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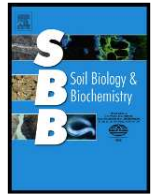
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Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil

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ABSTRACT

Aerated soils represent an important sink for atmospheric methane (CH₄), due to the effect of methanotrophic bacteria, thus mitigating current atmospheric CH₄ increases. Whilst rates of CH₄ 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 CH₄ sink. We used quasi-continuous automated chamber measurements of soil CH₄ and CO₂ 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 CH₄ 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 CO₂ efflux and CH₄ uptake. Results show a clear effect of the presence of live roots and ECM mycelium on soil CO₂ efflux and CH₄ uptake. The presence of ECM hyphae alone (without plant roots) showed intermediate fluxes of both CO₂ and CH₄ 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 CH₄ and CO₂ flux. We further found a surprising increase in soil CH₄ uptake during the night, and discuss diurnal fluctuations in atmospheric CH₄ (with higher concentrations during stable atmospheric conditions at night) as a potential driver of CH₄ 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 (CO₂) and methane (CH₄) play a pivotal role in global climate change (Ciais et al., 2013; Tian et al., 2016). Anthropogenically driven increases in atmospheric CO₂ from fossil fuel combustion and land-use change are the main drivers of climate change. Increasing atmospheric CH₄ concentrations are now thought to contribute 20% of the total greenhouse gas warming (Ciais et al., 2013; Myhre et al., 2013). For anthropogenic CH₄ emission sources, rice cultivation, ruminants, landfills, and gas evasion dur-

ing fossil fuel extraction dominate (Ciais et al., 2013; Myhre et al., 2013). Methane oxidation in upland soils represent an important sink for atmospheric CH₄, but poor constraints on the uptake of atmospheric CH₄ by soil microorganisms contributes to overall uncertainty in the global atmospheric CH₄ budget, and predictions of how soil-atmosphere feedbacks may modulate future changes in atmospheric CH₄ concentrations (Kirschke et al., 2013; Nisbet et al., 2014). Similarly, whilst the dynamics and drivers of CO₂ exchange from terrestrial ecosystems are reasonably well understood (Jung et al., 2011), there remain significant uncertainties around feedbacks between plants, soil microbes,

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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 CO₂ in particular, the role of temperature and soil water availability on heterotrophic decomposition 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 CH₄ 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 CH₄ oxidizers. This is because methanotrophs are able to subsist on other simple C1 compounds (e.g. methanol, formaldehyde, formate) when CH₄ 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 CH₄ 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 CH₄ 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 CH₄ oxidation has largely been obtained from laboratory incubations using high concentrations of CH₄, 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 CH₄ concentration effects under ambient conditions are far less common, because past work on atmospheric CH₄ 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 CH₄ concentrations significantly influence CH₄ uptake *in situ* because of the prevalence of other environmental drivers (e.g. moisture, temperature) and the narrow range over which atmospheric CH₄ 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 CO₂ and CH₄ 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 CO₂ and CH₄ 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 (H₂O) 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 m² 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 CO₂ 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 new ECM hyphal ingrowth in the *SM* treatment.

Both the amount of litter and the amount of precipitation entering collars was standardised to remove the influence of the considerable spatial heterogeneity on litter amounts and canopy through-fall. Collars were sheltered from through-fall using transparent shields of corrugated PVC (30 × 40 cm) suspended at about 25 cm above collars, and average amounts of rainfall (based on measurements on site) were added to collars every week.

2.3. Soil CO₂ and CH₄ flux measurements

From 5th May until 13th June 2009, soil surface fluxes of CO₂ and CH₄ were measured using 12 opaque multiplexed automatic chambers (LI-8100-101, Li-Cor, Lincoln, Nebraska, USA; approximately 20 cm diameter). Chambers were placed over PVC collars of respective treatments, sealing tightly around the outside of collars with a rubber gasket. CO₂ concentrations were measured using a LI-8100 (Li-Cor, Lincoln, Nebraska, USA), whilst CH₄ concentrations were measured using a Fast Greenhouse Gas analyser (FGGA; Los Gatos Research, Mount View, California, USA). The multiplexer sampled each chamber sequentially such that chambers were measured once an hour. During the measurements, each chamber was closed for 3 min only, ensuring that the enclosed soil area is subject to the same conditions as the surrounding soil.

2.4. Environmental measurements

Soil temperature and soil water content (SWC) were recorded every 10 min using PT100 thermistor probes and SM200 probes (Delta-T Devices, Cambridge, UK), respectively. Soil temperature measurements were at 0.05 and 0.1 m depths ($n = 3$ per depth) and SWC measurements ($n = 3$) were measured at 0.05 m depth. Atmospheric pressure was recorded continuously (1 Hz) by the (LI-8100). Photosynthetically Active Radiation (PAR) was measured every 10 min at a nearby canopy opening (QS5 PAR Quantum Sensor, Delta-T Devices, Cambridge, UK).

Additionally, SWC was measured inside all soil collars once a week prior to manual water addition (see above) using a hand-held probe (SM200, Delta-T Devices, Cambridge, UK). A spatial average of through-fall at the site were collected from the nine collectors (funnel diameter = 20 cm) once every week. These funnels were placed on the ground at random locations throughout the site.

Data for wind speed and wind gust speed were obtained from the UK Met-Office website (www.metoffice.gov.uk) for observations from Linton on Ouse, located approximately 20 km NW of the experimental plot. Note that despite the spatial separation, these data are used to allow a general characterisation of atmospheric mixing due to wind, not precise conditions at the site (see below).

2.5. Data processing and flux calculations

Fluxes of CO₂ and CH₄ were calculated from linear regression of the concentration measurements obtained during each 3 min chamber closure. The first 40 s of each measurement were removed to allow the complete mixing of chamber air, meaning that each regression used 140 data points spanning a 140 s period. The correlation coefficient (r^2), root mean square error (*RSME*) and *p* value were calculated for each linear regression.

