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Role of CH_4 oxidation, production and transport in forest soil CH_4 flux

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Abstract

Forest soils are an important sink for atmospheric CH_4 but the contribution of CH_4 oxidation, production and transport to the overall CH_4 flux is difficult to quantify. It is important to understand the role these processes play in CH_4 dynamics of forest soils, to enable prediction of how the size of this sink will respond to future environmental change. Methane oxidation, production and transport were investigated for a temperate forest soil, previously shown to be a net CH_4 consumer, to determine the extent to which physical and biological processes contributed to the net flux. The sum of oxidation rates for soil layers were significantly greater ($P < 0.05$) than for the intact soil cores from which the layers were taken. Combined with the immediate inhibition of CH_4 uptake on waterlogging soils, the findings suggested that soil CH_4 diffusion was an important regulator of CH_4 uptake. In support of this, a subsurface maximum for CH_4 oxidation was observed, but the exact depth of the maximum differed when rates were calculated on a mass or on an areal basis. Markedly varying potential CH_4 uptake activities between soil cores were masked in intact core rates. Potential CH_4 oxidation conformed well to Michaelis–Menten kinetics but V_{max}, K_t and a_A° values varied with depth, suggesting different functional methanotrophic communities were active in the profile. The presence of monophasic kinetics in fresh soil could not be used to infer that the soil was exposed only to CH_4 mixing ratios $\leq$ atmospheric, as challenging soils with 20% CH_4 in air did not induce low-affinity oxidation kinetics. Atmospheric CH_4 oxidation potentials exceeded production potentials by 10–220 times. The results show that the forest soil CH_4 flux was dominated by CH_4 oxidation and transport, methanogenesis played only a minor role.

Keywords: Methane oxidation; Methane consumption; Forest soil; Methane transport; Kinetics

1. Introduction

Atmospheric concentrations of CH_4, an important greenhouse gas (Prinn, 1994), have increased by approximately 2.45 fold since the Industrial Revolution (IPCC, 1995). Unsaturated soils represent the only net biological sink for atmospheric CH_4 and any effects on the size of this sink will markedly affect the rate of change in atmospheric CH_4 concentration (Reeburgh et al., 1993). In peatlands the CH_4 flux from the soil surface is often reported to be the resultant of CH_4 oxidation, CH_4 production and CH_4 transport within the soil (Moore and Dalva, 1997), and the same assumption is applicable to unsaturated soils. It is important to understand and quantify the role these three processes play in the CH_4 dynamics of unsaturated soils, to enable prediction of how the size of this sink will respond to future environmental change.

The characteristics of the CH_4 oxidising and producing communities and factors which affect these characteristics and CH_4 transport determine the magnitude of the surface CH_4 flux. The 'characteristics' include the potential activity and depth distribution of CH_4 oxidation and production and the factors may include events such as inorganic N fertilisation and changes in soil water content. For example, inorganic N additions can decrease CH_4 oxidation potentials (Gulledge and Schimel, 1998) and similar additions in the field have decreased soil surface CH_4 uptake (Steudler et al., 1989). Similarly, forest soils consume less CH_4 as the soil is made wetter (Castro et al., 1994) but rather than an inhibition of the soil CH_4 oxidation potential, the effect may be the result of slower CH_4 diffusion to the site of oxidation or a stimulation of CH_4 production. Slower CH_4 diffusion would be an important factor if a sub-surface maximum for CH_4 oxidation exists within the soil (Kruse et al., 1996), as CH_4 has to diffuse down through the soil and does so 10^4 times slower through water than air (Whalen et al., 1990). To

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determine whether changes in CH_4 production or rate of CH_4 diffusion are most important in reduced net soil CH_4 uptake in wetted soils, determination of the potential activities and vertical distribution of oxidation and production within the soil is useful.

The kinetic parameters for soil CH_4 oxidation also provide valuable information regarding the dynamics of CH_4 oxidation and production within a soil profile. By quantifying the kinetics, Bender and Conrad (1992) suggested that it could be determined whether a soil was exposed only to atmospheric and sub-atmospheric concentrations of CH_4, with high-affinity activity then dominating. The additional presence of low-affinity activity (Bender and Conrad, 1992, 1995; Conrad, 1996) would imply that a significant CH_4 source was present within the soil, possibly due to biological CH_4 production, as has been observed for many peat systems (Hall et al., 1996).

