RESEARCH ARTICLE

Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog

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Abstract

The relationship between biogeochemical process rates and microbial functional activity was investigated by analysis of the transcriptional dynamics of the key functional genes for methanogenesis (methyl coenzyme M reductase; *mcrA*) and methane oxidation (particulate methane monooxygenase; *pmoA*) and *in situ* methane flux at two peat soil field sites with contrasting net methane-emitting and -oxidizing characteristics. qPCR was used to quantify the abundances of *mcrA* and *pmoA* genes and transcripts at two soil depths. Total methanogen and methanotroph transcriptional dynamics, calculated from *mcrA* and *pmoA* gene : transcript abundance ratios, were similar at both sites and depths. However, a linear relationship was demonstrated between surface *mcrA* and *pmoA* transcript dynamics and surface flux rates at the methane-emitting and methane-oxidizing sites, respectively. Results indicate that methanotroph activity was at least partially substrate-limited at the methane-emitting site and by other factors at the methane-oxidizing site. Soil depth also contributed to the control of surface methane fluxes, but to a lesser extent. Small differences in the soil water content may have contributed to differences in methanogen and methanotroph activities. This study therefore provides a first insight into the regulation of *in situ*, field-level surface CH_4 flux at the molecular level by an accurate reflection of gene : transcript abundance ratios for the key genes in methane generation and consumption.

Introduction

Boreal and subarctic peatlands store approximately 15–30% of the world's carbon and are believed to contribute 1–15 g C m^{-2} $year^{-1}$ to the atmospheric methane budget (Mikaloff Fletcher *et al.*, 2004; Chen & Prinn, 2006; Limpens *et al.*, 2008). Net methane emission from peatlands results from a balance between methanogenesis, carried out under anaerobic conditions by *Euryarchaeota*, and methane oxidation, performed by *Alpha-* and *Gammaproteobacteria* at oxic–anoxic interfaces (Conrad, 1996; Ritchie *et al.*, 1997; Edwards *et al.*, 1998). Potential methane oxidation rates measured in soil slurries usually exceed the rates of potential methanogenesis (Segers, 1998) and it is generally assumed that most methane produced is recycled and does not reach the atmosphere (Conrad, 1996; Pearce & Clymo, 2001; Raghoebarsing *et al.*, 2005). Indeed, soils with high methane oxidation actively serve as methane sinks. Nevertheless, individual site-to-site specific estimates of the proportion of methane produced that escapes oxidation and is released into the atmosphere vary widely (MacDonald *et al.*, 1996; Whalen & Reeburgh, 2000; Pearce & Clymo, 2001). The reasons for site-to-site differences are largely unknown, but vegetation type and hydrological conditions are known to be major controlling factors (Joabsson *et al.*, 1999; Kettunen, 2003; Minkkinen & Laine, 2006; Niklaus *et al.*, 2006; Hornibrook *et al.*, 2008). Few studies have combined analysis of communities of both methanogens and methanotrophs with associated measurements of methane oxidation and production potentials (Edwards *et al.*, 1998; McDonald *et al.*, 1999) and, consequently, the relationships between methanogen and methanotroph communities and their activities with *in situ* flux rates are not well understood.

Increasingly, the abundance, dynamics and diversity of functional gene transcripts are providing information on *in situ* activities of functional microbial groups (Holmes *et al.*, 2004; Nicol *et al.*, 2005; Steunou *et al.*, 2006; Smith *et al.*, 2007; Nicol *et al.*, 2008; Nicolaisen *et al.*, 2008). *In situ*

activities, by inference, indicate process rates, but little information is available on the reliability of this approach. Previously, Freitag & Prosser (2009) proposed that mRNA gene : transcript abundance ratios, established by quantitative PCR (qPCR) and reverse transcription (RT)-qPCR of the key functional gene involved in methanogenesis (*mcrA*), are indicative of the methane production potential in peat slurries. The aim of this study was to determine whether gene : transcript abundance ratios of the *mcrA* gene and of the key functional gene involved in methane oxidation (particulate membrane-bound methane monooxygenase; *pmoA*) were effective predictors of *in situ* CH_4 flux rates. The study was carried out at two neighbouring sites with contrasting flux characteristics (net source vs. net sink) in a heather moorland at Lake Vyrnwy, North Wales, UK.