In order to separate valid flux measurements from possible artefacts (e.g. due to incomplete chamber closure, or leakage), we removed all CO₂ and CH₄ flux estimates where the r^2 value of the CO₂ measurement was below 0.9. This procedure removed approximately 19% of all data, most of which were associated with malfunctioning chambers during some of the observation period. Owing to the relatively smaller signal-to-noise ratio, small flux rates tended to show lower coefficients of variation (r^2). This was more pronounced for methane flux calculations, due to the smaller absolute concentration changes for this flux, and we did not apply the same rigorous r^2 threshold to fluxes as we did for CO₂. Instead, any CH₄ flux with an *RSME* of more than 0.02 μmol m⁻² s⁻¹ was also removed, affecting a further 1.8% of flux values. Concentrations of CO₂ and CH₄ c. 0.1 m above the soil surface were recorded from each chamber location immediately before chamber closure (initial 5 readings for each channel, i.e. before the concentrations had increased).

Small gaps in the data series of each chamber (less than six consecutive hours) were filled by using the average of fluxes four hours before and after the gap (from the same chamber). Larger data gaps were not filled. Flux values were calculated for each chamber separately and averaged according to treatment (*S*, *SM*, *SMR*), using each chamber as a true replicate.

2.6. Statistical methods

Cumulative flux sums were analysed by means of a two-way Analysis of Variance (ANOVA) for each chamber to look for a block and treatment effect, and a post-hoc Duncan's MRT test applied, if the data met the assumptions of homogeneity of variance and normality. All flux calculations and statistical analysis of cumulative flux values was carried out using SAS v8.01 (Statistical Analysis Software). Correlations between concentrations, fluxes and environmental variables were carried out using the Spearman's rank method (owing to non-normal distributions) in the SPSS Statistics software (Version 21; IBM Corp.).

The relationships between continuous environmental variables and trace gas fluxes were investigated using linear and/or multiple regressions and analysis of covariance. In some cases, autoregressive (AR) models were employed because gas fluxes and environmental variables showed temporal autocorrelations. Residuals from exploratory regression modelling revealed strong autocorrelation for all fluxes, as confirmed by autocorrelation function (ACF) plots and the Durbin Watson test (in all cases *p*-value < 0.001). It was found that a 2nd order AR model was optimal based on inspection of ACF plots. To facilitate comparisons between fitted coefficients, all variables were normalised by scaled to a mean of zero and a standard deviation of 1. The independent variables included in the regression models were: initial concentration of CO₂ or CH₄ (respectively), air pressure, air temperature, soil temperature (at 5 cm depth), solar radiation and soil water content.

3. Results

3.1. Soil respiration

Mean soil CO₂ flux (SMR) over the measuring period was $0.91 \pm 0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$. For the rhizosphere treatments, we found a significant effect of treatment but no effect of block ($F_{2,10} = 13.41$, $P < 0.002$). Treatment SMR showed significantly higher CO₂ fluxes than either of the other two treatments (Table 1).

The overall heterotrophic contribution to soil respiration averaged $55.2 \pm 0.3\%$ over the entire measurement period, with a tendency towards higher relative heterotrophic contributions towards the end of the observation period (Fig. 1c). Of the autotrophic contributions, about one-third could be attributed to ECM-mycelium CO₂ flux, with the remainder originating from roots ($15.8 \pm 0.3\%$ and $29.0 \pm 0.4\%$ of total soil CO₂ flux, respectively). Note that this is a simplistic presentation of flux contribution, based on flux differences to illustrate relative flux magnitudes. It assumes that flux contributions are independent and hence additive, thus excluding possible interactions between autotrophic and heterotrophic dynamics in the soil environment.

Over the course of the sampling period, soil CO₂ fluxes showed a gradual increase corresponding with seasonal changes in air and soil temperatures (Fig. 1d). At diurnal timescales, however, soil CO₂ flux showed lower rates at around midday, with flux rates reaching a peak at about 20:00 on average for the entire measurement period (Fig. 2b). The different rhizosphere treatments also show different diurnal patterns. For example, SMR and SM treatments show a more pronounced reduction in CO₂ flux during the middle of the day compared to the S treatment, resulting in greater diurnal amplitudes both in absolute and relative terms.

3.2. Soil CH₄ uptake

Mean CH₄ flux (SMR) over the measuring period was $-1.63 \pm 0.22 \text{ nmol m}^{-2} \text{ s}^{-1}$. Soil CH₄ flux varied significantly among rhizosphere treatments, but no significant effect of block was found (ANOVA $F_{2,10} = 14.39$, $P < 0.002$). The strongest sink was observed for the SMR treatment, followed by SM and S treatments ($P < 0.01$; Table 1).

Unlike CO₂ efflux, CH₄ uptake did not show a gradual seasonal increase with rising temperatures. Instead, the CH₄ sink strength showed short-term decreases following rain events and a gradual increase following the onset of drier conditions (Fig. 1e). On diurnal timescales, we observed a marked pattern of higher night-time CH₄ oxidation rates and lower daytime fluxes (Fig. 2a). In contrast to CO₂ dynamics, the daily oscillation in CH₄ fluxes did not vary among rhizosphere treatments. Atmospheric CH₄ concentrations measured above the soil surface showed lower daytime concentrations and higher night-time concentrations.

Table 1

Mean flux rates of methane and CO₂ for collars with contrasting access by roots and/or mycorrhizal hyphae: SMR = "soil, extraradical ECM hyphae & roots", SM = "soil 7 extraradical ECM hyphae", and S = heterotrophic soil CO₂ flux. Values are averages (± 1 St. Error) using temporal averages of flux rates from $n = 4$ collars as replicates. Different lower-case letters indicate significant differences between treatments for each of the gases.