Field studies showed a forest soil to be a consistent net CH_4 oxidiser across 1 year with the rate of CH_4 uptake primarily controlled by soil water potential (Bradford et al., 2001). Our purpose was to determine the extent to which CH_4 oxidation, production and transport contributed to the observed temporal changes in net CH_4 uptake of this soil. To achieve this the vertical distribution of CH_4 oxidation potentials and kinetic parameters within the soil profile were characterised to determine the depth of maximum oxidation and whether the CH_4 mixing ratio experienced *in situ* exceeded atmospheric concentrations. Oxidation kinetics were determined for soils experimentally challenged with 20% CH_4 in air to assess the validity of using kinetics to infer *in situ* mixing ratios. The potential of the soil to produce CH_4 was assessed by waterlogging intact soil cores and incubating sieved soil under an O_2 free atmosphere. In addition, it was hypothesised that if soil CH_4 diffusion was an important determinant of soil CH_4 flux, then the sum of the soil CH_4 fluxes for undisturbed soil layers would be greater than the CH_4 flux rate of an intact (i.e. not divided into soil layers) soil core.

2. Methods and materials

2.1. Soil

Soil cores were collected in 25 cm tall PVC cylinders (15 cm dia.) from Perridge Forest (NGR SX 869908), a temperate mixed deciduous woodland consisting predominantly of mature (ca. 80 years old) oak (*Quercus robur* L.). The soil was a freely draining, low-base status, acidic brown earth, mapped within the Denbigh 1 Association (Findlay et al., 1984). For a full site and soil description see Bradford et al. (2001).

2.2. Determining CH_4 flux

Methane flux for sieved soil (to 2 mm) was determined using a closed chamber technique, and for soil cores or undisturbed (i.e. non-sieved/unbroken) soil core layers using an open chamber technique. All experiments were performed at 20°C.

For the closed chamber technique, CH_4 concentrations were first standardised within a Wheaton bottle (120 ml) containing 10 g fresh weight (gfw) soil. A headspace gas sample was taken immediately upon sealing of the bottle. A second headspace sample was taken after 1 h, except for the kinetic determinations where repeated sampling over time was used. Although the soils follow first-order reaction kinetics (see Section 3 of this paper), a two-point rate calculation over a 1 h incubation provided rates representative of those calculated using rate constants derived from log-transformed time course data (M. A. Bradford, unpublished PhD thesis, Exeter University, 1999). Methane concentrations were determined on a Perkin Elmer Autosystem XL GC fitted with a FID operated at 200°C. Methane was separated isothermally on a Porapak Q (50–80 mesh) 2.5 m packed stainless steel column, with N_2 carrier gas flowing at 20 ml min^{-1}. The detector response was calibrated using certified gas standards (British Oxygen Company, Special Gases, UK). Rates of CH_4 oxidation for sieved soils are expressed as nmol CH_4 consumed g^{-1} dry weight (gdw) soil d^{-1}.

For the open chamber technique, PVC cylinders containing soil cores were sealed at the base and capped with a Perspex lid with two air inlets and one air outlet. External ambient air, supplied via a single mixing-chamber to ensure all cores received input air with the same CH_4 concentrations, was drawn through the chamber headspaces at 50 ml min^{-1}. The gas stream was automatically monitored for CH_4 by gas chromatography; for a full description of the gas analysis and data storage see Ineson et al. (1998).