Materials and methods

Site descriptions

The two sites (EC2 and EC6) were 0.5 km apart, located in the Afon Eiddew catchment of the upland Lake Vyrnwy RSPB reserve (North Wales, UK; 52°49′N, 3°35′E), and were part of a larger experiment studying the effects of vegetation type on net CH_4 flux from a typical UK upland area. The annual average air temperature was 6.4 °C and the annual average precipitation was 1501 mm at the Afon Eiddew catchment from 1961 to 1990. The sites were on an east-facing slope: EC2 at 475 m altitude and EC6 at 519 m. Plant coverage was common heather (*Calluna vulgaris*; 95%) with sparse hare's-tail cottongrass (*Eriophorum vaginatum*, 5%). The water table was near the soil surface and, at site EC6, just above the soil surface. The peat layer was at least 40 cm deep. At each of the two sites, the net CH_4 flux, green biomass of the plant species and gravimetric soil water content were determined at 15 equally spaced locations over an 8.5-m-long transect in August 2009. Four and five locations at EC2 and EC6, respectively, with the most highly contrasting CH_4 flux rates, were selected for molecular analysis. Locations were up to 6 m apart.

Methane flux measurements

Opaque polyvinyl chloride (PVC) rings (inner diameter 19 cm, height 20 cm) were inserted halfway into the soil. Net CH_4 fluxes were measured immediately within each ring using static, opaque chambers (30 cm high) covered with a reflective insulation foil to reduce heating during measurement. Gas-tight tygon tubing (inner diameter 1.6 mm, length 1.5 m) was connected to the top of each chamber to allow gas sampling with minimal disturbance, while the open tubing acted as a pressure vent between samplings. The chamber was connected to the PVC ring using an airtight rubber sealing band. At site EC2, areas with a high net CH_4 uptake were located earlier in the day using CH_4 flux measurements at a larger scale and with minimal disturbance (data not shown). This process identified areas where net CH_4 uptake dominated in site EC2 and subsequent measurements were taken using the smaller scale chamber approach. Uptake rates were determined by adding CH_4 to the headspace of the chambers at a concentration of 50 μM at the start of gas sampling, allowing the detection of any decrease in the CH_4 concentration in the headspace during subsequent GC. Gas samples were collected from each chamber every 30 min over a period of 120 min and stored in pre-evacuated Exetainers (Labco Limited, High Wycombe, UK). Methane concentrations of the gas samples were measured on a PerkinElmer-Arnel gas chromatograph (AutoSystem XL, PerkinElmer Instruments, Shelton, CT) equipped with a flame ionization detector and a 3.7-m Porapak Q 60/80 mesh column (N_2 carrier gas flow of 30 mL min^{-1}; and injector, column and detector temperatures of 120, 40 and 350 °C, respectively). The net CH_4 flux was determined from the slope of regressions of CH_4 concentrations with time in each chamber. Regressions with $r^2 < 0.90$ were rejected.

Soil and plant analyses

After the CH_4 measurements, above-ground vascular plant biomass was clipped within the ring at each location, sorted to the species level and divided into green and dead/woody biomass. Green above-ground dry weights of the two dominant plant species *C. vulgaris* and *E. vaginatum* within each core were determined after drying the green plant parts at 70 °C to a constant weight.

Following plant sampling, three subsamples of peat soil at 0–10 cm and 10–20 cm depths were collected from within each ring using a 2-cm auger, with samples within each ring being pooled and mixed for each depth. Samples were divided into two aliquots and immediately stored at 4 °C in the field. Subsamples for molecular analysis were frozen in liquid nitrogen within 4 h of collection and frozen at − 20 °C, before transfer to − 80 °C.

The second sets of aliquots were used to determine the gravimetric soil water content at 0–10 cm and 10–20 cm depths in each core. After removing roots from the soil samples, weight loss was measured after drying at 70 °C for at least 2 days.

