PERGAMON

Soil Biology & Biochemistry 33 (2001) 1695–1702

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

The effects of acid nitrogen and acid sulphur deposition on CH_4 oxidation in a forest soil: a laboratory study

M.A. Bradford[a,b,*], P. Ineson[a,2], P.A. Wookey[c], H.M. Lappin-Scott[b]

[a]*Institute of Terrestrial Ecology, Merlewood Research Station, Windermere Road, Grange-over-Sands, Cumbria, LA11 6JU, UK*
[b]*University of Exeter, Department of Biological Sciences, Prince of Wales Road, Exeter, Devon, EX4 4PS, UK*
[c]*Uppsala University, Institute of Earth Sciences, Physical Geography, Villavägen 16, S-752 36 Uppsala, Sweden.*

Received 27 June 2000; received in revised form 26 January 2001; accepted 6 April 2001

Abstract

Sieved soil and soil core experiments were performed to determine the potential sensitivity of forest soil CH_4 oxidation to oxidised N, reduced N and oxidised S atmospheric deposition. Ammonium sulphate was used to simulate reduced N deposition, HNO_3 oxidised N deposition and H_2SO_4 oxidised S deposition. The effects of NH_4^+, NO_3^-, SO_4^{2-} and H^+ on soil CH_4 flux were shown to be governed by the associated counter-anion or cation of the investigated ions. Ammonium sulphate, at concentrations greater than those that would be experienced in polluted throughfall, showed a low potential to cause inhibition of CH_4 oxidation. In contrast, HNO_3 strongly inhibited net CH_4 oxidation in sieved soils and also in soil cores. In addition, soil CO_2 production was inhibited and the organic and mineral soil horizons acidified in HNO_3 treated soil cores. This suggested that the HNO_3 effect on CH_4 flux might be indirectly mediated through aluminium toxicity. Sulphuric acid only inhibited CH_4 oxidation when added at pH 1. At concentrations more representative of heavily polluted throughfall, H_2SO_4 had no effect on soil CH_4 flux or CO_2 production from soil cores, even after 210 days of repeated addition. In contrast to HNO_3 additions, acidification of the soil was not marked and was only significant for the mineral soil. The findings suggest that the response of forest soil CH_4 oxidation to atmospheric acid deposition is strongly dependent on the form of acid deposition.

Keywords: Methane oxidation; Methane consumption; Acid deposition; Nitrogen fertilisation; Sulphur deposition; Forest soil

1. Introduction

The increasing atmospheric concentration of CH_4 is, in combination with the increase in other greenhouse gases, resulting in a change of global climate (IPCC, 1995). As forest soils represent a significant CH_4 sink (Dobbie and Smith, 1996), studies suggesting that anthropogenic N air pollution is bringing about a decrease in the forest soil sink for atmospheric CH_4 are cause for concern (Saari et al., 1997; Butterbach-Bahl et al., 1998). In contrast to acid N air pollution, studies by Sitaula et al. (1995); Bradford et al. (2001) suggest that acid S air pollution may actually increase the forest soil sink strength for atmospheric CH_4. It is important to understand better the potential for acid deposition to affect soil CH_4 uptake to enable prediction of how the size of this sink will respond to continuing anthropogenic acid deposition.

Not all studies support the observation that elevated atmospheric N deposition reduces the soil CH_4 sink strength and there are conflicting results for the effects of NH_4^+ and NO_3^- salts on forest soil CH_4 uptake (e.g. Schnell and King, 1994). King and Schnell (1998) report that the different patterns of inhibition can in part be explained by the associated counter-ion of the NH_4^+ or NO_3^- salt. Thus, studies assessing the potential of atmospheric N deposition to affect soil CH_4 oxidation must carefully consider which counter-ion to include in the N salt applied, and this is rarely done.

In contrast to N, very few studies have assessed the impact of elevated S deposition on CH_4 oxidation in forest soils, although S is still one of the main components of anthropogenic origin in rain (Barrett et al., 1995) and S pollution has the potential to cause soil acidification. Elevated acid S deposition has been shown to stimulate net CH_4 oxidation in forest soils (Sitaula et al., 1995; Bradford et al., 2001) and such air pollution can thus be considered to reduce the rate of increase of atmospheric CH_4.

* Corresponding author: NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot, Berks, SL5 7PY, UK.
E-mail address: m.a.bradford@ic.ac.uk (M.A. Bradford).
[2] Present address: The Department of Biology, University of York, PO Box 373, York, YO10 5YW, UK.

0038-0717/01/$ - see front matter
PII: S0038-0717(01)00091-8

Table 1
Summary of experiments showing type of experimental unit, the treatment, the number of times it was applied, how often, over what duration and whether a closed or dynamic chamber technique was used to determine CH_4 flux rate

Exp. no.	Experimental unit	Treatment	No. of additions	Addition interval (days)	Experiment duration (days)	CH_4 flux sampling technique
1	10 g sieved soil, Wheaton	NH_4Cl; $(NH_4)_2SO_4$	1	na[a]	0.04	Closed chamber
2	10 g sieved soil, Wheaton	HNO_3; H_2SO_4	1	na[a]	0.04	Closed chamber
3	100 g sieved soil, Duran	HNO_3; $NaNO_3$; NH_4NO_3	4	7	21	Closed chamber
4	100 g sieved soil, Duran	HNO_3; H_2SO_4	4	7	21	Closed chamber
5	Soil core	HNO_3	6	7	35	Dynamic chamber
6	Soil core	H_2SO_4	30	7	210	Dynamic chamber

[a] na, Not applicable.

