Title

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

Authors

Jens-Arne Subke[1], Catherine S. Moody[2], Timothy C. Hill[3], Naomi Voke[4], Sylvia Toet[5], Philip Ineson[4], Yit Arn Teh[6]

[1] Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, UK

[2] School of Geography, University of Leeds, Leeds LS2 9JT, UK

[3] College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4RJ, UK

[4] Department of Biology, University of York, York YO10 5DD, UK

[5] Environment Department, University of York, York YO10 5DD, UK

[6] Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 3UU, UK

Corresponding Author:

Jens-Arne Subke; jens-arne.subke@stir.ac.uk; Tel: 01786 467854

Abstract

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.

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 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 widows (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.

Methods

Study site

The field site is a 19-year-old (in 2009) forest stand dominated by *Pinus contorta* and *Pinus sylvestris* (approximate height: 6 to 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_2O) of the A_h horizon is approx. 3.5 (Heinemeyer et al., 2011).

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

145 measurements to allow for a re-establishment of soil microbial communities following
146 disturbance from collar installations, including the establishment of new ECM hyphal
147 ingrowth in the *SM* treatment.

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

153

154 *Soil CO_2 and CH_4 flux measurements*
155 From 5th May until 13th June 2009, soil surface fluxes of CO_2 and CH_4 were measured using
156 12 opaque multiplexed automatic chambers (LI-8100-101, Li-Cor, Lincoln, Nebraska, USA;
157 approximately 20 cm diameter). Chambers were placed over PVC collars of respective
158 treatments, sealing tightly around the outside of collars with a rubber gasket. CO_2
159 concentrations were measured using a LI-8100 (Li-Cor, Lincoln, Nebraska, USA), whilst CH_4
160 concentrations were measured using a Fast Greenhouse Gas analyser (FGGA; Los Gatos
161 Research, Mount View, California, USA). The multiplexer sampled each chamber
162 sequentially such that chambers were measured once an hour. During the measurements,
163 each chamber was closed for 3 minutes only, ensuring that the enclosed soil area is subject
164 to the same conditions as the surrounding soil.

165

166 *Environmental measurements*
167 Soil temperature and soil water content (SWC) were recorded every 10 minutes using PT100
168 thermistor probes and SM200 probes (Delta-T Devices, Cambridge, UK), respectively. Soil
169 temperature measurements were at 0.05 and 0.1 m depths (n = 3 per depth) and SWC
170 measurements (n = 3) were measured at 0.05 m depth m. Atmospheric pressure was
171 recorded continuously (1 Hz) by the (Li-8100). Photosynthetically Active Radiation (PAR) was

measured every 10 minutes 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 throughfall 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).

Data processing and flux calculations

Fluxes of CO_2 and CH_4 were calculated from linear regression of the concentration measurements obtained during each 3 minute chamber closure. The first 40 seconds of each measurement were removed to allow the complete mixing of chamber air, meaning that each regression used 140 data points spanning a 140 second 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 CO_2 and CH_4 flux estimates where the r^2 value of the CO_2 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 CO_2. Instead, any CH_4 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 CO_2 and CH_4 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.

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 Durban Watson test (in all cases p-value < 0.001). It was found that a 2^{nd} 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 CO_2 or CH_4 (respectively), air pressure, air temperature, soil temperature (at 5 cm depth), solar radiation and soil water content.

Results

Soil respiration

Mean soil CO_2 flux (*SMR*) over the measuring period was 0.91 ± 0.07 µmol m^{-2} 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 CO_2 fluxes than either of the other two treatments (Table 1).

The overall heterotrophic contribution to soil respiration averaged 55.2 ± 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 CO_2 flux, with the remainder originating from roots (15.8 ± 0.3% and 29.0 ± 0.4% of total soil CO_2 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 CO_2 fluxes showed a gradual increase corresponding with seasonal changes in air and soil temperatures (Fig. 1d). At diurnal timescales, however, soil CO_2 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 patters. For example, *SMR* and *SM* treatments show a more pronounced reduction in CO_2 flux during the middle of the day compared to the *S* treatment, resulting in greater diurnal amplitudes both in absolute and relative terms.

Soil CH_4 uptake

Mean CH_4 flux (*SMR*) over the measuring period was -1.63 ± 0.22 nmol m^{-2} s^{-1}. Soil CH_4 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 CO_2 efflux, CH_4 uptake did not show a gradual seasonal increase with rising temperatures. Instead, the CH_4 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 CH_4 oxidation rates and lower daytime fluxes (Fig. 2a). In contrast to CO_2 dynamics, the daily oscillation in CH_4 fluxes did not vary among rhizosphere treatments. Atmospheric CH_4 concentrations measured above the soil surface showed lower daytime concentrations and higher night-time concentrations.