2.3. CH_4 flux of intact soil cores, and undisturbed and sieved soil layers

This experiment was performed to determine: (1) the depth profile of soil CH_4 oxidation; (2) if soil CH_4 diffusion was an important determinant of soil CH_4 flux. Due to CH_4 flux sampling constraints, the experiment was performed in two halves ($A + B$). In parts A and B, CH_4 fluxes of intact soil cores were monitored and the cores were then split with a knife into defined soil horizons/layers (see below) and the CH_4 flux rates of these horizons/layers assessed. In part A, the intact cores were first split into the two constituent soil horizons, H and A. These two horizons were monitored, and then the H horizon was split into the H_t (t = top; 4 cm deep) and H_b (b = bottom; ca. 6 cm deep) soil layers and monitored. Three control cores were not split. In part B, intact cores were split into H_b and A_t (top 4 cm of A horizon) layers and monitored. Intact soil cores were analysed as controls. Lastly, to determine the depth profile of CH_4 oxidation for the upper 25 cm of soil, the H_t, H_b, A_t and A_b (bottom ca. 9 cm of A horizon) soil layers were sieved and their CH_4 flux rates determined using the described closed chamber technique. In addition, 5 gfw of the litter layer was finely cut

and analysed as for soil to determine CH_4 flux. Empty Wheaton bottles were analysed as blanks.

2.4. Kinetics of CH_4 oxidation

Sieved soils from the H_t, H_b and A_t layers of 20 cores were thoroughly mixed by layer and 10 gfw soil placed into Wheaton bottles. Six CH_4 oxidation rates were measured at increasing initial CH_4 mixing ratios in air (to 132 $\mu l\, l^{-1}$) and from these rates the kinetic parameters K_t and V_{max} were determined using Lineweaver–Burk plots. Three replicates were sampled per time point for each initial CH_4 concentration; rate constants were calculated from log-transformed time course data. Incubation time increased from 2 to 10 h as the initial CH_4 concentration was increased, facilitating measurement of a marked and repeatable decrease in headspace CH_4 concentration. Soil water content was determined gravimetrically and maintained by deionised water addition. Control soils were not exposed to higher than ambient CH_4 mixing ratios; ambient oxidation rates of controls were compared with soils exposed to higher than ambient CH_4 concentrations to ensure oxidation activities did not change over the course of the experiment.

V_{max} is defined here as the maximum specific uptake rate and K_t the half saturation concentration for uptake, after converting maximum uptake rates to units of CH_4 accumulated g^{-1} soil f.w. h^{-1}, similar to the methodology applied by Button (1991) for whole-cell uptake kinetics. V_{max} is reached at the substrate concentration where an organism can no longer increase its substrate utilisation rate and, thus, is a measure of the maximal rate of substrate uptake per unit of biomass. K_t is generally used as a measure for affinity of an organism for a substrate but is often misleading (Button, 1991) and so specific affinity ($a_A^{\circlearrowleft}$) was also determined. $a_A^{\circlearrowleft}$ is the best indicator of the ability of microorganisms to collect substrate from a dilute solution (Button, 1991) and is calculated from the relationship: $a_A^{\circlearrowleft} = V_{max}/K_t$ (Schut et al., 1993). Soil water CH_4 concentrations were calculated using the Ideal Gas Law and the Bunsen solubility coefficient for CH_4 at 20°C and 0‰ salinity (Yamamoto et al., 1976).

To determine the threshold (*Th*) for CH_4 oxidation, the point at which the substrate is at too low a concentration to be assimilated, soil bottles initially at ambient CH_4 mixing ratios were sampled after 2, 3, 5 and 15 days.

To evaluate the CH_4 producing potential of the soil, four additional Wheaton bottles per soil layer were flushed with N_2 gas and the headspace CH_4 concentrations re-sampled after 5 days. Anaerobic indicator (Don Whitley Scientific Ltd, W. Yorkshire, UK) was used to establish whether O_2-free conditions persisted in five control bottles containing soil.

Soil from H_b and A_t layers was autoclaved for 1 h at 121°C to determine if oxidation was a biological process. These soils were chosen because they exhibited the highest CH_4 oxidation potentials. Oxidation rates were assessed at the start and end of the kinetic experiment and autoclaved, soil free Wheaton bottles were analysed as blanks.