However, further investigations are required to determine the potential sensitivity of the soil sink to atmospheric S deposition so that we can better predict how the sink may respond in the long term. One way to do this is to challenge soils with significantly higher S concentrations than would normally be found in atmospheric deposition, although the interpretation of results must be judicious.

The overall objective of our study was to determine the potential sensitivity of forest soil CH_4 consumption to oxidised N, reduced N and oxidised S atmospheric deposition. An earlier field study (Bradford et al., 2001) showed that the CH_4 sink strength of the soil under investigation was increased by chronic H_2SO_4 deposition, while the chronic deposition of HNO_3 and $(NH_4)_2SO_4$ had no significant effect. The overall objective of the current study can be divided into three aims.

The first aim was to determine the maximum potential response of CH_4 oxidation to the two N pollutants used in the field study. This was achieved by exposing the uppermost mineral horizon (the main methanotrophic layer) to very high N concentrations, more typical of fertiliser inputs than polluted throughfall. Second, the effect of the N pollutants on CH_4 uptake was assessed at concentrations more typical of those experienced in very heavily polluted throughfall. When using this second approach, treatment additions were made to larger amounts of sieved soil than when using 'fertiliser type' treatment concentrations (the first approach), to prevent soil from drying out while it was stored and repeated treatment additions were made. Nitrogen treatment additions were also made to parallel soil cores to more closely simulate the effect the treatments would have on CH_4 flux in the natural environment. In sieved soil experiments, additional N salts were included to determine if substitution of the associated anion or cation influenced the effects on CH_4 oxidation. The third aim was to determine the response of CH_4 oxidation to significantly higher H_2SO_4 concentrations than used in the field study, using sieved soil and intact cores as described above for N.

Soil CH_4 consumption is commonly referred to as net soil CH_4 oxidation. Both terms refer to the net uptake of CH_4 by a soil, for example from the atmosphere. The uptake is referred to as *net* because both the processes of CH_4 production and oxidation occur in soils. The magnitude of each process determines the net flux of CH_4 either into or out of a soil; if production dominates the soil will be a net producer, if oxidation dominates the soil will be a net oxidiser/consumer. Well-drained, upland soils tend to be net CH_4 oxidisers or consumers; CH_4 production is generally absent (Conrad, 1996). A decrease in net CH_4 oxidation will decrease the soil sink strength for CH_4.

2. Materials and methods

Our study can be divided into six separate experiments and, for clarity, these are summarised in Table 1.

2.1. Soil

Soils were obtained from Perridge Forest (NGR SX 869908), a temperate mixed deciduous woodland consisting predominantly of mature (c. 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 and natural deposition data for NH_4^+-N, NO_3^--N, SO_4^{2-}-S and H^+ see Bradford et al. (2001).

2.2. Sieved soil experiments (experiments 1–4)

In all 'sieved soil' experiments, soil was used from the top 4 cm of mineral horizon, the depth of maximum CH_4 oxidation potential (M.A. Bradford, unpublished PhD thesis, Exeter University, 1999). This soil was sieved to 2 mm, mixed and soil water content determined gravimetrically by drying at 105°C to constant weight. Fresh sieved soil was placed into 120 cm^3 Wheaton bottles (10 g soil) or 1 l Duran bottles (100 g soil). Bottles were covered with a polythene bag to prevent excessive drying of the soil and incubated at 20°C. Either 1 cm^3 of deionised water (control) or an aqueous solution of $(NH_4)_2SO_4$, NH_4Cl, H_2SO_4 or HNO_3 was added once to the soils in the Wheaton bottles. A second control received no additions. Solution concentrations were 5 and 50 mM for $(NH_4)_2SO_4$ and H_2SO_4, and 10 and 100 mM for NH_4Cl and HNO_3; providing 1 and 10 μmol NH_4^+ gfw (gram fresh weight) $soil^{-1}$, or H^+ gfw $soil^{-1}$, as appropriate. The NH_4^+ and H^+ addition

experiments were performed separately due to CH_4 flux sampling constraints. Due to the fact that $(NH_4)_2SO_4$ showed little potential to cause inhibition of CH_4 oxidation it was not assessed in subsequent experiments. Note that when equimolar concentrations of $(NH_4)_2SO_4$ and NH_4Cl are compared, the anion concentration for $(NH_4)_2SO_4$ is half that of NH_4Cl. The same is true when comparing H_2SO_4 and HNO_3.

For soils in Duran bottles, 20 cm^3 of solution was added every 7 days for 21 days; treatments were deionised water and aqueous solutions of HNO_3, $NaNO_3$, NH_4NO_3 and H_2SO_4. The NO_3^- solution concentrations were 3.2 mM and provided 0.64 μmol NO_3^- gfw $soil^{-1}$; the SO_4^{2-} solution concentration was 1.4 mM and provided 0.28 μmol SO_4^{2-} gfw $soil^{-1}$. All solutions were added as a fine jet and distributed throughout the sample by gentle mixing. The pH values of soils from the Duran bottles were determined at the end of the experiment, following Grimshaw (1989) (soil:water ratio of 1:2 by volume). Due to CH_4 flux sampling constraints the Duran bottle additions were made across two separate experiments. Nitric acid, $NaNO_3$ and NH_4NO_3 were added in the first experiment and HNO_3 and H_2SO_4 in the second experiment.