Spearman's rank correlation analysis indicated that there was a significant correlation between the rate of CH_4 uptake in the soil and CH_4 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 m^3 m^{-3}; $r = -0.493$; $p < 0.001$; $n = 262$). Variation in CH_4 concentration in the atmosphere above the soil surface was found to correlate in turn with wind speed (Fig. 3b).

Relationship between trace gas fluxes and environmental variables

The AR model indicates a significant effect of SWC dynamics on fluxes of CO_2 and CH_4 for all treatments (Table 2). For CO_2, fluxes increased with rising soil moisture, while the opposite pattern was true for CH_4 (i.e. reduced CH_4 uptake with increasing SWC). AR analysis also indicated that soil temperature at the 5 cm depth was a good predictor of soil CO_2 fluxes among all the rhizosphere treatments, while air temperature was found to be a good predictor of CH_4 fluxes (Table 2). Furthermore, a significant negative correlation was found between solar radiation and CO_2 fluxes (Table 2).

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 CH_4 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 CO_2 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 CH_4 oxidation of 12.7 and 14.5%, respectively, compared to the 1-hour 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.

Discussion

Rhizosphere effects on soil CO_2

Results from the root and extraradical ECM mycelium exclusion treatments suggest a significant effect of root and ECM presence on CO_2 flux. Higher soil CO_2 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 CO_2 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 ± 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 CO_2 compared to the *S* treatment. The mesh-collar approach we chose for this study selects in-growth

312 based on hyphal diameter only, but we acknowledge that it creates further selection of ECM
313 species based on their "exploration types" (Tedersoo and Smith, 2013); whilst species
314 classified as long to medium distance explorers (*sensu* Tedersoo & Smith, 2013) are likely to
315 dominate in *SM* treatments, 'contact' and short-distance explorer types of ECM are likely to
316 be underrepresented.

317

318 *Rhizosphere effects on soil CH_4*

319 What was more intriguing, however, was the distinct pattern in CH_4 uptake among the root
320 & ECM exclusion treatments. In the presence of a fully intact rhizosphere (*SMR* treatment),
321 net CH_4 uptake was almost 3 times that of the bulk soil; while in the presence of ECM
322 hyphae, net CH_4 uptake was approximately 40% higher than in the bulk soil (Table 1).
323 Although some of this variation in fluxes may be attributable to differences in soil moisture
324 content among the treatments (see section on the role of environmental drivers below), we
325 believe it is unlikely that soil moisture was the principal cause for this pattern because the
326 absolute difference in soil moisture content among the treatments was small compared to
327 the difference in fluxes (e.g. soil moisture varied by only 1.5-13.0 %, whereas CH_4 fluxes
328 varied by as much as 300 % among treatments). Other measured environmental variables
329 did not vary significantly between treatments. This suggests that the observed pattern was
330 due to some other biotic or environmental factor that we did not measure, or the result of
331 fundamental underlying differences in microbial methanotrophic populations among
332 treatments. With respect to the latter, we propose that soil with an intact rhizosphere may
333 promote a more vigorous methanotrophic community by supplying methanotrophs with
334 alternate sources of labile C (e.g. methanol, formaldehyde, formate) and/or by providing a
335 greater sources of nutrients for methanotroph growth (Hanson and Hanson, 1996; Bodelier
336 and Laanbroek, 2004; Veraart et al., 2015). Highest fine root densities in this forest occur
337 throughout the organic horizon and superficial mineral horizons; soil methanotrophic
338 bacteria are generally assumed to occur mainly in the upper mineral horizons in coniferous
339 forests (Saari et al., 1998), so the close spatial proximity makes it possible that rhizosphere
340 derived C1 compounds support the population size of also methanotrophs. In addition,
341 roots and extraradical ECM hyphae can also promote macropore and aggregate formation

342 (Angers and Caron, 1998; Six et al., 2006), which may facilitate transport of CH_4 to
343 methanotrophs by improving soil structure and overall pore connectivity.