To establish whether low-affinity CH_4 oxidising activity could develop when the soil was exposed to high CH_4 mixing ratios, A_t layer soil (chosen because it represented the maximum for CH_4 oxidation on a volume basis) was challenged with 20% CH_4 in air for 40 days and the kinetic parameters V_{max}, K_t and $a_A^{\circlearrowleft}$ determined as above. For the 40 days incubation, four replicates of 130 gfw soil aliquots in 1.7 l Kilner jars were used for the ambient and elevated CH_4 treatments. Soils were exposed to laboratory air for 15 min every 2–3 days to restore O_2 concentrations to those in ambient air and then headspace CH_4 concentrations were readjusted to restore treatments. Soil water content was determined gravimetrically and maintained by deionised water addition.

2.5. Waterlogging soil cores

The potential for soil to produce CH_4 under waterlogged conditions was determined by comparing CH_4 flux from four waterlogged soil cores and four non-waterlogged soil cores (control). Soils were monitored to quantify baseline CH_4 flux rates prior to treatment assignation, to enable blocking in the experimental design. Waterlogged cores were maintained in polythene buckets filled with deionised water to the height of the top of the soil core. The height of the water was maintained for 30 days during which soil CH_4 flux was periodically sampled.

2.6. Statistical analysis

All data analyses and statistical comparisons were performed using SAS (SAS Institute, 1988). Repeated measures blocked ANOVA were used to assess the effects of waterlogging cores and of splitting intact cores and soil horizons. Paired t-tests, or blocked ANOVA without repeated measures, were performed for sieved soil experiments and for comparing means for split core units assessed for CH_4 flux at separate times. Frequency distributions of the CH_4 fluxes or model residuals were tested for normality ($\alpha = 0.1$) using the Shapiro–Wilk test. Non-normal data were normalised by $\log_{10}$-transformation prior to analysis. Coefficients of variation (CV) were calculated for CH_4 flux data in the core splitting experiments to provide a measure of the spatial heterogeneity of flux rates.

3. Results

3.1. CH_4 flux of intact soil cores, and undisturbed and sieved soil layers

The sum of net CH_4 oxidation rates for constituent soil layers of whole soil cores and H horizons were significantly greater ($P < 0.05$) than the measured oxidation rates of intact whole soil cores and intact whole H horizons (Fig. 1). When

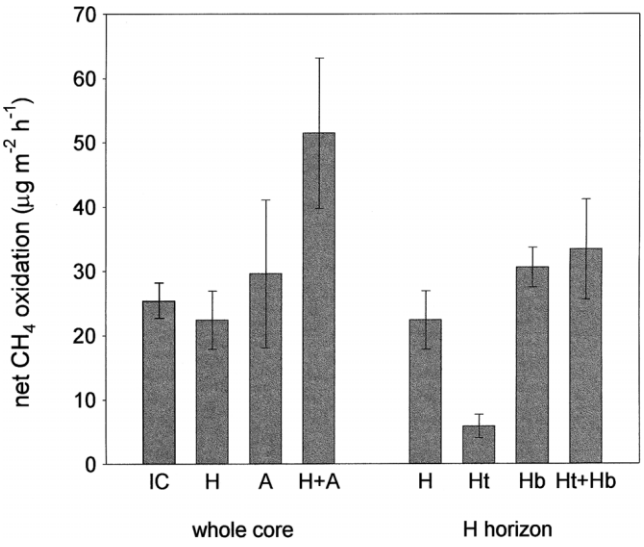

Fig. 1. Net CH_4 oxidation of intact soil core (IC) and undisturbed H horizon and the individual and summed fluxes from the constituent soil layers (H, A; H_t, H_b). The sum of the fluxes from the constituent layers (H + A; $H_t + H_b$) were significantly greater ($P < 0.05$) than the fluxes from the soil units from which they were taken (IC; H). Flux rates are means ±1 SEM ($n = 3$). H_t, top of H horizon; H_b, bottom of H horizon.