2.3. Soil core experiments (experiments 5 and 6)

Either 189 cm^3 of deionised water (control), or 189 cm^3 of HNO_3 at one of two concentrations, was added every 7 days for 35 days to three replicate soil cores (25 cm deep, 15 cm diameter) and excess solution that drained from the base of cores was discarded. All solutions were added slowly, care being taken to ensure the entire core surface received even application. The HNO_3 concentrations were 3.2 mM and 1.3 mM, equivalent to 45 and 18 mg N l^{-1}, respectively. To facilitate comparison with sieved soil experiments, this provided 0.21 μmol NO_3^- and H^+ gdw $soil^{-1}$ for the higher HNO_3 treatment and 0.08 μmol NO_3^- and H^+ gdw $soil^{-1}$ for the lower HNO_3 treatment. Note that values are expressed by gram dry weight soil, rather than fresh weight. The mean moisture content in sieved soil experiments was 27%. The pH of separate soil layers was determined at the end of the experiment, using the same method described for soils in Duran bottles. Soil layers are defined as: H_t, top 4 cm of H horizon; H_b, remaining 6 cm of H horizon; A_t, top 4 cm of A horizon (the first mineral horizon in the soil profile). All soil cores were intact with undisturbed organic and mineral horizons, devoid of vegetation and incubated in the dark at 20°C.

A separate experiment, assessing the effect of repeated H_2SO_4 deposition on CH_4 flux from soils, was performed exactly as for the HNO_3 soil core experiment above, except additions were continued for 210 days. Sulphuric acid treatment concentrations were 1.4 mM and 0.56 mM, equivalent to 45 and 18 mg S l^{-1}, respectively. To facilitate comparison with sieved soil experiments, this provided 0.09 μmol SO_4^{2-} and 0.18 μmol H^+ gdw $soil^{-1}$ for the higher H_2SO_4 treatment and 0.036 μmol SO_4^{2-} and 0.072 μmol H^+ gdw $soil^{-1}$ for the lower H_2SO_4 treatment.

2.4. Determining soil CH_4 flux

Methane flux from sieved soil was determined using a closed chamber technique and from soil cores using a dynamic chamber technique. All measurements were made at 20°C. Net methanotrophic rates for soils in Duran bottles and cores were determined prior to treatment allocation, to facilitate blocking in the experimental design. Replicates were blocked on the basis of these rates, with treatments being randomised within blocks.

For the closed chamber technique, CH_4 concentrations were first standardised within bottles by flushing with compressed air (CH_4 concentration in this air ranged between 1.79 and 1.91 μl l^{-1}). A headspace gas sample was taken immediately upon sealing of the bottle using butyl rubber septa and Al-crimps or a Duran bottle lid modified to accommodate a size 17 Suba-Seal (W.H. Freeman Co., Barnsley, UK). A second headspace gas sample was taken after 1 h. The 1 h incubation was the minimum time required to produce repeatable rate measurements. Although the soils follow first-order reaction kinetics (Bradford, loc. cit.), a two-point rate calculation was used because we were concerned only with relative treatment effects in samples with the same initial methane concentration and equal assay duration (see Gulledge et al., 1997). However, these rates were representative of those calculated using rate constants derived from log-transformed time course data (Bradford, loc. cit.). The decrease in headspace CH_4 concentration ranged between −0.05 and 0.45 μl l^{-1} across all replicates.

Methane concentrations were determined on a Shimadzu 14-B GC fitted with a FID operated at 120°C. Methane was separated isothermally on a 2 m Haysep-D packed glass column at 50°C, with N_2 carrier gas flowing at 40 cm^3 min^{-1}. The detector response was calibrated using certified gas standards (British Oxygen Company, Special Gases, UK), nominally containing 10.2 μl l^{-1} CH_4 in air. Rates of CH_4 oxidation are expressed as nmol CH_4 consumed per gram dry weight (gdw) soil per day.

For the dynamic 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 headspace gas concentrations, was drawn through the chamber headspaces at 50 cm^3 min^{-1}. The gas stream was automatically monitored for CH_4, CO_2 and N_2O by gas chromatography. Flux rates were determined by analysing the difference in the gas concentrations in the inlet and outlet gas flows. For a full description of the gas analysis and data storage see Ineson et al. (1998).

2.5. Statistical analysis

All data analyses and statistical comparisons were

performed using SAS (SAS Institute, 1988). ANOVA, with repeated measures and blocked analysis in the ANOVA models where applicable, were used to assess the impacts of treatment addition on trace gas flux and soil pH. The pH data were transposed to $\mu eq\ H^+\ l^{-1}$ prior to being tested with ANOVA but means are presented as pH in the text. Frequency distributions of the trace gas fluxes or model residuals were tested for normality ($\alpha = 0.1$) using the Shapiro–Wilk test. Non-normal data (CO_2 and N_2O flux after repeated HNO_3 addition) were ranked prior to analysis. Ranking was carried out within blocks before repeated measures blocked ANOVA were performed, the basic procedure being equivalent to Friedman's two-way analysis for block designs used to analyse non-parametric data.

3. Results

3.1. Sieved soil experiments

Addition of $(NH_4)_2SO_4$ caused a significant inhibition ($P < 0.05$) of net CH_4 oxidation but only at the 10 μmol NH_4^+ gfw $soil^{-1}$ concentration (Fig. 1). Ammonium chloride, added at 1 μmol NH_4^+ gfw $soil^{-1}$, caused an equivalent inhibition of oxidation and at 10 μmol NH_4^+ gfw $soil^{-1}$, a significantly ($P < 0.05$) greater reduction of CH_4 oxidation than the equivalent concentration of $(NH_4)_2SO_4$ (Fig. 1). There was no significant difference ($P > 0.05$) between the CH_4 uptake rate of soils receiving deionised water or no solution additions (data not shown).

