



# The effects of acid nitrogen and acid sulphur deposition on CH<sub>4</sub> oxidation in a forest soil: a laboratory study

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## Abstract

Sieved soil and soil core experiments were performed to determine the potential sensitivity of forest soil CH<sub>4</sub> oxidation to oxidised N, reduced N and oxidised S atmospheric deposition. Ammonium sulphate was used to simulate reduced N deposition, HNO<sub>3</sub> oxidised N deposition and H<sub>2</sub>SO<sub>4</sub> oxidised S deposition. The effects of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and H<sup>+</sup> on soil CH<sub>4</sub> 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<sub>4</sub> oxidation. In contrast, HNO<sub>3</sub> strongly inhibited net CH<sub>4</sub> oxidation in sieved soils and also in soil cores. In addition, soil CO<sub>2</sub> production was inhibited and the organic and mineral soil horizons acidified in HNO<sub>3</sub> treated soil cores. This suggested that the HNO<sub>3</sub> effect on CH<sub>4</sub> flux might be indirectly mediated through aluminium toxicity. Sulphuric acid only inhibited CH<sub>4</sub> oxidation when added at pH 1. At concentrations more representative of heavily polluted throughfall, H<sub>2</sub>SO<sub>4</sub> had no effect on soil CH<sub>4</sub> flux or CO<sub>2</sub> production from soil cores, even after 210 days of repeated addition. In contrast to HNO<sub>3</sub> 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<sub>4</sub> oxidation to atmospheric acid deposition is strongly dependent on the form of acid deposition. © 2001 Elsevier Science Ltd. All rights reserved.

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

## 1. Introduction

The increasing atmospheric concentration of CH<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>. It is important to understand better the potential for acid deposition to affect soil CH<sub>4</sub> 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<sub>4</sub> sink strength and there are conflicting results for the effects of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> salts on forest soil CH<sub>4</sub> 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<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> salt. Thus, studies assessing the potential of atmospheric N deposition to affect soil CH<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>.

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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<sub>4</sub> flux rate

Exp. no.	Experimental unit	Treatment	No. of additions	Addition interval (days)	Experiment duration (days)	CH <sub>4</sub> flux sampling technique
1	10 g sieved soil, Wheaton	NH <sub>4</sub> Cl; (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1	na <sup>a</sup>	0.04	Closed chamber
2	10 g sieved soil, Wheaton	HNO <sub>3</sub> ; H <sub>2</sub> SO <sub>4</sub>	1	na <sup>a</sup>	0.04	Closed chamber
3	100 g sieved soil, Duran	HNO <sub>3</sub> ; NaNO <sub>3</sub> ; NH <sub>4</sub> NO <sub>3</sub>	4	7	21	Closed chamber
4	100 g sieved soil, Duran	HNO <sub>3</sub> ; H <sub>2</sub> SO <sub>4</sub>	4	7	21	Closed chamber
5	Soil core	HNO <sub>3</sub>	6	7	35	Dynamic chamber
6	Soil core	H <sub>2</sub> SO <sub>4</sub>	30	7	210	Dynamic chamber

<sup>a</sup> 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<sub>4</sub> consumption to oxidised N, reduced N and oxidised S atmospheric deposition. An earlier field study (Bradford et al., 2001) showed that the CH<sub>4</sub> sink strength of the soil under investigation was increased by chronic H<sub>2</sub>SO<sub>4</sub> deposition, while the chronic deposition of HNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> oxidation. The third aim was to determine the response of CH<sub>4</sub> oxidation to significantly higher H<sub>2</sub>SO<sub>4</sub> concentrations than used in the field study, using sieved soil and intact cores as described above for N.

Soil CH<sub>4</sub> consumption is commonly referred to as net soil CH<sub>4</sub> oxidation. Both terms refer to the net uptake of CH<sub>4</sub> by a soil, for example from the atmosphere. The uptake is referred to as *net* because both the processes of CH<sub>4</sub> production and oxidation occur in soils. The magnitude of each

process determines the net flux of CH<sub>4</sub> 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<sub>4</sub> oxidisers or consumers; CH<sub>4</sub> production is generally absent (Conrad, 1996). A decrease in net CH<sub>4</sub> oxidation will decrease the soil sink strength for CH<sub>4</sub>.