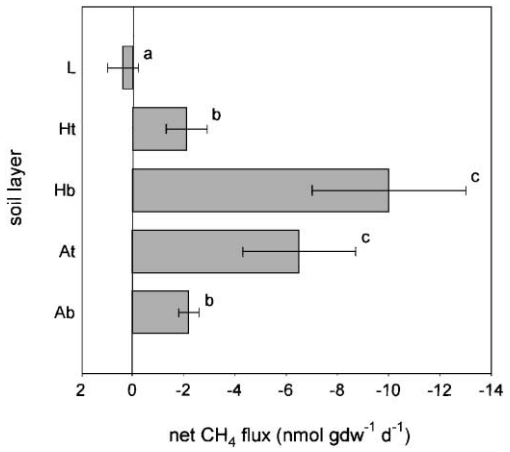

Fig. 2. Depth profile of potential net CH_4 flux (net CH_4 oxidation is shown by negative values). Bars marked with different letters (a–c) have significantly different flux rates ($P < 0.05$). Changes in headspace CH_4 concentrations in soil free bottles were not significantly different from changes in L layer bottles ($P > 0.05$). Flux rates are means ±1 SEM ($n = 4$). *L*, litter layer; H_t and H_b, top and bottom of H horizon; A_t and A_b, top and bottom of A horizon.

other soil layers apart from H_b and A_t were discarded (Part *B*), an increased CH_4 consumption for the sum of these two layers over the intact core rate was observed (data not shown), but the increase was not significant ($P = 0.09$). No changes in flux rates of parallel intact control cores were observed and, thus, the shift in rates was associated with the splitting of cores and horizons. High potential CH_4 oxidation rates in one layer of a soil core corresponded with high potential oxidation in another layer of the same core (data not shown); the same was true for low potential rates.

The CVs for net CH_4 oxidation rates across replicates markedly increased as soil cores were split into horizons and then into layers (Table 1).

Except for the litter layer, all sieved soil layers consistently oxidised CH_4 under the study conditions (Fig. 2). Change in headspace CH_4 concentrations in litter layer bottles were not significantly different to those for soil free bottles ($P > 0.05$) and were significantly lower ($P < 0.05$) than for all other soil layers. The H_b and A_t soil layers had the highest oxidation potentials, which were significantly greater ($P < 0.05$) than the measured potentials for the H_t and A_b layers. Although sieved H_b layer soil had a higher oxidation potential than sieved A_t layer soil (Fig. 2), there was no significant difference between the rates ($P > 0.05$). On a mass basis in undisturbed soil, oxidation potentials were also highest for H_b soil and this was significant ($P < 0.05$; Table 2), but on an areal basis, the maximum potential CH_4 oxidation occurred in A_t soil ($P < 0.05$; Table 2). Oxidation potentials on a mass basis for H_b and A_t soil increased when assessed for sieved rather than undisturbed soil (Table 2).

3.2. Kinetics of CH_4 oxidation

Potential CH_4 oxidation in H_t, H_b and A_t soils conformed

Table 1
Coefficients of variation (CV) for net CH_4 oxidation rates of intact soil cores (IC), undisturbed H and A horizons and the constituent soil layers (A_t, H_t, H_b) of the horizons, ($n = 3$). The experiment was performed in two halves (A + B). CVs increased as soils were broken down from intact cores into horizons and layers (A_t, top of A horizon; H_t, top of H horizon; H_b, bottom of H horizon)

Soil layer	CV part *A*	CV part *B*
IC	19.5	19.8
H	34.6	ND
A	27.4	ND
A_t	ND	39.1
H_t	66.5	ND
H_b	41.1	56.4

Table 2
Net CH_4 oxidation by area (μg CH_4 m^{-2} h^{-1}) and by mass (nmol CH_4 gdw^{-1} d^{-1}) of undisturbed and sieved soil layers (H_b, A_t; $n = 4$). (Values linked by different superscript letters are significantly different within the same row ($P < 0.05$). Oxidation was significantly greater by mass for sieved A_t soil than undisturbed A_t soil ($P < 0.05$); no significant difference between sieved and undisturbed H_b soil was observed. (H_b, bottom of H horizon; A_t, top of A horizon)

Soil layer	H_b	A_t
Net CH_4 oxidation ± 1 SEM		
By area (undisturbed soil)	25.6 ± 3.3^a	35.4 ± 3.1^b
By mass (undisturbed soil)	4.9 ± 0.1^a	1.1 ± 0.6^b
By mass (sieved soil)	10.0 ± 3.0	6.5 ± 2.2