Addition of HNO_3 and H_2SO_4 at a concentration of 10 μmol H^+ gfw $soil^{-1}$ caused a marked inhibition of net CH_4 oxidation (Fig. 2) but this inhibition was significantly greater for the HNO_3 treatment ($P < 0.05$). A HNO_3 addition at 1 μmol H^+ gfw $soil^{-1}$ caused an equivalent inhibition to that observed for the higher H_2SO_4 addition but H_2SO_4 at the same concentration caused no significant inhibition of CH_4 uptake when compared to the control ($P > 0.05$; Fig. 2). There was no significant difference ($P > 0.05$) between the CH_4 uptake rate of soils receiving deionised water or no solution additions (data not shown).

In the first Duran bottle experiment, all three separate NO_3^- salt additions caused a significant reduction in net CH_4 oxidation (Fig. 3) within 14 days of the first addition ($P < 0.01$) and the effect was more pronounced by the next and final sampling ($P < 0.001$). In addition, all three species caused significant ($P < 0.001$) soil acidification (Table 2). In the second Duran bottle experiment, HNO_3 had the same effects as in the first experiment and H_2SO_4 had no significant effects ($P > 0.05$) on CH_4 oxidation or soil acidity (data not shown).

3.2. Soil core experiments

Both concentrations of added HNO_3 caused a significant reduction ($P < 0.05$) in net CH_4 oxidation and net CO_2 production after six additions and a marked but non-signifi-

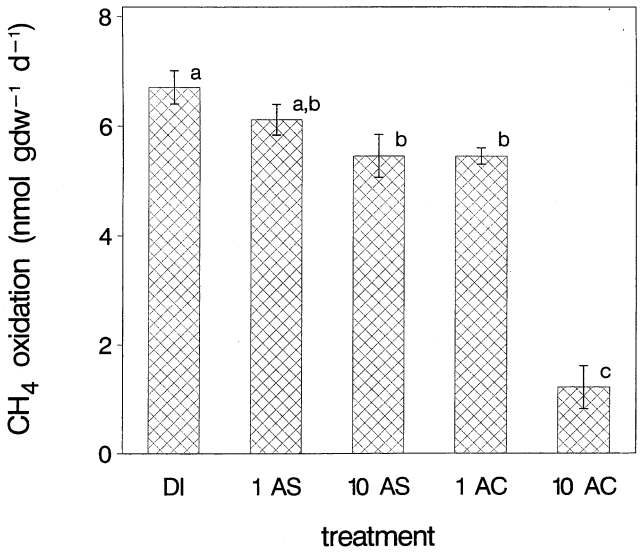

Fig. 1. Soil CH_4 oxidation 3 days after a single addition of deionised water (DI), $(NH_4)_2SO_4$ (AS) or NH_4Cl (AC), at 1 or 10 μmol NH_4^+ gfw $soil^{-1}$. Different letters denote significant differences ($P < 0.05$) between treatments. Flux rates are means ±1 SEM ($n = 4$).

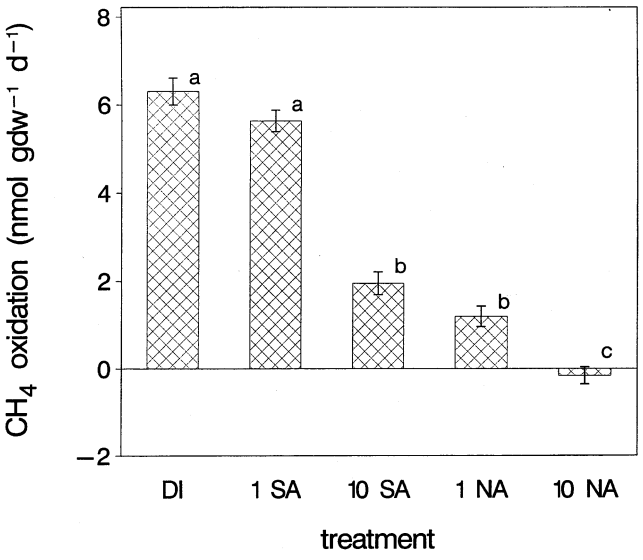

Fig. 2. Soil CH_4 oxidation 3 days after a single addition of deionised water (DI), H_2SO_4 (SA) or HNO_3 (NA), at 1 or 10 μmol H^+ gfw soil [1]. Different letters denote significant differences ($P < 0.05$) between treatments. Flux rates are means ± 1 SEM ($n = 4$).

Table 2
pH of sieved A horizon soil after four repeated treatments with deionised water (DI) and aqueous HNO_3, $NaNO_3$ and NH_4NO_3. One addition was made every 7 days and provided 0.64 μmol NO_3^- gfw $soil^{-1}$. Mean pH values are shown ($n = 4$)

Treatment	DI	HNO_3	$NaNO_3$	NH_4NO_3
pH[a]	3.4a	2.9b	3.2b	3.2b

[a] Values with different letters are significantly different ($P < 0.001$). These a posteriori comparisons were only performed after a significant overall effect was detected using blocked ANOVA ($P < 0.001$).