## 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<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, SO<sub>4</sub><sup>2-</sup>-S and H<sup>+</sup> 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<sub>4</sub> 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<sup>3</sup> 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<sup>3</sup> of deionised water (control) or an aqueous solution of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>Cl, H<sub>2</sub>SO<sub>4</sub> or HNO<sub>3</sub> 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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub>, and 10 and 100 mM for NH<sub>4</sub>Cl and HNO<sub>3</sub>; providing 1 and 10 μmol NH<sub>4</sub><sup>+</sup> gfw (gram fresh weight) soil<sup>-1</sup>, or H<sup>+</sup> gfw soil<sup>-1</sup>, as appropriate. The NH<sub>4</sub><sup>+</sup> and H<sup>+</sup> addition

experiments were performed separately due to  $\text{CH}_4$  flux sampling constraints. Due to the fact that  $(\text{NH}_4)_2\text{SO}_4$  showed little potential to cause inhibition of  $\text{CH}_4$  oxidation it was not assessed in subsequent experiments. Note that when equimolar concentrations of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NH}_4\text{Cl}$  are compared, the anion concentration for  $(\text{NH}_4)_2\text{SO}_4$  is half that of  $\text{NH}_4\text{Cl}$ . The same is true when comparing  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ .

For soils in Duran bottles,  $20\text{ cm}^3$  of solution was added every 7 days for 21 days; treatments were deionised water and aqueous solutions of  $\text{HNO}_3$ ,  $\text{NaNO}_3$ ,  $\text{NH}_4\text{NO}_3$  and  $\text{H}_2\text{SO}_4$ . The  $\text{NO}_3^-$  solution concentrations were 3.2 mM and provided  $0.64\text{ }\mu\text{mol NO}_3^- \text{ gfw soil}^{-1}$ ; the  $\text{SO}_4^{2-}$  solution concentration was 1.4 mM and provided  $0.28\text{ }\mu\text{mol SO}_4^{2-} \text{ gfw soil}^{-1}$ . 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  $\text{CH}_4$  flux sampling constraints the Duran bottle additions were made across two separate experiments. Nitric acid,  $\text{NaNO}_3$  and  $\text{NH}_4\text{NO}_3$  were added in the first experiment and  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  in the second experiment.

### 2.3. Soil core experiments (experiments 5 and 6)

Either  $189\text{ cm}^3$  of deionised water (control), or  $189\text{ cm}^3$  of  $\text{HNO}_3$  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  $\text{HNO}_3$  concentrations were 3.2 mM and 1.3 mM, equivalent to 45 and  $18\text{ mg N l}^{-1}$ , respectively. To facilitate comparison with sieved soil experiments, this provided  $0.21\text{ }\mu\text{mol NO}_3^-$  and  $\text{H}^+ \text{ gdw soil}^{-1}$  for the higher  $\text{HNO}_3$  treatment and  $0.08\text{ }\mu\text{mol NO}_3^-$  and  $\text{H}^+ \text{ gdw soil}^{-1}$  for the lower  $\text{HNO}_3$  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:  $\text{H}_t$ , top 4 cm of H horizon;  $\text{H}_b$ , remaining 6 cm of H horizon;  $\text{A}_t$ , 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  $20^\circ\text{C}$ .

A separate experiment, assessing the effect of repeated  $\text{H}_2\text{SO}_4$  deposition on  $\text{CH}_4$  flux from soils, was performed exactly as for the  $\text{HNO}_3$  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  $18\text{ mg S l}^{-1}$ , respectively. To facilitate comparison with sieved soil experiments, this provided  $0.09\text{ }\mu\text{mol SO}_4^{2-}$  and  $0.18\text{ }\mu\text{mol H}^+ \text{ gdw soil}^{-1}$  for the higher  $\text{H}_2\text{SO}_4$  treatment

and  $0.036\text{ }\mu\text{mol SO}_4^{2-}$  and  $0.072\text{ }\mu\text{mol H}^+ \text{ gdw soil}^{-1}$  for the lower  $\text{H}_2\text{SO}_4$  treatment.