Extraction of nucleic acids

Before the extraction of nucleic acids, peat cores were homogenized by cryomilling. In brief, frozen peat cores, double-bagged in strong polyethylene plastic bags, were precooled in liquid N_2 and shattered into small pieces by impact between a 2 lb hammer and an anvil. Small pieces were transferred into an IKA A11 analytical mill (VWR

International, Lutterworth, UK) and homogenized by impact grinding at liquid N_2 temperatures. Total nucleic acids were extracted by transferring aliquots (0.5 g) of frozen ground peat in duplicate into liquid N_2-chilled, 2-mL screw-cap Blue Matrix Ribolyser tubes (Hybaid, Ashford, Middlesex, UK), preloaded with 0.5 mL CTAB extraction buffer (Griffiths *et al.*, 2000). Loaded Ribolyser tubes were brought to ice temperatures until thawing of ground peat was visible by a colour change, before the addition of cold Tris-buffered phenol and chloroform/isoamylalcohol. Extraction and purification of nucleic acids, removal of genomic DNA and reverse transcription for RNA aliquots were carried out as described previously (Freitag & Prosser, 2009).

qPCR

Quantification of *mcrA* and *pmoA* DNA and mRNA sequence fragments was carried out using qPCR and RT-qPCR, respectively. *McrA* DNA and cDNA sequence fragments were amplified with the ML primer pair (Luton *et al.*, 2002; Juottonen *et al.*, 2006) as described previously (Freitag & Prosser, 2009), with some modifications. In brief, all qPCR reactions were carried out in 25-μL reactions with 5 μL of template DNA or cDNA added to a 20-μL qPCR reaction mixture containing 2.5 U AccuSure polymerase (Bioline, London, UK), 1 × AccuSure reaction buffer, including 2 mmol $MgSO_4$, 500 μmol of each dNTP and SYBR Green I (Invitrogen, 10 000 × concentration) at a final concentration of 0.4 × DMSO (5% final concentration, Sigma-Aldrich, Poole, UK), betaine (0.65 M, Sigma-Aldrich), linear acrylamide (100 ng μL^{-1}) and BSA (200 ng μL^{-1}) were added to the qPCR master mix to increase efficiency and sensitivity. The ML forward and reverse primers were used at 1.2 μM concentration each. *McrA* qPCR was carried out using a hot-start protocol with an initial denaturation step of 10 min at 95 °C and 40 cycles of 95 °C denaturation for 30 s, 56 °C annealing for 45 s and 68 °C extension for 45 s, followed by fluorescence quantification at the end of a 81 °C step for 8 s, to allow the dissociation of possible primer dimers and unspecific amplification products. Standard curves for calibration of *mcrA* qPCR were created from a 782-bp PCR amplicon spanning the *mcrA* region of *Methanosarcina barkeri* Schnellen 1947 (DSM 804) as described previously (Freitag & Prosser, 2009). *PmoA* qPCR was performed using the *pmoA* A189f–mb661r primer pair. The *pmoA* A189f–mb661r primer pair has a high target specificity and thus low interference from highly similar *amoA* sequence types (Costello & Lidstrom, 1999; Bourne *et al.*, 2001), but does not detect *Methylocella palustris* type II methanotrophs (Dedysh *et al.*, 2003). The PCR temperature profile was initially optimized for maximum amplicon specificity by temperature gradient qPCR with subsequent melt curve analysis and standard agarose gel electrophoresis. The qPCR reaction mixture was as described above, but with a 0.8 μM concentration of forward and reverse primers each, also containing 4 ng μL^{-1} of a single-strand binding protein (Sigma-Aldrich). *PmoA* qPCR was carried out using a hot-start protocol with an initial denaturation step of 10 min at 95 °C and 40 cycles of 95 °C denaturation for 30 s, 64 °C annealing for 45 s and 68 °C extension for 45 s, followed by fluorescence quantification at the end of an 86.5 °C step for 16 s, to allow the dissociation of possible primer dimers and unspecific amplification products. Standard curves for the calibration of *pmoA* qPCR were created using triplicate 10-fold dilution series covering seven orders of magnitude from 10^1 to 10^7 gene copies per qPCR reaction during each run. The standard consisted of a 709-bp PCR amplicon spanning the *pmoA* region of *Methylosinus trichosporium* OB3b (NCIMB 11131) created with primers M.trich_f-TTCTGACGCT GCTGTTTCTG and M.trich_r-TTCCGAGGAACCAAAGA GAA. *Methylosinus trichosporium* primer sequences were aligned to the *pmo*-operon sequence of *M. trichosporium* (NCBI U31650) using the EPRIMER3 primer prediction software (Rice *et al.*, 2000). qPCR reactions were performed in duplicate per DNA or cDNA template and were carried out using a MyIQ5 Real-Time PCR detection system (BioRad, Hemel Hempstead, UK). DNA and cDNA qPCR *mcrA* and *pmoA* copy numbers were expressed g^{-1} soil (dry weight) and the relative gene expression was calculated as gene : transcript abundance $\log_2$ ratios (Yun *et al.*, 2006). Additionally, the qPCR amplification efficiency of peat samples was assessed by subjecting serial dilutions of DNA to *mcrA* and *pmoA* qPCR as described above. Efficiency was calculated as the slope of linear regression of derived copy numbers against the dilution factor.