Treatment	Mean CO ₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Mean CH ₄ flux ($\text{nmol m}^{-2} \text{s}^{-1}$)
SMR	0.9061 ± 0.0705^a	-1.626 ± 0.221^a
SM	0.6521 ± 0.0317^b	-0.8180 ± 0.1216^b
S	0.5352 ± 0.0454^b	-0.5877 ± 0.0530^c

Spearman's rank correlation analysis indicated that there was a significant correlation between the rate of CH₄ uptake in the soil and CH₄ concentrations measured in the atmosphere above the soil surface (Fig. 3a). This correlation was significant for the entire data set ($r = -0.237$; $p < 0.01$; $n = 759$), but was dominated by a strong dependence of fluxes on concentration at low soil water content (SWC = $0.22-0.35 \text{ m}^3 \text{ m}^{-3}$; $r = -0.493$; $p < 0.001$; $n = 262$). Variation in CH₄ concentration in the atmosphere above the soil surface was found to correlate in turn with wind speed (Fig. 3b).

3.3. Relationship between trace gas fluxes and environmental variables

The AR model indicates a significant effect of SWC dynamics on fluxes of CO₂ and CH₄ for all treatments (Table 2). For CO₂, fluxes increased with rising soil moisture, while the opposite pattern was true for CH₄ (i.e. reduced CH₄ uptake with increasing SWC). AR analysis also indicated that soil temperature at the 5 cm depth was a good predictor of soil CO₂ fluxes among all the rhizosphere treatments, while air temperature was found to be a good predictor of CH₄ fluxes (Table 2). Furthermore, a significant negative correlation was found between solar radiation and CO₂ fluxes (Table 2).

3.4. Sampling frequency analysis

Re-sampling the data set to simulate results that would have been obtained under contrasting sampling scenarios show a generally lower apparent CH₄ oxidation flux rate, with an apparent reduction by up to 14.5% for fortnightly sampling frequencies from the SMR treatment, 12.5% for alternate days in the S treatment, and 23.2% for fortnightly sampling from the SM treatment (Fig. 4). The CO₂ reduction in apparent flux was up to 13.8%, 17.9% and 12% for weekly sampling of SMR, SM and S treatments, respectively. The standard deviation associated with different sampling frequencies increases with decreasing frequency, owing to the lower number of sampling events for lower frequencies. Sampling frequencies of 1 and 2 weeks would have resulted in an under-estimation of mean CH₄ oxidation of 12.7 and 14.5%, respectively, compared to the 1-h results in the SMR treatment. The uncertainty of estimates measured by the observed standard deviation of measurements for contrasting sampling intensities was similar for frequencies down to bi-weekly samplings. For less frequent intervals, standard deviations increased by approximately 25 and 50% for 1 and 2-week intervals, respectively.

4. Discussion

4.1. Rhizosphere effects on soil CO₂

Results from the root and extraradical ECM mycelium exclusion treatments suggest a significant effect of root and ECM presence on CO₂ flux. Higher soil CO₂ efflux in the SMR treatment can be expected, and has been documented exhaustively elsewhere in other soil respiration partitioning studies (Subke et al., 2006). The enhanced soil CO₂ flux in the SMR treatment reflects the respiration of live roots and mineralization of root-derived organic materials in the rhizosphere, and the proportion of heterotrophic respiration ($51.1 \pm 13.6\%$) falls within the range observed in other temperate forest sites (Subke et al., 2006; Bond-Lamberty and Thomson, 2010). The lack of a significant difference between SM and S treatments, while surprising, may reflect the fact that the mycorrhizal biomass in SM treatments was not large enough to produce significantly greater amounts of CO₂ compared to the S treatment. The mesh-collar approach we chose for this study selects in-growth based on hyphal diameter only, but we acknowledge that it creates further selection of ECM species based on their "exploration types" (Tedersoo and Smith, 2013); whilst species classified as