Table 3
Flux of CH_4, CO_2 and N_2O of soil cores after repeated additions of 1289 μM (low N) and 3226 μM (high N) HNO_3 solution. Controls received deionised water. Treatment additions (six in total) were made every 7 days. Flux rates are means ± 1 SEM ($n = 3$)

Trace gas flux	Treatment[a]		
	Deionised water	Low N	High N
CH_4 oxidation ($\mu g\ m^{-2}\ h^{-1}$)	33.0 ± 2.0a	11.2 ± 1.0b	5.1 ± 0.0b
CO_2 production ($mg\ m^{-2}\ h^{-1}$)	781.9 ± 196.6a	340.0 ± 63.8b	287.7 ± 22.7b
N_2O production ($\mu g\ m^{-2}\ h^{-1}$)	93.1 ± 6.6a	375.9 ± 118.1a	203.0 ± 37.0a

[a] Values with different letters are significantly different ($P < 0.05$). These a posteriori comparisons were only performed after a significant overall effect was detected using repeated measures blocked ANOVA.

Table 4
pH of soil layers after repeated additions of 1289 μM (low N) and 3226 μM (high N) HNO_3 solution. Controls received deionised water. Treatment additions (six in total) were made every 7 days. Mean pH values are shown ($n = 3$)

Soil layer[a]	Treatment[b]		
	Deionised water	Low N	High N
H_t	4.1a	4.0a	3.4b
H_b	4.0a	4.0a	3.4b
A_t	3.9a	3.8a	3.4b

[a] H_t, top 4 cm of H horizon; H_b, remaining 6 cm of H horizon; A_t, top 4 cm of A horizon.
[b] Within the same soil layer, values with different letters are significantly different ($P < 0.001$). These a posteriori comparisons were only performed after a significant overall effect was detected using blocked ANOVA.

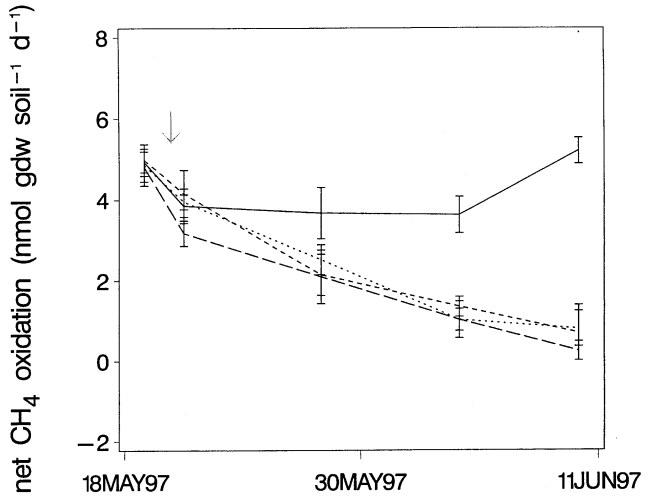

Fig. 3. Effect of repeated deionised water (——), $NaNO_3$ (– – – –), HNO_3 (·····) and NH_4NO_3 (– · – · –) additions on net soil CH_4 oxidation (↓ = 1st treatment addition). One addition was made every 7 days and provided 0.64 μmol NO_3^- gfw $soil^{-1}$. All three NO_3^- salts caused significant inhibition of net CH_4 oxidation relative to the deionised water control ($P < 0.001$). Flux rates are means ± 1 SEM ($n = 4$).

Table 5
Flux of CH_4 and CO_2 of soil cores after repeated additions of 564 μM (low S) and 1408 μM (high S) H_2SO_4 solution. Controls received deionised water. Treatment additions (30 in total) were made every 7 days. Flux rates are means ± 1 SEM ($n = 3$). There was no significant difference between treatments ($P > 0.05$)

Trace gas flux	Treatment		
	Deionised water	Low S	High S
CH_4 oxidation ($\mu g\ m^{-2}\ h^{-1}$)	20.3 ± 4.2	16.0 ± 3.5	17.4 ± 3.5
CO_2 production ($mg\ m^{-2}\ h^{-1}$)	717.7 ± 15.8	613.6 ± 34.1	684.2 ± 12.5

Table 6
pH of soil layers after repeated additions of 564 μM (low S) and 1408 μM (high S) H_2SO_4 solution. Controls received deionised water. Treatment additions (30 in total) were made every 7 days. Mean pH values are shown ($n = 3$)

Soil layer[a]	Treatment[b]		
	DI	Low S	High S
H_t	4.5	4.4	4.4
H_b	4.5	4.3	4.3
A_t	4.4a	4.3a	4.1b

[a] H_t, top 4 cm of H horizon; H_b, remaining 6 cm of H horizon; A_t, top 4 cm of A horizon.
[b] Within the same soil layer, values with different letters are significantly different ($P < 0.001$). These a posteriori comparisons were only performed after a significant overall effect was detected using blocked ANOVA.

cant ($P = 0.09$) increase in N_2O production (Table 3). The inability to detect a significant effect on N_2O production was probably the result of the high spatial variability in N_2O flux (Sitaula and Bakken, 1993) and the low replicate number of cores used. In contrast to the Duran bottle experiment, the inhibition of CH_4 consumption by the lower concentration HNO_3 treatment did not correspond to a significant acidification of the soil ($P > 0.05$). However, the higher HNO_3 concentration caused significant ($P < 0.05$) acidification throughout the soil profile (Table 4).

Thirty-one repeated additions of H_2SO_4 had no significant effect ($P > 0.05$) on net CH_4 oxidation or CO_2 production (Table 5). The most concentrated H_2SO_4 treatment caused significant acidification of the soil ($P < 0.05$) but this was restricted to the A_t layer soil (Table 6).