Statistical analysis

Data are expressed as the mean of biological (sites and depths) replicates from the means of technical replicates (extractions, qPCRs; $n = 8$) and SEs. Simple linear regression analysis was performed to describe the relationships of gene : transcript abundance $\log_2$ ratios with CH_4 flux data. ANOVA was used to test the significance of regression coefficients against a slope of 0, and the significance in the mean differences between different sites or depth was established by a simple one-way ANOVA. *F*-ratio test significance was only considered when not influenced by individual data points.

Results and discussion

CH_4 flux rates

The mean methane flux rates at the cover box locations selected for analysis were significantly different between

Lake Vyrnwy heather sites EC2 and EC6 (Table 1). Flux rates were negative at the four selected EC2 cover box locations, demonstrating net methane oxidation (range: -0.5 to -1.8 mg CH_4 m^{-2} h^{-1}) with values similar to those observed for a drained Finnish fen (Minkkinen & Laine, 2006). Methane emissions from the five selected locations at site EC6 were between 8.3 and 11.9 mg CH_4 m^{-2} h^{-1} and thus in a range similar to observations from an ombrotrophic peatland in the United Kingdom (Greenup *et al.*, 2000; Laine *et al.*, 2007). The mean net methane emission and methane oxidation rates at the selected sites corresponded to mean higher and lower water contents, respectively, in the 0–10-cm-depth soil cores. However, the water content was more variable at the methane-emitting site EC6 and differences were only significant between 0–10 cm and 10–20 cm depth at site EC6 ($P = 0.03$; Table 1). Green above-ground biomass of *C. vulgaris* and *E. vaginatum* within the cores did not differ significantly between the two sites (data not shown).

Molecular analysis

The total nucleic acid yields were similar for the selected locations at EC2 and EC6 sampling sites for RNA and DNA, but yields were significantly higher at 0–10 cm depth (Table 1). As reported previously, replicate extractions were more reproducible for RNA than for DNA, with respective average SEM values of 0.33 and 1.9, but yields were lower (Freitag & Prosser, 2009). Amplification of qPCR calibration standards was linear (regression coefficient 0.991–0.995) for both *mcrA* and *pmoA* qPCR assays from 10^2 to 10^7 copies of template per reaction. qPCR efficiency in all assays was between 85% and 97%, allowing confident enumeration of > 10 *mcrA* copies per assay. Unspecific amplification was observed during *pmoA* qPCR assays at template concentrations below 10^3 copy numbers per reaction, necessitating a high fluorescence read temperature close to the melting temperature of the *pmoA* amplicon, also resulting in a higher detection limit of $> 10^2$ *pmoA* copies per assay. Peat sample *mcrA* and *pmoA* qPCR efficiencies calculated from the slope of linear regressions of template DNA dilution series were within the same range as qPCR standard efficiencies, suggesting an unbiased enumeration of the environmental *mcrA* and *pmoA* sequence types, although *pmoA* qPCR sample efficiencies were more variable than for the *mcrA* assay. Amplicons were generated from all DNA and cDNA templates and the mean *mcrA* gene and transcript copy numbers were higher than reported previously (Freitag & Prosser, 2009), with the highest values of 2.2×10^9 and 4.2×10^9 *mcrA* genes and transcripts g^{-1} (dry

Table 1. CH_4 flux, gravimetric water content, total DNA and RNA yields, log *mcrA* and *pmoA* gene and transcript abundances and $\log_2$ gene : transcript abundance ratios