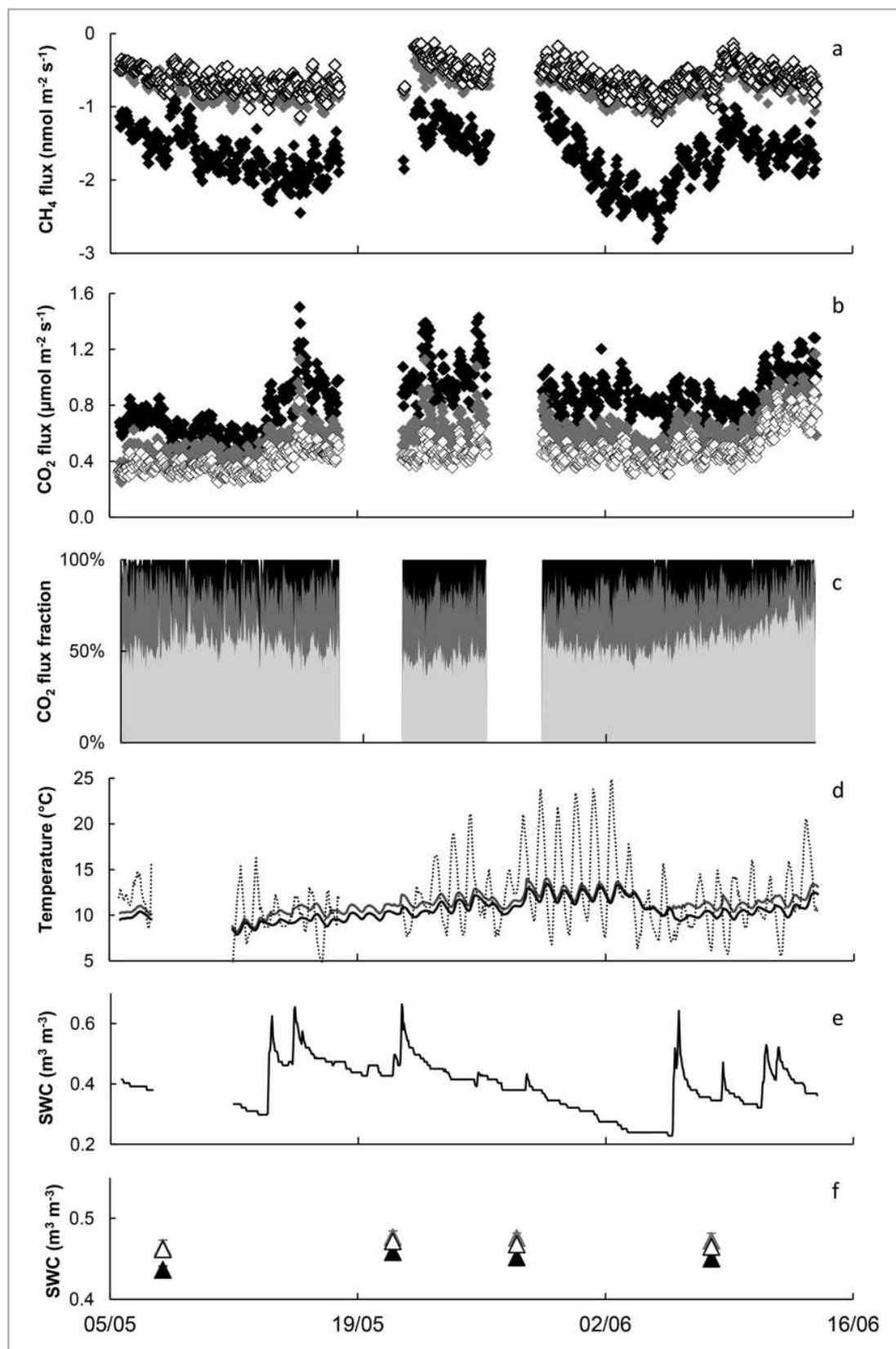


Fig. 1. Overview of flux dynamics and environmental parameters during the measuring period. (a) CH_4 flux and (b) CO_2 flux from SMR (black), SM (grey) and S (open) collars. (c) Apparent CO_2 flux fractions from decomposition (light grey), extraradical ECM hyphae (dark grey) and “true” root respiration (black), based on flux difference between collar treatments. (d) Temperatures measured in the soil at 5 cm (grey line) and 10 cm (black line) and in the air above the soil surface (dashed line). Soil water content was measured continuously (n = 3) (e), and periodically for different treatments (f). All error bars represent 1 standard error (n = 4).

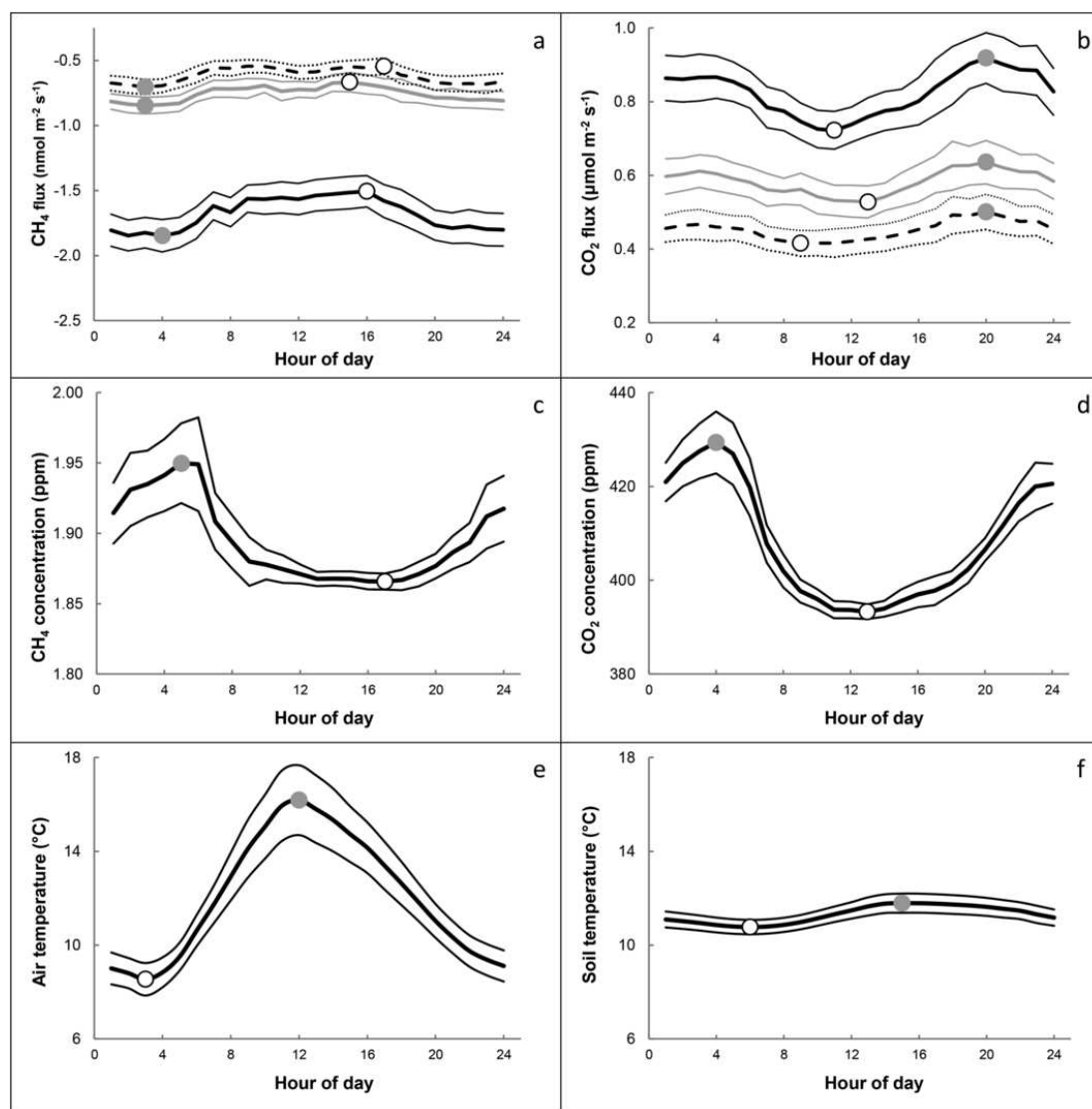


Fig. 2. Mean diurnal dynamics of CH_4 and CO_2 fluxes and key environmental parameters. Data are means averaged over the entire measuring period, thin lines indicate the 95% confidence intervals; maximum and minimum values are indicated by grey and white circles, respectively (meaning min. and max. *negative* fluxes for CH_4). Mean hourly fluxes of CH_4 (a) and CO_2 (b) for the three collar types are shown alongside CH_4 and CO_2 concentrations above the soil surface (c, d). Collar treatments are shown separately in panels a and b: SMR (solid black lines), SM (grey lines), and S (dashed black lines). Also shown are diurnal courses of air temperature (e), and soil temperature at 5 cm depth (f).