344

345 *Environmental regulation of CO_2 flux*
346 Mean CO_2 flux (0.91 ± 0.07 µmol m^{-2} s^{-1}) is close to the mean of boreal forests (1.01 ± 0.60
347 µmol m^{-2} s^{-1}), but in the lower range of annual temperate forest rates (1.97 ± 1.11
348 µmol m^{-2} s^{-1} (Bond-Lamberty and Thomson, 2010). Both soil temperature and soil water
349 content (with the exception of the *Soil* treatment) significantly influenced the dynamics of
350 soil CO_2 efflux, consistent with studies in other forest ecosystems (Wu et al., 2011).
351 However, what was surprising is an apparent negative correlation between radiation and
352 soil CO_2 efflux (Table 2). The temporal shift in peak soil CO_2 efflux, which occurs between
353 18:00 and 20:00 h, may in part explain this correlation, as periods of high radiation are
354 associated with low CO_2 flux, and peak fluxes occurred close to the time of sun set.
355 However, the autoregressive model showed a strong influence of soil temperature, which
356 also peaked between 18:00 and 20:00, so that the additional influence of radiation remains
357 unexplained. We note that the *S* treatment (which does not experience direct influence of
358 belowground allocation of C by plants) does not show any statistically significant effect of
359 radiation, which suggests that the inverse radiation-CO_2 flux relationship is influenced by
360 autotrophic C supply. Why this should have a negative sign is however less clear, as previous
361 studies have established a clear and direct relationship between radiation (and hence
362 photosynthetic activity) and belowground CO_2 fluxes (Mencuccini and Hölttä, 2010; Martin
363 et al., 2012). One possible explanation is that night-time depletion of sugars or other
364 carbohydrate stores may suppress carbohydrate utilisation (and consequently, respiration)
365 during the first half of the day, leading to the apparent negative relationship between
366 radiation and root respiration earlier in the day (Gibon et al., 2004). Subsequent
367 accumulation of photosynthate may release this biochemical inhibition, leading to higher
368 respiration rates during the evening and night.

369 Lags between C assimilation in the canopy and utilisation in the rhizosphere are a further
370 possibility to explain shifts in fluxes with regards to drivers. The meta-analysis of transport
371 times of sugars fixed during photosynthesis to root via the phloem by Mencuccini and Hölttä

372 (2010) indicates that for an approximate 10 m path length (tree height plus root length), a
373 lag of between 1 and 3 days is likely. However, the observation that peak CO_2 flux in the S
374 treatment coincides with that in other (autotroph-influenced) treatments (Fig. 2b) suggests
375 that, whilst the magnitude of response is impacted by photosynthate supply, the timing is
376 more likely to relate to lags in soil diffusion.

377

378 *Environmental regulation of CH_4 flux*

379 The magnitude of CH_4 uptake in intact soil collars over the sampling period
380 (1.63 ± 0.22 nmol m^{-2} s^{-1}, Table 1) is similar to fluxes reported from mixed deciduous
381 woodlands in Scotland (0.14 to 2.39 nmol m^{-2} s^{-1} (Dobbie et al., 1996), but relatively high
382 when compared to fluxes across other European temperate forests (uptake rates of 0.18 to
383 1.43 nmol m^{-2} s^{-1} averaged over an entire year; (Grunwald et al., 2012). Our results indicate
384 a significant influence of soil moisture and air temperature on CH_4 flux over the
385 measurement period, confirming findings from another temperate coniferous site (Ueyama
386 et al., 2015). Unlike CO_2, the rate of CH_4 uptake was inversely related to both soil moisture
387 and air temperature; i.e. the positive correlation between CH_4 flux and soil moisture or air
388 temperature represents an inverse relationship with CH_4 uptake because more negative
389 fluxes denote higher rates of CH_4 oxidation while more positive fluxes denote lower rates of
390 CH_4 oxidation. For example, over the moisture range observed in this experiment, CH_4
391 uptake declined in response to rising soil moisture content (i.e. CH_4 flux became more
392 positive with increasing soil moisture). Progressive drying of soil probably increased soil
393 pore connectivity and facilitated more rapid transport of CH_4 from the atmosphere to sites
394 of methanotrophic activity (see late May, early June in Fig. 1). Likewise, increases in air
395 temperature were associated with a decline in rates of CH_4 uptake (i.e. CH_4 flux also became
396 more positive with increasing air temperature). This trend may reflect the effect of
397 temperature on CH_4 dissolution and substrate supply to methanotrophs. Methane is a
398 poorly soluble hydrophobic compound, and its dissolution into the aqueous phase is closely
399 linked to temperature. Higher air temperatures may reduce rates of CH_4 dissolution,
400 subsequently reducing the supply of aqueous-phase CH_4 to methanotrophs and thus
401 suppressing rates of atmospheric CH_4 uptake (Teh et al., 2006; Templeton et al., 2006).