	Depth (cm)	Site EC2	Site EC6	*P*-value	
CH_4 flux (mg m^{-2} h^{-1})	/	-0.95 (0.29)	10.2 (0.6)	< 0.0001	
				Site	Depth
Soil water content (% gravimetric content)	0–10	83.4 (0.28)	88.2 (1.16)	0.82	0.06
	10–20	84.7 (0.38)	78.7 (3.6)		
Total DNA (μg g^{-1} soil)	0–10	7.2 (1.4)	8.6 (1.7)	0.86	0.04
	10–20	4.7 (0.35)	4.1 (2.0)		
Total RNA (μg g^{-1} soil)	0–10	7.8 (1.8)	8.3 (1.9)	0.71	0.009
	10–20	4.8 (0.3)	2.9 (1.3)		
Log *mcrA* template abundance (g^{-1} soil)	0–10	7.9 (0.51)	8.9 (0.13)	0.03	0.04
	10–20	7.1 (0.42)	8.1 (0.59)		
Log *mcrA* transcript abundance (g^{-1} soil)	0–10	8.5 (0.37)	9.25 (0.08)	0.006	0.28
	10–20	7.7 (0.48)	9.0 (0.31)		
Log *pmoA* template abundance (g^{-1} soil)	0–10	7.1 (0.67)	8.7 (0.11)	0.04	0.3
	10–20	7.2 (0.58)	7.7 (0.45)		
Log *pmoA* transcript abundance (g^{-1} soil)	0–10	5.4 (1.0)	6.7 (0.3)	0.04	0.59
	10–20	5.8 (0.75)	7.0 (0.24)		
Gene : transcript abundance ratios					
McrA	0–10	1.059 (0.02)	1.048 (0.02)	0.94	0.07
	10–20	1.097 (0.003)	1.126 (0.06)		
PmoA	0–10	0.742 (0.07)	0.765 (0.04)	0.19	0.04
	10–20	0.796 (0.04)	0.923 (0.05)		
pmoA : *mcrA*	0–10	0.66 (0.06)	0.74 (0.03)	0.11	0.173
	10–20	0.73 (0.034)	0.83 (0.02)		

Data are presented as mean and SE (in parentheses, $n = 4$–5) for each soil core depth at each sampling site. *P*-values are from *t*-test comparisons of means between sites.

weight) soil, respectively. *PmoA* gene abundances were in the same order of magnitude as *mcrA* gene abundances and more than one order of magnitude higher than reported from peat soils with and without heather vegetation (Kolb *et al.*, 2005; Chen *et al.*, 2008). However, *pmoA* transcript abundances were lower by one to two orders of magnitude than gene abundances and close to the detection limit of qPCR in some samples. The mean *mcrA* gene and transcript abundances were significantly higher in the methane-emitting site EC6, and for both sites, gene and transcript abundances were higher in the 0–10-cm-depth cores. For *pmoA*, the mean gene and transcript abundances were also significantly higher in the methane-emitting site EC6, and at both sites, transcript abundances were also higher in 10–20-cm-depth cores, but the differences were not significant (Table 1). Methanotroph abundance and activity may, however, have been underestimated due to the exclusion of *M. palustris* type II target sequences (Dedysh, 2002; Dedysh *et al.*, 2003).