long to medium distance explorers (*sensu* Tedersoo and Smith, 2013) are likely to dominate in SM treatments, ‘contact’ and short-distance explorer types of ECM are likely to be underrepresented.

4.2. Rhizosphere effects on soil CH_4

What was more intriguing, however, was the distinct pattern in CH_4 uptake among the root & ECM exclusion treatments. In the presence of a fully intact rhizosphere (SMR treatment), net CH_4 uptake was almost 3 times that of the bulk soil; while in the presence of ECM hyphae, net CH_4 uptake was approximately 40% higher than in the bulk soil (Table 1). Although some of this variation in fluxes may be attributable to differences in soil moisture content among the treatments (see section on the role of environmental drivers below), we believe it is unlikely that soil moisture was the principal cause for this pattern because the absolute difference in soil moisture content among the treatments was small compared to the difference in fluxes (e.g. soil moisture varied by only 1.5–13.0%, whereas CH_4 fluxes varied by as much as 300% among treatments). Other measured environmental variables did not

vary significantly between treatments. This suggests that the observed pattern was due to some other biotic or environmental factor that we did not measure, or the result of fundamental underlying differences in microbial methanotrophic populations among treatments. With respect to the latter, we propose that soil with an intact rhizosphere may promote a more vigorous methanotrophic community by supplying methanotrophs with alternate sources of labile C (e.g. methanol, formaldehyde, formate) and/or by providing a greater sources of nutrients for methanotroph growth (Hanson and Hanson, 1996; Bodelier and Laanbroek, 2004; Veraart et al., 2015). Highest fine root densities in this forest occur throughout the organic horizon and superficial mineral horizons; soil methanotrophic bacteria are generally assumed to occur mainly in the upper mineral horizons in coniferous forests (Saari et al., 1998), so the close spatial proximity makes it possible that rhizosphere derived C1 compounds support the population size of also methanotrophs. In addition, roots and extraradical ECM hyphae can also promote macropore and aggregate formation (Angers and Caron, 1998; Six et al., 2006), which may facilitate transport of CH_4 to methanotrophs by improving soil structure and overall pore connectivity.