4. Discussion

The potential for $(NH_4)_2SO_4$ to cause inhibition of CH_4 oxidation in the study soil, at concentrations that might be experienced in polluted throughfall, is low at least in the short term. Similarly, Gulledge et al. (1997) reported no short-term impact of $(NH_4)_2SO_4$ on CH_4 uptake in sieved soils from two upland boreal forests. In the current study,

slight inhibition (rate 85% of control) of net oxidation was observed at the highest concentration of $(NH_4)_2SO_4$. Considering the widely reported inhibitory effect of NH_4^+ on soil CH_4 oxidation (e.g. Steudler et al., 1989), these findings were unexpected.

In contrast to $(NH_4)_2SO_4$, NH_4Cl was a potent inhibitor of CH_4 oxidation and at equimolar NH_4^+ concentrations, NH_4Cl caused significantly greater inhibition of oxidation than $(NH_4)_2SO_4$. This suggests that the sensitivity of oxidation in our study soil to NH_4^+ salts is regulated by the associated anion. King and Schnell (1998) hypothesised that NH_4^+ was responsible for the actual inhibition but that the anion would modify this inhibition depending on its ability to desorb or adsorb NH_4^+ from, or onto, cation exchange sites. They attributed the greater inhibition of CH_4 oxidation by NH_4Cl than $(NH_4)_2SO_4$ to increased NH_4^+ adsorption by SO_4^{2-}, although Cl^- can also promote NH_4^+ desorption (King and Schnell, 1998). The possibility of a Cl^- effect to explain the different inhibition patterns between NH_4Cl and $(NH_4)_2SO_4$ was not dismissed and substituting Na^+ for NH_4^+ in the salts may have helped to explain these differences. However, there is always the complication that NH_4^+ may be released from clays because of Na^+ exchange (see Adamsen and King, 1993).

A single addition of HNO_3 and H_2SO_4 at equivalent H^+ concentrations (assuming full dissociation of the acids) had different impacts on net CH_4 oxidation, with HNO_3 acting as a potent inhibitor of the process at both treatment levels (equivalent to pH 1 and 2). The reduction in CH_4 oxidation at pH 1 is consistent with the observation that additions of aqueous solutions of pH values <2 inhibit soil biological activity (P. Ineson, unpublished PhD thesis, Liverpool University, 1983).

The absence of a response of CH_4 oxidation to H_2SO_4 at pH 2 is surprising because this pH is far below the range of pH optima reported for CH_4 oxidation in acidic environments (4.5–7.0; Dunfield et al., 1993; Bender and Conrad, 1995; Dedysh et al., 1998). However, work by Bender and Conrad (1995) did suggest that some soil methanotrophs are strongly tolerant of acidic pH, with low-affinity activity still found at soil pH values as low as 2.3. It is unlikely that any negative impact on methanotrophy from the H^+ treatment would have been counterbalanced by an inhibition of methanogenesis by acidity (Topp and Hanson, 1991; Dunfield et al., 1993) or SO_4^{2-} (Oremland and Polcin, 1982; Nedwell and Watson, 1995). This is because our study soil had a very low methanogenic potential (Bradford, loc. cit.). It is possible that, following King and Schnell (1998), SO_4^{2-} may have increased NH_4^+ adsorption within the soil and this may have negated any inhibition of CH_4 uptake by elevated acidity. Also, the mineral soil showed a marked buffering capacity to repeated additions of H_2SO_4 both as sieved soil in Duran bottles and within intact soil cores. This buffering capacity may have protected the soil methanotrophs from the acidity of the H_2SO_4 additions.

If the SO_4^{2-} did not counter any inhibition of methanotrophy caused by elevated H^+, then the strong inhibition by HNO_3 at pH 2 suggests that CH_4 oxidation in our study soil is not particularly sensitive to elevated H^+ concentrations and that NO_3^- is a potent inhibitor of the process. Inhibition by NO_3^- (but as a salt with Group 1 elements other than H) has been observed in other in vitro studies with forest soils (Adamsen and King, 1993; Priemé and Christensen, 1997). In support of these studies, we observed significant inhibition of net CH_4 oxidation 14 days after the first NO_3^- salt addition to sieved soil in Duran bottles. The acidification of the soil in our study may also have played a role in the observed inhibition of CH_4 oxidation and, thus, our work suggests that NO_3^- or elevated acidity directly or indirectly inhibits methanotrophy.

The inhibition of CH_4 oxidation by soil cores exposed to HNO_3 may have been caused by a direct or indirect inhibition of methanotrophy. If indirect, a possible mechanism might be that the high H^+ concentrations in the HNO_3 treatment could have released NH_4^+ from cation exchange sites due to H^+ having a much greater affinity for cation exchange sites than NH_4^+ (Aber and Melillo, 1991).

Aluminium toxicity may have been another possible cause of the inhibition of CH_4 oxidation in HNO_3 treated cores. Our study soil, with a pH of 4.0, was situated in the upper region of the Al buffer range (pH 2.8–4.2; Ulrich, 1987) and, thus, when the HNO_3 treatment caused soil acidification, Al^{3+} was presumably released from cation exchange sites in the soil. Carnol et al. (1997) showed that the soils most sensitive to acidification, through factors such as enhanced ion deposition, are those with low base saturation and a pH in the lower Ca or Al buffer ranges. Therefore, the Perridge soil, with a low base status and pH, will be particularly sensitive to enhanced acid deposition and any acidification might reduce the CH_4 sink strength of this soil.