Gene : transcript abundance ratios

The gene : transcript abundance ratio reflects transcript abundance per cell and has been suggested as a more direct measure of physiological activity then absolute abundance (Kolb *et al.*, 2005; Yun *et al.*, 2006; Knorr *et al.*, 2008a, b; Nicolaisen *et al.*, 2008; Freitag & Prosser, 2009). At the CH_4-emitting site, EC6, the mean *mcrA* and *pmoA* gene : transcript abundance ratios were higher, but differences were significant only between depths for *pmoA* (Table 1). Gene : transcript abundance ratios spanned 0.5–8.1 transcripts per cell for *mcrA*, but the ratios were significantly lower for *pmoA* due to lower *pmoA* transcript abundances. Values were also lower than reports from methane-oxidizing forest soils (Kolb *et al.*, 2005) that also showed up to threefold lower negative CH_4 flux rates. The *mcrA* gene : transcript ratio was constant over all samples at both sites ($r^2 = 0.79$; $P = 0.003$). The equivalent *pmoA* ratio varied at the EC6 site ($r^2 = 0.1$; $P = 0.76$), but not at the methane-oxidizing EC2 site ($r^2 = 0.91$; $P = 0.002$). Pure culture studies on thermophilic methanogens have demonstrated 50–400 *mcrA* transcripts per cell (Hennigan & Reeve, 1994), and the low ratios for both *mcrA* and *pmoA* therefore suggest low overall activity, underestimation of gene transcripts and/or high numbers of inactive or dead cells. Whereas methanogens are presumably present and active below the water table in waterlogged peat soils (Galand *et al.*, 2002), active methane-oxidizing bacteria are assumed to be confined to a soil depth above the oxic–anoxic interface or in close proximity to oxygen-conducting plant tissues, where methane concentrations are sufficient to support energy gain (Schimel *et al.*, 1993; Henckel *et al.*, 2000). The homogenization over a soil depth of 10 cm during extraction may have integrated small regions of high activities over larger areas where oxygen supply was not sufficient to maintain methane oxidation, potentially resulting in lower total *pmoA* transcript abundances. However, the total *pmoA* abundance and transcriptional activity were not significantly different at a soil depth below 10 cm at both sites, and Jaatinen *et al.* (2005) also found high methane-oxidizing potentials below the water table in a Finnish mire and decreased methanotroph activity in drained soil.

In the 0–10-cm soil cores at the CH_4-emitting site, EC6, the CH_4 flux rate increased linearly with the *mcrA* gene : transcript abundance ratio (Table 2, Fig. 1a), while there was no evidence for a linear relationship between CH_4 fluxes and *pmoA* ratios. In contrast, at the CH_4-oxidizing site, EC2, the CH_4 flux rate was inversely related to the *pmoA* gene : transcript abundance ratio, while *mcrA* ratios showed no relationship (Table 2, Fig. 1a). For both sites, trends in relationships between CH_4 flux rates and gene : transcript abundance ratios were similar in 10–20-cm-depth soil cores (Fig. 1b), but were not significant.

CH_4 flux showed similar trends with transcriptional activity (*pmoA* for site EC2 and *mcrA* for site EC6) and gene abundance, but the relationships were more variable than those with ratios, and regression coefficients were not significant. At the methane-emitting site, EC6, *mcrA* transcript abundance, but not gene abundance, increased with CH_4 flux. Trends with transcriptional activity were less evident in the 10–20 cm depth than the 0–10 cm depth (data not shown). The relationships between CH_4 flux and gene and transcript abundances over the entire data were only significant for *mcrA* transcripts ($r^2 = 0.42$; $P = 0.007$).

The dependence of methanotroph activity on substrate supply by methanogens was also explored by analysing the relationships between gene abundance, transcript abundance and the gene : transcript ratio of *pmoA* and *mcrA*. A significant correlation was found only for the relationship between *pmoA* transcript and *mcrA* transcript abundances in the 0–10 cm depth at the methane-emitting site EC6 ($r^2 = 0.78$; $P = 0.048$; Fig. 2). This suggests that methanotroph activity at site EC6 was at least partially dependent on

Table 2. Correlation coefficients from linear regressions of CH_4 flux rates with $\log_2$ gene : transcript abundance ratios

	Site	Depth	r^2	*P*-value
mcrA ratio over CH_4 flux	EC6	0–10	0.91	0.03
		10–20	0.51	0.18
	EC2	0–10	0.13	0.25
		10–20	0.02	0.86
pmoA ratio over CH_4 flux	EC6	0–10	0.45	0.21
		10–20	0.21	0.43
	EC2	0–10	0.99	0.01
		10–20	0.88	0.06

Data are presented as mean and SE (in parentheses, $n = 4$–5) for each soil core depth at each sampling site. *P*-values are from the *F*-ratio test of ANOVA against a slope of 0 for linear regressions.