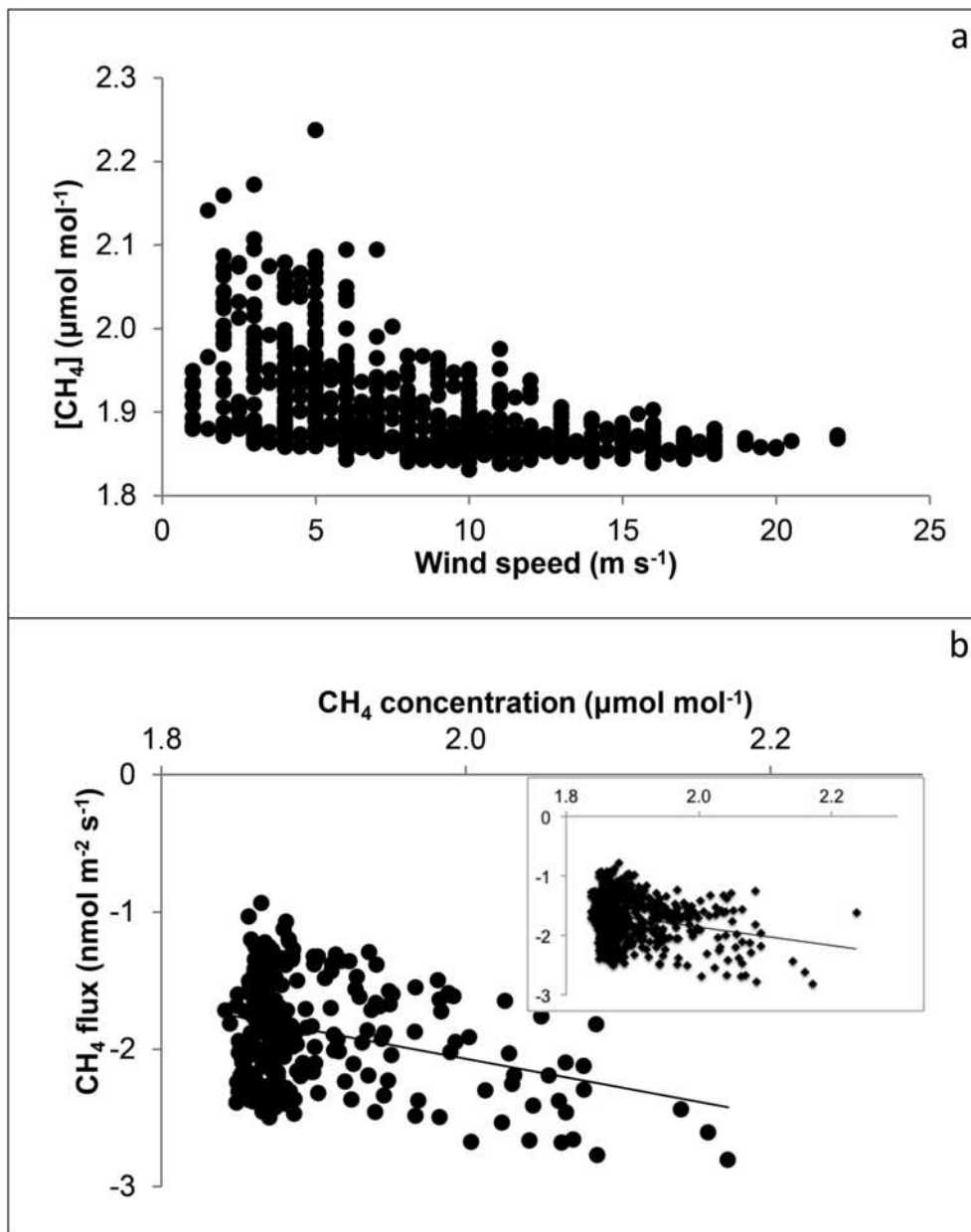


Fig. 3. (a) Relationship between CH₄ concentration above the soil surface and wind speed. (b) Correlation between CH₄ concentration above the soil surface and instantaneous CH₄ flux in SMR treatment. The main graph shows correlation of driest conditions (Soil Water Content between 0.22 and 0.35 m³ m⁻³), inset shows all data.

4.3. Environmental regulation of CO₂ flux

Mean CO₂ flux ($0.91 \pm 0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$) is close to the mean of boreal forests ($1.01 \pm 0.60 \mu\text{mol m}^{-2} \text{s}^{-1}$), but in the lower range of annual temperate forest rates ($1.97 \pm 1.11 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Bond-Lamberty and Thomson, 2010). Both soil temperature and soil water content (with the exception of the *Soil* treatment) significantly influenced the dynamics of soil CO₂ efflux, consistent with studies in other forest ecosystems (Wu et al., 2011). However, what was surprising is an apparent negative correlation between radiation and soil CO₂ efflux (Table 2). The temporal shift in peak soil CO₂ efflux, which occurs between 18:00 and 20:00 h, may in part explain this correlation, as periods of high radiation are associated with low CO₂ flux, and peak fluxes occurred close to the time of sun set. However, the autoregressive model showed a strong influence of soil temperature, which also

peaked between 18:00 and 20:00, so that the additional influence of radiation remains unexplained. We note that the *S* treatment (which does not experience direct influence of belowground allocation of C by plants) does not show any statistically significant effect of radiation, which suggests that the inverse radiation-CO₂ flux relationship is influenced by autotrophic C supply. Why this should have a negative sign is however less clear, as previous studies have established a clear and direct relationship between radiation (and hence photosynthetic activity) and belowground CO₂ fluxes (Mencuccini and Hölttä, 2010; Martin et al., 2012). One possible explanation is that night-time depletion of sugars or other carbohydrate stores may suppress carbohydrate utilisation (and consequently, respiration) during the first half of the day, leading to the apparent negative relationship between radiation and root respiration earlier in the day (Gibon et al., 2004). Subsequent accumulation of photosynthate may release this biochemical inhibition, leading to higher respiration rates during the evening and night.

Table 2

Coefficients from the autoregressive (AR) model. Coefficients of each parameter are shown along with the standard error (S.E.). Significance Coefficients are highlighted in bold with the level of significance indicated: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*) and for marginally insignificant Coefficients $p < 0.1$ (#). Note that all variables were scaled to a mean of zero and a standard deviation of 1.

		Intercept	AR (1)	AR (2)	Initial CO ₂	Initial CH ₄	SWC	Pressure	Radiation	T _{air}	T _{soil}	Adj-R ²
F _{CO2,S}	Coeff	0.021	0.629***	0.298***	-0.026		0.031#	-0.017	-0.028	-0.004	0.055**	0.88***
	S.E.	0.014	0.038	0.038	0.017		0.016	0.020	0.018	0.024	0.021	
F _{CO2,MS}	Coeff	0.041*	0.596***	0.220***	-0.001		0.080***	-0.002	- 0.063**	0.001	0.095***	0.80***
	S.E.	0.018	0.040	0.040	0.022		0.022	0.025	0.022	0.030	0.027	
F _{CO2,RMS}	Coeff	0.049**	0.610***	0.171***	0.031		0.079***	-0.035#	- 0.077***	0.009	0.139***	0.86***
	S.E.	0.015	0.039	0.038	0.019		0.018	0.020	0.018	0.023	0.023	
F _{CH4,S}	Coeff	0.013	0.589***	0.269*	-	0.007	0.071**	0.035	-0.032	0.083*	-0.045	0.74***
	S.E.	0.022	0.038	0.038		0.023	0.027	0.031	0.027	0.034	0.028	
F _{CH4,MS}	Coeff	0.008	0.524***	0.330***	-	0.002	0.049#	0.027	- 0.056*	0.107**	-0.043	0.72***
	S.E.	0.022	0.038	0.037		0.023	0.026	0.032	0.027	0.034	0.029	
F _{CH4,RMS}	Coeff	-0.002	0.610***	0.308***	-	0.015	0.052**	0.014	0.021	0.057*	-0.046*	0.88**
	S.E.	0.015	0.037	0.036		0.015	0.019	0.021	0.018	0.023	0.019	