Table 3
Kinetic parameters (V_{max}, a_A°, K_t, *Th*) of potential net CH_4 oxidation for fresh soil layers (H_t, H_b, A_t) and the ratio of potential atmospheric CH_4 oxidation to potential CH_4 production ($n = 3$) (H_t, top of H horizon; H_b, bottom of H horizon; A_t, top of A horizon)

Soil layer (parameter)	H_t	H_b	A_t
V_{max} (nmol gdw^{-1} h^{-1})	0.7	13.0	0.3
a_A° (l gdw^{-1} h^{-1})	0.02	0.19	0.03
K_t (nM or $\mu l\,l^{-1}$)[a]	36 or 25	69 or 48	9 or 6
Th[b] ($\mu l\,l^{-1}$) ± 1 SEM	0.14 ± 0.03	0.04 ± 0.03	0.04 ± 0.02
Oxidation/production	10	221	105

[a] nM in soil water or $\mu l\,l^{-1}$ in headspace.
[b] $P > 0.05$; no significant difference between *Th* values was observed.

well to typical Michaelis–Menten kinetics ($r^2 > 0.98$ in all cases). The V_{max}, K_t and a_A° values all markedly varied with depth, with the highest values being observed in the H_b layer soil (Table 3). Although V_{max} and K_t values were higher in the H_t than A_t soil, the a_A° was greater in the A_t than H_t soil (Table 3). There was no significant difference ($P > 0.05$) in *Th* values between soils (Table 3).

Oxidation potentials exceeded production potentials in all soils (Table 3). Highest CH_4 production potentials were observed in H_t soil and had a mean (±1 SEM) rate of 4.4 ± 0.4 pmol CH_4 $gdw^{-1}\,h^{-1}$, which was significantly greater ($P < 0.05$) than the production potentials for H_b and A_t soils. The highest CH_4 production rate, combined with the lowest potential CH_4 oxidation, resulted in H_t soil having the lowest ratio of oxidation to production (Table 3). H_b soil produced significantly more CH_4 than A_t soil ($P < 0.05$; data not shown), but the greater oxidation potential in the lower organic layer resulted in a much greater ratio of potential oxidation to production (Table 3).

Ambient oxidation rates in control soils and soils exposed to CH_4 concentrations up to 132 $\mu l\,l^{-1}$ did not significantly differ ($P > 0.05$) at the start or end of the kinetic experiments (data not shown). The net CH_4 oxidation potential for autoclaved soil was significantly lower than that for non-autoclaved control soil, 24 h and 14 days after autoclaving ($P < 0.001$). Change in headspace CH_4 concentrations for autoclaved soil bottles were not significantly different ($P > 0.05$) from those in soil free bottles (blanks; data not shown). The complete inhibition of CH_4 oxidation through autoclaving confirmed that oxidation within the soil was biologically mediated, as reported by Crill (1991) and Bender and Conrad (1992, 1995).

3.3. Kinetics of CH_4 oxidation for CH_4-enriched soil

Both V_{max} and K_t values for soil CH_4 uptake were greater after exposure to a 20%, rather than an ambient, CH_4 headspace, but the a_A° remained approximately the same (Table 4). There was no significant difference between the ambient CH_4 oxidation rate of the two treatments ($P > 0.05$), the mean values being equal (Table 4).

Table 4
Kinetic parameters (V_{max}, a_A°, K_t, *Th*) and rates of potential atmospheric CH_4 oxidation (pmol gdw^{-1} h^{-1}) for A_t layer soil incubated with ambient and 20% CH_4 in the headspace for 40 days ($n = 4$)

Soil incubation (parameter)	Ambient CH_4	20% CH_4
V_{max} (nmol gdw^{-1} h^{-1})	0.4	0.7
a_A° (l gdw^{-1} h^{-1})	0.04	0.04
K_t (nM or $\mu l\,l^{-1}$)[a]	13 or 9	25 or 17
Oxidation at ambient ± 1 SEM[b]	60 ± 1	60 ± 1

[a] nM in soil water or $\mu l\,l^{-1}$ in headspace.
[b] $P > 0.05$; no significant treatment effect was detected.

