is accepted as an important driver of soil metabolic activity (Högberg et al., 2001; Singh et al., 2004). There is further an increasing acceptance of the significance of ectomycorrhizal (ECM) hyphae as recipients of autotrophic C supply in belowground carbon cycling of temperate forests (Subke et al., 2011; Heinemeyer et al., 2012). Soil C priming, whereby plant-derived substrates enhance heterotrophic SOM decomposition by soil micro-organisms, has also been described in a wide range of soil conditions (Kuzyakov et al., 2000; Subke et al., 2004), underlining an important interaction between autotrophic and heterotrophic soil C turnover. For CH$_4$ dynamics, there is a lack of knowledge regarding the interaction with belowground plant C supply. Whilst the influence of soil conditions such as water content, redox potential and (to a lesser extent) temperature are generally well described, we lack field-based data for interactions of methane oxidation with autotrophic C supply in upland soils. It is known that low molecular weight compounds (i.e. single carbon, or 'C1' molecules) exuded from roots or ectomycorrhizal hyphae support a diverse bacterial community in the rhizosphere (Fransson et al., 2016), potentially including atmospheric CH$_4$ oxidizers This is because methanotrophs are able to subsist on other simple C1 compounds (e.g. methanol, formaldehyde, formate) when CH$_4$ is scarce (Hanson and Hanson, 1996). As a consequence, the greater diversity and availability of labile C compounds in the rhizosphere may buffer methanotrophic populations during periods when CH$_4$ availability is low. Moreover, mineralization of nutrients from soil organic matter in the rhizosphere may alleviate nutrient limitation among methanotrophs, promoting larger and more active methanotrophic populations (Bodelier and Laanbroek, 2004; Veraart et al., 2015).

One of the main methodological challenges lies in understanding how trace gas fluxes respond to changes in biotic and abiotic variables that fluctuate over relatively short timescales (e.g. hours to days) (Groffman et al., 2009; Savage et al., 2014). These phenomena are difficult to study because of the limitations imposed by conventional low frequency sampling techniques. For example, transient weather phenomena – such as rainfall events, atmospheric pressure variations, or changes in wind speed – can profoundly alter soil-atmosphere fluxes by affecting gas transport processes (Tokida et al., 2007; Yano et al., 2014; Redeker et al., 2015) or rates of biological activity (Groffman et al., 2009; Liptzin et al., 2011; Heinemeyer et al., 2012; Yano et al., 2014). Diurnal fluctuations in temperature, moisture, irradiance, or atmospheric conditions can also modulate trace gas fluxes through direct or indirect effects on the metabolic activity of plants and microorganisms (Subke and Bahn, 2010; Baldocchi et al., 2012; Hatala et al., 2012; Wang et al., 2013). Sporadic trace gas measurements run the risk of systematic bias of true flux estimates, as fluctuations in drivers are not captured appropriately, and specific times of day when measurements are typically carried out (e.g. around midday) represent only a partial sample of diurnal conditions or flux dynamics. Whilst there are some investigations of impacts of sampling intervals and bias from limited diurnal sampling windows (Savage et al., 2014; Ueyama et al., 2015), a further quantification of uncertainty associated with manual/sporadic vs. automated/continuous measurements is necessary to capture site specific conditions and inform comparisons among studies.

Methane oxidation in well-drained soils, in particular, is significantly affected by CH$_4$ availability (Bender and Conrad, 1992; Hanson and Hanson, 1996; Tate et al., 2012), which may rapidly fluctuate based on local meteorological conditions (Baldocchi et al., 2012; Redeker et al., 2015). However, evidence for a concentration-based effect on atmospheric CH$_4$ oxidation has largely been obtained from laboratory incubations using high concentrations of CH$_4$, which exceed values normally observed in well-drained, aerobic soils, mimicking instead microaerophilic or near-anaerobic wetland conditions (Bender and Conrad, 1992; Teh et al., 2006; Templeton et al., 2006; Tate et al., 2012; Malghani et al., 2016). Field studies of CH$_4$

concentration effects under ambient conditions are far less common, because past work on atmospheric CH$_4$ oxidation has focused on isotope fractionation effects rather than on uptake kinetics (King et al., 1989; Reeburgh et al., 1997). Thus, it is unclear if fluctuations in atmospheric CH$_4$ concentrations significantly influence CH$_4$ uptake *in situ* because of the prevalence of other environmental drivers (e.g. moisture, temperature) and the narrow range over which atmospheric CH$_4$ concentrations typically vary.

Here we present the results from a quasi-continuous automated flux chamber experiment that investigated the effects of rapid, short-term fluctuations (i.e. hourly) in environmental variables and the presence or absence of plant roots and/or extra radical ECM mycelium in modulating soil-atmosphere fluxes of CO$_2$ and CH$_4$ from a temperate forest soil. The aim of this research was to: (a) establish if the presence of an intact rhizosphere significantly altered rates of trace gas exchange; (b) determine if rapid, short-term fluctuations in environmental variables influenced CO$_2$ and CH$_4$ fluxes in temperate forest soils; and (c) identify potential measurement bias from discontinuous sampling strategies.

2. Methods

2.1. Study site

The field site is a 19-year-old (in 2009) forest stand dominated by *Pinus contorta* and *Pinus sylvestris* (approximate height: 6–8 m) with occasional *Betula pendula* but no ground cover, situated approximately 8 km south of York, UK (53°54′38″N 0°59′54″W). The site has a well-draining sandy gley podzol overlain by a thin (c. 3 cm on average) organic horizon and a litter layer of between 1 and 2 cm. The pH (H$_2$O) of the A$_h$ horizon is approx. 3.5 (Heinemeyer et al., 2011).

2.2. Experimental design

To address the influence of root and rhizosphere C supply to soil, we included three contrasting rhizosphere treatments (n = 4 per treatment): 1) a *Soil only* treatment (hereafter referred to as 'S'); a *Soil plus extramatrical ECM mycelium* treatment (hereafter referred to as 'SM'); and a *Soil plus roots plus extramatrical ECM mycelium* treatment (hereafter referred to as 'SMR').

For the *S* treatment, PVC pipe sections (20 cm diameter, 35 cm long) were inserted into the soil to a depth of 30 cm. Each of these pipe sections had four windows (5 cm high x 6 cm wide) cut into the sides, which was covered by 1 μm nylon mesh (Normesh Ltd., Oldham, UK). The windows were positioned such that after insertion to the soil, they were just below the soil surface, and extending throughout the organic horizon into the mineral soil. The same design of pipe sections with windows was used for the *SM* treatment, but mesh size was increased to 41 μm. This aperture size allows fungal mycelium to penetrate into the soil enclosed within pipe sections from surrounding soil, but prevents ingress of roots (Heinemeyer et al., 2012). For the *SMR* treatment (i.e. intact rhizosphere control), we used shorter pipe sections (20 cm diameter, 8 cm length) inserted into the organic soil layer to about 2 cm depth. The emplacement of the PVC pipe sections for all treatments resulted in about 5–6 cm of pipe length extending above the soil surface (from here referred to as 'collars'), from where gas exchange with the atmosphere could be measured.

Collar locations were randomized within an area of approximately 300 m^2 within the forest stand, with a requirement of individual locations being between 50 and 200 cm from tree stems, and a minimum distance of 100 cm between collars. The different rhizosphere treatments were randomly allocated according to a block design (based on soil CO$_2$ efflux measurements from the soil surface prior to treatment allocation) in order to account for localized environmental effects. All collars were established 12 months prior to the flux measurements to allow for a re-establishment of soil microbial communities following disturbance from collar installations, including the establishment of