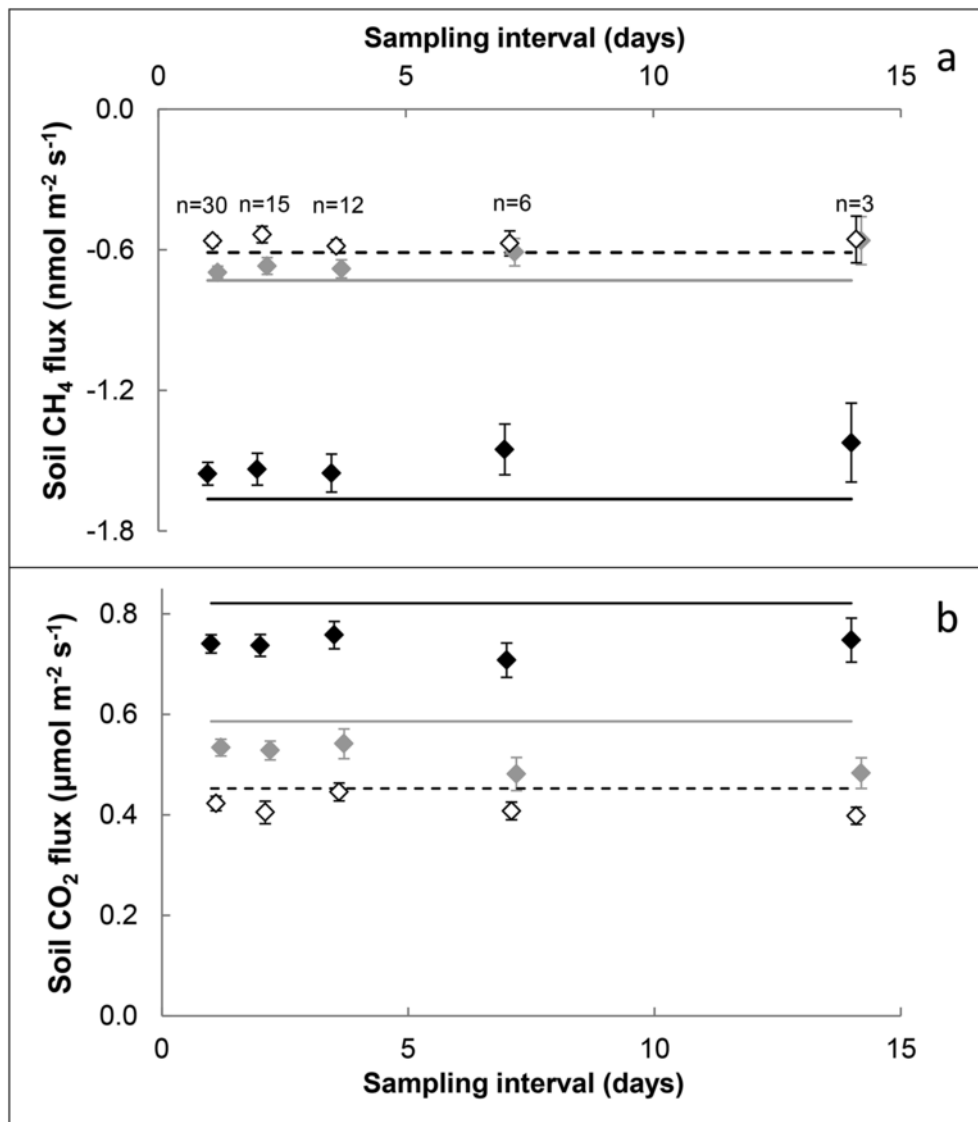


Fig. 4. Mean CH₄ (a) and CO₂ (b) flux estimate for all three treatments over the 6-week observation period based on increasing sampling intervals. Horizontal lines give the “true” average flux based on hourly observations. Black symbols & solid lines: SMR, grey symbols and lines: SM, open symbols and hatched lines: S; error bars show standard errors. Numbers of temporal replicates for each sampling interval (identical for all collar treatments and both gases) is indicated in the upper panel.

Lags between C assimilation in the canopy and utilisation in the rhizosphere are a further possibility to explain shifts in fluxes with regards to drivers. The meta-analysis of transport times of sugars fixed during photosynthesis to root via the phloem by Mencuccini and Hölttä (2010) indicates that for an approximate 10 m path length (tree height plus root length), a lag of between 1 and 3 days is likely. However, the observation that peak CO₂ flux in the S treatment coincides with that in other (autotroph-influenced) treatments (Fig. 2b) suggests that, whilst the magnitude of response is impacted by photosynthate supply, the timing is more likely to relate to lags in soil diffusion.

4.4. Environmental regulation of CH₄ flux

The magnitude of CH₄ uptake in intact soil collars over the sampling period ($1.63 \pm 0.22 \text{ nmol m}^{-2} \text{ s}^{-1}$, Table 1) is similar to fluxes reported from mixed deciduous woodlands in Scotland (0.14–2.39 $\text{nmol m}^{-2} \text{ s}^{-1}$) (Dobbie et al., 1996), but relatively high when compared to fluxes across other European temperate forests (uptake rates of 0.18–1.43 $\text{nmol m}^{-2} \text{ s}^{-1}$ averaged over an entire year;

Grunwald et al., 2012). Our results indicate a significant influence of soil moisture and air temperature on CH₄ flux over the measurement period, confirming findings from another temperate coniferous site (Ueyama et al., 2015). Unlike CO₂, the rate of CH₄ uptake was inversely related to both soil moisture and air temperature; i.e. the positive correlation between CH₄ flux and soil moisture or air temperature represents an inverse relationship with CH₄ uptake because more negative fluxes denote higher rates of CH₄ oxidation while more positive fluxes denote lower rates of CH₄ oxidation. For example, over the moisture range observed in this experiment, CH₄ uptake declined in response to rising soil moisture content (i.e. CH₄ flux became more positive with increasing soil moisture). Progressive drying of soil probably increased soil pore connectivity and facilitated more rapid transport of CH₄ from the atmosphere to sites of methanotrophic activity (see late May, early June in Fig. 1). Likewise, increases in air temperature were associated with a decline in rates of CH₄ uptake (i.e. CH₄ flux also became more positive with increasing air temperature). This trend may reflect the effect of temperature on CH₄ dissolution and substrate supply to methanotrophs. Methane is a poorly soluble hydrophobic com-

pound, and its dissolution into the aqueous phase is closely linked to temperature. Higher air temperatures may reduce rates of CH₄ dissolution, subsequently reducing the supply of aqueous-phase CH₄ to methanotrophs and thus suppressing rates of atmospheric CH₄ uptake (Teh et al., 2006; Templeton et al., 2006). Alternatively, the apparent inverse relationship between air temperature and CH₄ flux may be a result of the concurrent diurnal fluctuations in atmospheric CH₄ concentrations (Fig. 2), which may obscure a confounding impact of substrate limitations underlying the CH₄ flux response (see below).