3.4. Waterlogging soil cores

Net CH_4 oxidation rates were significantly greater ($P < 0.01$) in control than waterlogged cores, with mean uptake rates (±1 SEM) of -30 ± 3 and -2 ± 1 μg CH_4 $m^{-2}\,h^{-1}$ across the 30 d period, respectively. Net CH_4 oxidation dropped immediately on waterlogging and no consistent emissions of CH_4 were observed over the 30 d study (data not shown).

4. Discussion

4.1. CH_4 flux of intact soil cores, and undisturbed and sieved soil layers

Kruse et al. (1996) predicted that CH_4 diffusion was an important controller of CH_4 oxidation rate in forest soils similar to Perridge. We hypothesised that if CH_4 supply to oxidisers was important then the sum of the CH_4 uptake rates for soil horizons and soil layers would be greater than the oxidation rates for the intact cores or H horizons from which they were taken. Greater uptake was observed and, also, oxidation potentials were greater in sieved than undisturbed H_b and A_t soils. As well as improved CH_4 supply to methanotrophs as soils were broken down into horizons, then layers and then sieved, O_2 supply would also have improved. Due to the fact that Mancinelli (1995) proposed that O_2 is not likely to be limiting in systems where the CH_4 source is atmospheric, we suggest that the increased oxidation was caused by increased CH_4 supply.

Variability between net CH_4 oxidation rates for separated soil units increased as they were divided from cores, to horizons, to layers (Table 1). Thus, the full spatial heterogeneity in potential CH_4 oxidation rates for specific soil layers was not exhibited in intact core CH_4 uptake rates. We propose that environmental constraints, such as soil water status, regulate net CH_4 uptake so efficiently that markedly different potential uptake activities between cores are masked. Thus, in the field, soil methanotrophic activity must operate far below its optimum due to environmental constraints. Such constraints may, in part, explain

the prolonged recovery of methanotrophic activity after soil disturbances (Ojima et al., 1993).

The high rates of net CH_4 oxidation in the H_b and A_t layers, when compared to the H_t layer, suggest that net CH_4 consumption in the Perridge soil is mainly driven by lower soil layers (i.e. below the H_t layer). This corresponds with the findings of many workers who report distinct subsurface maxima for potential CH_4 oxidation in temperate and boreal forest soils. The greatest activity has been found either in the uppermost mineral layers (Crill, 1991; Koschorreck and Conrad, 1993; Saari et al., 1997), or, as in our study, in the lower organic and the upper mineral layers (Adamsen and King, 1993; Czepiel et al., 1995; Kruse et al., 1996; Amaral and Knowles, 1997; Saari et al., 1997).

Our study showed that a deciduous litter layer did not exhibit net methanotrophic activity. This finding is supported by Saari et al. (1997) who observed no CH_4 uptake by coniferous litters and even net CH_4 production.

The depth of maximum CH_4 oxidation potential differed when potentials were calculated on a mass or area basis (Table 2). The higher by mass potential of the H_b layer suggests that it has a larger, or more active, methanotrophic community per gram of soil than the A_t layer. However, the A_t layer had higher potential oxidising activity by area (or volume). Roslev et al. (1997), using isotopically labelled CH_4 as a biomarker, demonstrated that the depth of maximum CH_4 assimilation within intact soil cores corresponded to the depth of the maximum CH_4 oxidation potential by volume. This suggests that the A_t layer in the Perridge soil would dominate CH_4 oxidation in intact soil profiles and questions the ecological relevance of determining depth profiles of CH_4 oxidation potential using only sieved soil (Saari et al., 1997).

4.2. Kinetics of CH_4 oxidation

All fresh soil layers exhibited Michaelis–Menten saturation curves, typical of high-affinity methanotrophic activity (Bender and Conrad, 1992). Indeed, the values of K_t and *Th* observed by us were within or just below the range reported by Conrad (1996) for soils where the atmosphere was the largest source of CH_4 (K_t: 20–200 nM; *Th*: 30–700 pM $\equiv$ 21–480 nl l^{-1} under the conditions of our study).