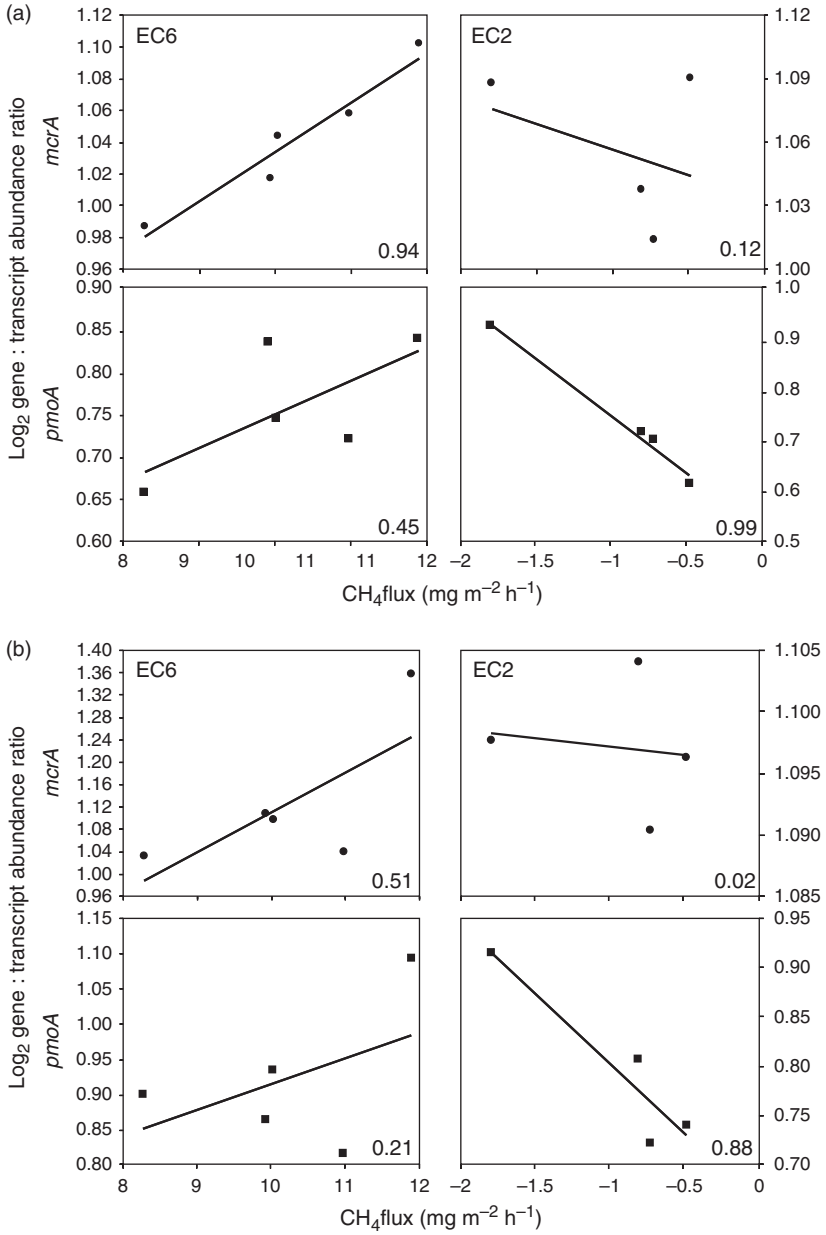

Fig. 1. Activity of Lake Vyrnwy peat methanogen and methanotrophs from soil depths (a) 0–10 cm and (b) 10–20 cm assessed as *mcrA* (●) and *pmoA* (■) $\log_2$ gene : transcript abundance ratios plotted against corresponding CH_4 flux rates. Left panels show the CH_4-emitting site EC6 and right panels show the CH_4-oxidizing site EC2.

the substrate produced by methanogens. However, at site EC6, CH_4 flux rates were related to *mcrA* gene : transcript abundance ratios, while the relationship with the *pmoA* ratio was not significant. The relatively high *pmoA* gene and transcript abundances and their ratios at site EC6 also suggest significant CH_4 oxidation, but the surface CH_4 flux is not modulated by *pmoA* activity, as at site EC2.

Conversely, the inverse linear relationship between the CH_4 flux rate and the *pmoA* ratio at the methane-oxidizing site EC2 suggests the direct influence of methanotrophs, but not methanogens, on the modulation of surface CH_4 flux. As methanotrophy is not coupled to methanogen activity, oxygen supply, as the second major factor controlling methanotroph activity (Henckel *et al.*, 2000), is likely to control the surface CH_4 flux.