The observed influence of soil moisture on CH₄ uptake slightly complicates a direct interpretation of rhizosphere treatments. Manual soil moisture measurements showed a significant (although numerically small) influence of treatment on soil moisture, with the SMR treatment having consistently lower soil moisture than the other two treatments. This artefact from deep collar methods has been reported before (Heinemeyer et al., 2012), and is likely to be caused by the absence of root water uptake in SM and S treatments. However, the magnitude of the treatment effect on soil moisture, whilst statistically significant, is small (between 0.01 and 0.03 m³ m⁻³ for a soil water content range of between 0.23 and 0.66 m³ m⁻³ over the measuring period). The relatively consistent contributions of autotrophic sources to soil CO₂ efflux (Fig. 1c) suggest that the soil moisture variations were insufficient to impact on plant productivity and rhizosphere C allocation, so that microbial supply of plant-derived C did not seemingly change significantly over the measurement period, notwithstanding an apparent reduction in root and ECM flux contributions in the last week in Fig. 1c.

Interestingly, there was also a significant and well-constrained influence of CH₄ concentration on CH₄ uptake, with CH₄ uptake increasing (i.e. fluxes becoming more negative) with increasing CH₄ concentration. Diurnal changes in CH₄ concentration were therefore associated with predictable diurnal shift in CH₄ uptake. For example, daytime mean concentrations of CH₄ were consistently around 1.86 ppm between the hours of 9:00 and 20:00, but night-time concentrations showed progressively increasing concentrations, with a peak of c. 1.95 ppm at 6:00. This diurnal variation in CH₄ concentrations coincides with an overall shift towards higher CH₄ uptake rates at night. The underlying cause for this shift towards higher nighttime CH₄ concentrations are atmospheric mixing effects. Collapse of the atmospheric boundary layer at night and poorer atmospheric mixing leads to the localized accumulation of atmospheric CH₄ (Baldocchi et al., 2012). However, given the consistent and comparatively strong soil CH₄ sink, the nighttime increase in local atmospheric CH₄ concentrations above the global tropospheric average is surprising. We can only speculate that the increase in concentration could be caused by local hotspots of CH₄ production located away from the immediate measurement plot (Baldocchi et al., 2012). For example, CH₄ production from local anaerobic hotspots (Baldocchi et al., 2012) or soil-derived CH₄ emissions transported through trees (Covey et al., 2012; Wang et al., 2016) may enhance local atmospheric CH₄ concentrations under stable nighttime atmospheric conditions. Irrespective of the actual source of CH₄ underlying the increase during periods of low atmospheric mixing, there is a clear response in the strength of CH₄ uptake and atmospheric concentration, in good agreement in diurnal patterns (Fig. 2a and c). This finding is potentially significant, as it suggests that soil microbial oxidizers may represent a potential negative feedback to rising atmospheric CH₄ concentrations. Our observations are supported by a number of laboratory-based studies that have found clear methane oxidation dependencies when large concentration gradients are applied (Bender and Conrad, 1992; Tate et al., 2012; Malghani et al., 2016). Experimental ranges in these studies exceed concentration ranges normally encountered in the boundary layer above the soil surface; concentration ranges in cited publications are 40–570 ppm in Tate et al. (2012), 30–60 ppm in Malghani et al. (2016) or even 5% in Bender and Conrad (1992). That methane oxidation rates respond to much smaller varia-

tions in concentration detectable in the field is however a novel observation. Of course, one important caveat is that the AR model did not identify CH₄ concentration as a significant predictor of CH₄ flux, despite the strong correlation. As mentioned before, there is a possibility that confounding covariance of air temperature and CH₄ concentrations may obscure actual relationships between CH₄ flux and driving variables, and field-based experimental manipulations of methane concentrations and temperature are needed to resolve this point.

4.5. Insights obtained from quasi-continuous chamber measurements

Quasi-continuous, automated sampling of soil gas exchange provides the most comprehensive data to estimate soil or ecosystem greenhouse gas budgets. The sampling frequency exercise we performed indicated that manual chamber sampling, assuming that manually sampled fluxes were collected during mid-day, under-estimate soil CO₂ and CH₄ fluxes from our temperate forest study site by 12–15%. This is because manual sampling during day-time hours would not have accounted for diurnal changes in gas flux, in particular periods when gas fluxes were heightened (e.g. enhanced soil respiration between 18:00–20:00 and elevated CH₄ uptake from 20:00–6:00). Continuous atmospheric flux measurements (such as the eddy covariance technique) provide a further powerful tool to investigate short-term temporal flux variations and dependence on environmental drivers (Phillips et al., 2017), but chamber based studies like ours provide critical process understanding from manipulations that can not be captured by eddy covariance.

It should be noted that these are not universal values that can be applied to correct manual gas sampling estimates obtained in other temperate forest locations. Rather, it serves to illustrate that diurnal fluctuations in soil gas exchange should be obtained for studies otherwise relying on periodic gas sampling in order to estimate seasonal or annual budgets in order to account for fluxes that may be partially driven by recurring (e.g. diurnal) shifts in environmental conditions or circadian patterns.

A key insight gained from the use of this continuous sampling approach is that we have identified temporal trends in the data that may point to new or previously unidentified controls on CH₄ and CO₂ fluxes. The mid-day depression in soil respiration and the subsequent rise in fluxes from 18:00–20:00 may suggest a physiological control on autotrophic respiration linked to the internal carbohydrate status of plant tissues (Gibon et al., 2004), whilst the night-time increase in soil CH₄ uptake, coincident with the rise in atmospheric CH₄ concentrations, may indicate that high-affinity CH₄ oxidising bacteria are sensitive to small and short-term variations in substrate availability, a phenomenon not described before.

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