The characteristics of the functional methanotrophic community changed with depth. The highest V_{max} and K_t values were measured in the H_b layer, as were the highest methanotrophic potentials on a mass basis. The highest a_A° was also observed in the H_b layer, suggesting that the methanotrophs in this layer were the most efficient scavengers of CH_4 (Button, 1991). Only *Th* values were roughly equivalent between the three layers, indicating that the methanotrophs within each layer were all capable of oxidising CH_4 at extremely low partial pressures.

4.3. Kinetics of CH_4 oxidation for CH_4-enriched soil

The observed kinetic values and the monophasic form of the kinetics means that low-affinity methanotrophic activity was not present in the Perridge soil. Bender and Conrad (1992), after determining similar kinetic profiles for the A_h horizons from a number of sites, proposed that such soils were exclusively exposed to CH_4 mixing ratios below atmospheric values. Bender and Conrad (1992) only observed biphasic kinetics, the second phase approximating to low-affinity kinetics, when soils had been exposed to concentrations of CH_4 significantly higher than atmospheric. In contrast, low-affinity CH_4 oxidising activity was not induced when our soil was challenged with 20% CH_4 in air. Our observation opens to question whether the detection of only high-affinity kinetics within a soil can be used to reliably predict that high concentrations of CH_4 do not develop within that soil and, thus, that CH_4 production is insignificant.

The V_{max} and K_t of the high-affinity activity markedly increased after incubation of soils under a 20% CH_4 headspace, as observed by Bender and Conrad (1992), but the a_A° remained unchanged. This suggests that the methanotrophic community increased in size during exposure to elevated CH_4 but that the methanotroph community did not undergo a functional shift. Despite the increase in V_{max} and K_t observed in our study, the atmospheric CH_4 oxidising potential for the CH_4-enriched soils did not increase. This suggests that CH_4 uptake was still limited by factors other than the 'true potential' of the soil methanotrophic community for CH_4 oxidation. Thus, methods commonly used to assess oxidation potentials of soils do not provide optimal conditions for CH_4 uptake by soil methanotrophs. Physical conditions, such as soil moisture, may still influence CH_4 supply to an extent that efficiently masks differences in potential methanotrophic activity. This should be considered when oxidation potentials are reported.

4.4. Waterlogging soil cores

The immediate drop in net CH_4 oxidation rates of soil cores on waterlogging suggests that the inhibition was largely a physical process. This is consistent with the hypothesis that the regulation of CH_4 uptake by soil water status is mediated by diffusional limitation of CH_4 to the methanotrophs, rather than due to stimulation of methanogenic activity.

4.5. Conclusion

Net CH_4 flux in the Perridge soil was dominated by CH_4 oxidation, methanogenesis played a minor role. The data support the contention that rate of soil CH_4 diffusion to the site of CH_4 oxidation explains the strong regulation of net CH_4 oxidation by soil water potential at this site (Bradford et al., 2001). The full spatial heterogeneity in potential CH_4 oxidation rates for specific soil layers was not exhibited in intact core CH_4 uptake rates. Based on this observation we suggest that environmental constraints regulate net CH_4 uptake so efficiently as to mask markedly different potential

uptake activities in intact soils. The H_b layer contained the most active methanotrophic community and was best able to oxidise CH_4 at low substrate concentrations. However, the A_t layer soil exhibited the greatest oxidation potential for an equivalent area and on the basis of the work by Roslev et al. (1997), the A_t layer dominated *in situ* CH_4 oxidation. Our study demonstrates that by using a suite of approaches, that include potential activity, depth profile and kinetic studies, important insights into the CH_4 dynamics of a soil can be obtained. If these approaches are conducted in isolation care must be taken in the interpretation of the data. The insights generated from using a suite of approaches can be used to more confidently predict how and why soil CH_4 flux may respond to future environmental change, such as altered soil moisture.

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