Alternatively, the apparent inverse relationship between air temperature and CH_4 flux may be a result of the concurrent diurnal fluctuations in atmospheric CH_4 concentrations (Fig. 2), which may obscure a confounding impact of substrate limitations underlying the CH_4 flux response (see below).

The observed influence of soil moisture on CH_4 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 $m^3\ m^{-3}$ for a soil water content range of between 0.23 and 0.66 $m^3\ m^{-3}$ over the measuring period). The relatively consistent contributions of autotrophic sources to soil CO_2 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 CH_4 concentration on CH_4 uptake, with CH_4 uptake increasing (i.e. fluxes becoming more negative) with increasing CH_4 concentration. Diurnal changes in CH_4 concentration were therefore associated with predictable diurnal shift in CH_4 uptake. For example, daytime mean concentrations of CH_4 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 CH_4 concentrations coincides with an overall shift towards higher CH_4 uptake rates at night. The underlying cause for this shift towards higher nighttime CH_4 concentrations are atmospheric mixing effects. Collapse of the atmospheric boundary layer at night and poorer atmospheric mixing leads to the localized accumulation of atmospheric CH_4 (Baldocchi et al. 2012). However, given the consistent and comparatively strong soil CH_4 sink, the nighttime increase in local atmospheric CH_4 concentrations above the global tropospheric average is surprising. We can

only speculate that the increase in concentration could be caused by local hotspots of CH_4 production located away from the immediate measurement plot (Baldocchi et al., 2012). For example, CH_4 production from local anaerobic hotspots (Baldocchi et al., 2012) or soil-derived CH_4 emissions transported through trees (Covey et al., 2012; Wang et al., 2016) may enhance local atmospheric CH_4 concentrations under stable nighttime atmospheric conditions. Irrespective of the actual source of CH_4 underlying the increase during periods of low atmospheric mixing, there is a clear response in the strength of CH_4 uptake and atmospheric concentration, in good agreement in diurnal patterns (Fig. 2a & 2c). This finding is potentially significant, as it suggest that soil microbial oxidizers may represent a potential negative feedback to rising atmospheric CH_4 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 & Conrad (1992). That methane oxidation rates respond to much smaller variations in concentration detectable in the field is however a novel observation. Of course, one important caveat is that the AR model did not identify CH_4 concentration as a significant predictor of CH_4 flux, despite the strong correlation. As mentioned before, there is a possibility that confounding covariance of air temperature and CH_4 concentrations may obscure actual relationships between CH_4 flux and driving variables, and field-based experimental manipulations of methane concentrations and temperature are needed to resolve this point.

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 CO_2 and CH_4 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 CH_4 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 CH_4 and CO_2 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 CH_4 uptake, coincident with the rise in atmospheric CH_4 concentrations, may indicate that high-affinity CH_4 oxidising bacteria are sensitive to small and short-term variations in substrate availability, a phenomenon not described before.

Acknowledgements

The research was funded by the UK Natural Environment Research Council (grant no. NE/H01182X/1). We are grateful to the Biology Department Electronic Workshop for construction of the multiplexer unit and other technical support.

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638 **Table 1** Mean flux rates of methane and CO_2 for collars with contrasting access by roots
639 and/or mycorrhizal hyphae: *SMR* = "soil, extraradical ECM hyphae & roots", *SM* = "soil 7
640 extraradical ECM hyphae", and *S* = heterotrophic soil CO_2 flux. Values are averages (± 1 St.
641 Error) using temporal averages of flux rates from n = 4 collars as replicates. Different lower-
642 case letters indicate significant differences between treatments for each of the gases.

Treatment	Mean CO_2 flux ($\mu mol\ m^{-2}\ s^{-1}$)	Mean CH_4 flux ($nmol\ m^{-2}\ s^{-1}$)
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}

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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	

Figure captions

Figure 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).

Figure 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).

Figure 3

(a) Relationship between CH_4 concentration above the soil surface and wind speed. (b) Correlation between CH_4 concentration above the soil surface and instantaneous CH_4 flux in *SMR* treatment. The main graph shows correlation of driest conditions (Soil Water Content between 0.22 and 0.35 $m^3\ m^{-3}$), inset shows all data.

Figure 4

Mean CH_4 (a) and CO_2 (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.