For the lower concentration HNO_3 treatment applied to the soil cores, where significant soil acidification was not observed, the mode of action might have been via Al^{3+} release within methanotrophic microsites, or may suggest a direct NO_3^- impact on CH_4 oxidation within the Perridge soil. The reduction in CO_2 release, in combination with a decrease in CH_4 uptake, has also been observed by Ineson et al. (pers. comm.) for a forest soil subjected to repeated $NaNO_3$ addition. As the methanotrophs are unlikely to be associated with a significant proportion of the soil respiration at Perridge, the HNO_3 impact is probably unspecific and affects a large proportion of the soil microbial biomass. For this reason, we favour the Al^{3+} hypothesis, rather than NO_3^- solely having a direct impact on the methanotrophs. Given the low treatment concentrations used in the current study, the effects are not likely to be associated with the acute effects on general soil microbial activity observed during artificial soil acidification (Lettl, 1985). Whatever the HNO_3 inhibition mechanism, our study suggests that elevated atmospheric deposition of HNO_3 could reduce the soil CH_4 sink strength.

The high concentration H_2SO_4 treatment added to soil

cores represented a S concentration of 45 mg l^{-1} and a pH of 2.6, with the low treatment having a S concentration of 18 mg l^{-1} and a pH of 2.9. Although the H^+ concentration in the high H_2SO_4 treatment approached that for the high HNO_3 treatment, the S treatment only significantly acidified the mineral soil, whereas the higher HNO_3 treatment also acidified the organic horizon. In addition, the acidification was slight in the H_2SO_4 experiment relative to the acidification caused by HNO_3 and additions were made over a time scale about three times longer in the H_2SO_4 core experiment. Therefore, the ability of the Perridge soil to buffer acid deposition appears to be determined by which anion is associated with the proton and this is probably due to the way in which NO_3^- and SO_4^{2-} behave in soil. Sulphate will adsorb onto soil surfaces but NO_3^- generally shows no specific interactions with the soil surface and is instead held in the soil solution (Mott, 1988). Erisman and Draaijers (1995) state that the acidity generating potential of HNO_3 is greater than its acid load, whereas for H_2SO_4, potential and actual acidification are roughly equivalent.

In the absence of soil acidification, it was expected that CH_4 oxidation in soil cores would increase in response to the elevated SO_4^{2-} deposition, as it did in an associated field experiment at the Perridge site (Bradford et al., 2001), but no increase was observed in the current study. See Sitaula et al. (1995) and Bradford et al. (2001) for discussion of possible mechanisms as to why soil CH_4 oxidation increased in response to field additions of chronic H_2SO_4. The absence of a response in the laboratory may have been because the H_2SO_4 additions were for too short a period, or because the current laboratory study may not have adequately mimicked in situ soil conditions necessary for H_2SO_4 additions to stimulate CH_4 uptake. If the latter was the case, this may suggest that the stimulation of field soil CH_4 oxidation rates observed by Sitaula et al. (1995) and Bradford et al. (2001) was the result of indirect in situ effects, rather than direct effects on soil CH_4 oxidisers. Alternatively, the SO_4^{2-} concentrations used in the current study, which were markedly higher than those used in the field experiment, may have been too high to stimulate net CH_4 oxidation. General toxic effects of the elevated acid deposition on methanotrophs were considered unlikely because inhibition of overall soil respiration was not observed in the soil cores. Thus, effects associated with soil acidification would have been unlikely to mask any stimulatory effect of elevated H_2SO_4 deposition on CH_4 oxidation.

5. Conclusion

The effect of wet deposited N and S on net CH_4 oxidation at the Perridge site is governed by the associated anion and cation of the elevated pollutant. Ammonium sulphate, at concentrations greater than those that would be experienced in polluted throughfall, showed little potential to cause inhibition of CH_4 uptake. The presence of SO_4^{2-} as the associated anion might have counteracted any inhibitory impact of NH_4^+ on CH_4 oxidation. Additionally, deposition of H_2SO_4 at concentrations likely to be experienced in heavily acid S polluted throughfall, is unlikely to have a significant direct impact on soil CH_4 oxidation in systems similar to Perridge Forest, at least in the short term. In contrast, CH_4 oxidation in the forest soil was strongly inhibited by elevated HNO_3 deposition.

Acknowledgements

The authors thank the UK Natural Environment Research Council for funding, Sir Henry Studholme for use of Perridge Forest, and two anonymous reviewers for their constructive comments.

References

Aber, J.D., Melillo, J.M., 1991. Terrestrial Ecosystems. Saunders College Publishing, Philadelphia.

Adamsen, A.P.S., King, G.M., 1993. Methane consumption in temperate and subarctic forest soils: rates, vertical zonation, and responses to water and nitrogen. Applied and Environmental Microbiology 59, 485–490.

Barrett, K., Seland, Ø., Foss, A., Mylona, S., Sandnes, H., Styve, H., Tarrasón, L., 1995. European Transboundary Acidifying Air Pollution: EMEP (Co-operative Programme for Monitoring and Evaluation of the Long Range Transmission of Air Pollutants in Europe) Report 1/95. EMEP, MSC-W, Oslo.

Bender, M., Conrad, R., 1995. Effect of CH_4 concentrations and soil conditions on the induction of CH_4 oxidation activity. Soil Biology & Biochemistry 27, 1517–1527.

Bradford, M.A., Wookey, P.A., Ineson, P., Lappin-Scott, H.M., 2001. Controlling factors and effects of chronic nitrogen and sulphur deposition on methane oxidation in a temperate forest soil. Soil Biology & Biochemistry 33, 93–102.

Butterbach-Bahl, K., Gasche, R., Huber, Ch., Kreutzer, K., Papen, H., 1998. Impact of N-input by wet deposition on N-trace gas fluxes and CH_4-oxidation in spruce forest ecosystems of the temperate zone in Europe. Atmospheric Environment 32, 559–564.