These results support the hypothesis that in the peat soils studied, CH_4 flux rates are primarily controlled by methanotroph activity: the potential for CH_4 production and oxidation activity is present throughout, but in sites with positive flux rates, oxidation does not compensate for

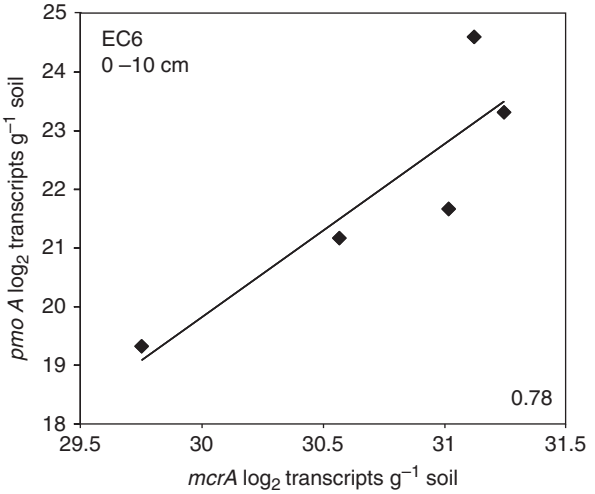

Fig. 2. Linear regression of *pmoA* and *mcrA* $\log_2$ transcripts g^{-1} soil at depth 0–10 cm at the methane-emitting site EC6 ($P = 0.048$).

methanogen activity. At low or negative CH_4 flux rates, methanogen activity may also be high, but CH_4-oxidizer activity is less limited by other constraining factors and fluxes are mainly controlled by *pmoA* activity. The potential for oxidation exceeds the amount of CH_4 produced, obscuring any relationship between *pmoA* and *mcrA* and leading to a direct correlation between the CH_4 flux rate and the *pmoA* gene : transcript abundance ratio. The total *mcrA* and *pmoA* gene and transcript abundances also suggest that the total CH_4 production and consumption were similar at the two sites at both depths and only the linear relationships between the CH_4 flux rate and *mcrA* or *pmoA* gene : transcript abundance ratios effectively reflect the difference between sites. It is thus likely that methanogenesis and methane oxidation rates established under laboratory conditions as potentials are also similar and thus may mask site-specific characteristic CH_4 turnover and flux rates (Knorr *et al.*, 2008a, b).

Gene transcripts, abundances and their ratios in the 10–20-cm-depth cores (Table 1, Fig. 1b) demonstrate similar, if somewhat weaker, trends to the data from 0–10-cm-depth cores. In particular, the correlation of lower soil depth *mcrA* activity with surface CH_4 fluxes at locations with net CH_4 emissions suggests that greater soil depths also play a role in the control of surface CH_4 fluxes and contribute to the total soil methane budget when methane fluxes are not mediated by methane oxidizer activity (McLain *et al.*, 2002). Similarly, the high *pmoA* gene and transcript abundances and the correlation of *pmoA* activity with net CH_4 in the deeper soil layer at EC2 suggest an influence of methanotroph activity at greater soil depth on surface CH_4 fluxes. However, the presence and activity of methanotrophs in deeper soil layers is usually associated with oxygen-conducting plant tissues, but is surprising in a heather-dominated waterlogged peat soil. The vegetation type, density and geographical characteristics were similar between sites and differences in the soil water content were small and not significant. Higher soil water saturation usually indicates decreasing oxygen availability and thus favours conditions for anaerobic processes. If the observed maximum difference of 5% soil water content is sufficient for a complete reversal of CH_4-emission characteristics, the balance between conditions favouring methanogenesis or methane oxidation is rather delicate, as such small changes in water content are likely to be affected by single rainfall events or a slight variation in water drainage. Conversely, EC6 soil water content at the methane-emitting site was also negatively correlated with *mcrA* and *pmoA* gene : transcript abundance ratios ($r^2 = 0.67$ and 0.69; $P = 0.004$ and 0.003 for *mcrA* and *pmoA*, respectively). In total, the limited size of the data set analysed does not allow a conclusive inference on the major driving forces in the observed differences in activities and CH_4 flux as additional factors indirectly affected by or independent of the soil water content (e.g. availability of degradable organic matter, history of water table movements) may also be responsible for changes in methanogenesis and methane oxidation activities.

In conclusion, *in situ* CH_4 flux rates were accurately reflected by gene : transcript abundance ratios for the key genes in methane production and oxidation even at sites with contrasting characteristics. This is the first study demonstrating the regulation of *in situ* surface CH_4 flux on the molecular level.

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