Carnol, M., Ineson, P., Dickinson, A.L., 1997. Soil solution nitrogen and cations influenced by $(NH_4)_2SO_4$ deposition in a coniferous forest. Environmental Pollution 97, 1–10.

Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiological Reviews 60, 609–640.

Dedysh, S.N., Panikov, N.S., Tiedje, J.M., 1998. Acidophilic methanotrophic communities from *Sphagnum* peat bogs. Applied and Environmental Microbiology 64, 922–929.

Dobbie, K.E., Smith, K.A., 1996. Comparison of CH_4 oxidation rates in woodland, arable and set aside soils. Soil Biology & Biochemistry 28, 1357–1365.

Dunfield, P., Knowles, R., Dumont, R., Moore, T.R., 1993. Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. Soil Biology & Biochemistry 25, 321–326.

Erisman, J.W., Draaijers, G.P.J., 1995. Atmospheric Deposition in Relation to Acidification and Eutrophication. Studies in Environmental Science 63. Elsevier, Netherlands.

Findlay, D.C., Colborne, G.J.N., Cope, D.W., Harrod, T.R., Hogan, D.V., Staines, S.J., 1984. Soils and their use in south west England. Soil Survey of England and Wales. Bulletin No. 14. Harpenden, Herts, UK.

Grimshaw, H.M., 1989. Soil analysis. In: Allen, S.E. (Ed.). Chemical Analysis of Ecological Materials. 2nd ed. Blackwell Scientific Publications, Oxford, pp. 7–45.

Gulledge, J., Doyle, A.P., Schimel, J.P., 1997. Different NH_4^+-inhibition patterns of soil CH_4 consumption: a result of distinct CH_4-oxidizer populations across sites? Soil Biology & Biochemistry 29, 13–21.

Ineson, P., Coward, P.A., Hartwig, U.A., 1998. Soil gas fluxes of N_2O, CH_4 and CO_2 beneath *Lolium perenne* under elevated CO_2: the Swiss free air carbon dioxide enrichment experiment. Plant and Soil 198, 89–95.

IPCC (1995) Climate Change 1995. The Science of Climate Change. Contribution of Working Group 1 to the Second Assessment Report of the Intergovernmental Panel on Climate Change. Houghton, J.T., Meira Filho, L.G., Callander, B.A., Harris, N., Kattenberg, A., Maskell, K. (Eds.). Cambridge University Press, Cambridge.

King, G.M., Schnell, S., 1998. Effects of ammonium and non-ammonium salt additions on methane oxidation by *Methylosinus trichosporium* OB3b and Maine forest soils. Applied and Environmental Microbiology 64, 253–257.

Lettl, A., 1985. SO_2 pollution. I. Influence of acidification on bacterial communities of forest soils. Ekologia (CSSR) 4, 19–31.

Mott, C.J.B., 1988. Surface chemistry of soil particles. In: Wild, A. (Ed.). Russell's Soil Conditions and Plant Growth. 11th ed. Longman Scientific and Technical, John Wiley & Sons Inc, New York, pp. 240–281.

Nedwell, D.B., Watson, A., 1995. CH_4 production, oxidation and emission in a UK ombrotrophic peat bog: influence of SO_4^{2-} from acid rain. Soil Biology & Biochemistry 27, 893–903.

Oremland, R.S., Polcin, S., 1982. Methanogenesis and sulfate reduction: Competitive and noncompetitive substrates in estuarine sediments. Applied and Environmental Microbiology 44, 1270–1276.

Priemé, A., Christensen, S., 1997. Seasonal and spatial variation of methane oxidation in a Danish spruce forest. Soil Biology & Biochemistry 29, 1165–1172.

Saari, A., Martikainen, P.J., Ferm, A., Ruuskanen, J., de Boer, W., Troelstra, S.R., Laanbroek, H.J., 1997. Methane oxidation in soil profiles of Dutch and Finnish coniferous forests with different soil texture and atmospheric nitrogen deposition. Soil Biology & Biochemistry 29, 1625–1632.

SAS Institute, 1988. SAS/STAT™ User's Guide Release 6.03 Edition. SAS Institute Inc, Cary, NC, USA.

Schnell, S., King, G.M., 1994. Mechanistic analysis of ammonium inhibition of atmospheric methane consumption in forest soils. Applied and Environmental Microbiology 60, 3514–3521.

Sitaula, B.K., Bakken, L.R., 1993. Nitrous oxide release from spruce forest soil: relationships with nitrification, methane uptake, temperature, moisture and fertilization. Soil Biology & Biochemistry 25, 1415–1421.

Sitaula, B.K., Bakken, L.R., Abrahamsen, G., 1995. CH_4 uptake by temperate forest soil: Effect of N input and soil acidification. Soil Biology & Biochemistry 27, 871–880.

Steudler, P.A., Bowden, R.D., Melillo, J.M., Aber, J.D., 1989. Influence of nitrogen fertilisation on methane uptake in temperate forest soils. Nature 341, 314–316.

Topp, E., Hanson, R.S., 1991. Metabolism of radiatively important trace gases by methane-oxidising bacteria. In: Rogers, J.E., Hanson, R.S. (Eds.). Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes. American Society for Microbiology, Washington, DC, pp. 71–90.

Ulrich, B., 1987. Stability, Elasticity and Resilience of Terrestrial Ecosystems with Respect to Organic Matter balance. Ecological Studies 61. Springer-Verlag, Berlin.