### 2.4. Determining soil $\text{CH}_4$ 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  $20^\circ\text{C}$ . 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,  $\text{CH}_4$  concentrations were first standardised within bottles by flushing with compressed air ( $\text{CH}_4$  concentration in this air ranged between  $1.79$  and  $1.91\text{ }\mu\text{l l}^{-1}$ ). 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 Gullledge 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  $\text{CH}_4$  concentration ranged between  $-0.05$  and  $0.45\text{ }\mu\text{l l}^{-1}$  across all replicates.

Methane concentrations were determined on a Shimadzu 14-B GC fitted with a FID operated at  $120^\circ\text{C}$ . Methane was separated isothermally on a 2 m Haysep-D packed glass column at  $50^\circ\text{C}$ , with  $\text{N}_2$  carrier gas flowing at  $40\text{ cm}^3 \text{ min}^{-1}$ . The detector response was calibrated using certified gas standards (British Oxygen Company, Special Gases, UK), nominally containing  $10.2\text{ }\mu\text{l l}^{-1}$   $\text{CH}_4$  in air. Rates of  $\text{CH}_4$  oxidation are expressed as  $\text{nmol CH}_4$  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  $50\text{ cm}^3 \text{ min}^{-1}$ . The gas stream was automatically monitored for  $\text{CH}_4$ ,  $\text{CO}_2$  and  $\text{N}_2\text{O}$  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  $\mu\text{eq H}^+ \text{ l}^{-1}$  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 ( $\text{CO}_2$  and  $\text{N}_2\text{O}$  flux after repeated  $\text{HNO}_3$  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  $(\text{NH}_4)_2\text{SO}_4$  caused a significant inhibition ( $P < 0.05$ ) of net  $\text{CH}_4$  oxidation but only at the  $10 \mu\text{mol NH}_4^+ \text{ gfw soil}^{-1}$  concentration (Fig. 1). Ammonium chloride, added at  $1 \mu\text{mol NH}_4^+ \text{ gfw soil}^{-1}$ , caused an equivalent inhibition of oxidation and at  $10 \mu\text{mol NH}_4^+ \text{ gfw soil}^{-1}$ , a significantly ( $P < 0.05$ ) greater reduction of  $\text{CH}_4$  oxidation than the equivalent concentration of  $(\text{NH}_4)_2\text{SO}_4$  (Fig. 1). There was no significant difference ( $P > 0.05$ ) between the  $\text{CH}_4$  uptake rate of soils receiving deionised water or no solution additions (data not shown).

Addition of  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  at a concentration of  $10 \mu\text{mol H}^+ \text{ gfw soil}^{-1}$  caused a marked inhibition of net  $\text{CH}_4$  oxidation (Fig. 2) but this inhibition was significantly greater for the  $\text{HNO}_3$  treatment ( $P < 0.05$ ). A  $\text{HNO}_3$  addition at  $1 \mu\text{mol H}^+ \text{ gfw soil}^{-1}$  caused an equivalent inhibition to that observed for the higher  $\text{H}_2\text{SO}_4$  addition but  $\text{H}_2\text{SO}_4$  at the same concentration caused no significant inhibition of  $\text{CH}_4$  uptake when compared to the control ( $P > 0.05$ ; Fig. 2). There was no significant difference ( $P > 0.05$ ) between the  $\text{CH}_4$  uptake rate of soils receiving deionised water or no solution additions (data not shown).

In the first Duran bottle experiment, all three separate  $\text{NO}_3^-$  salt additions caused a significant reduction in net  $\text{CH}_4$  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,  $\text{HNO}_3$  had the same effects as in the first experiment and  $\text{H}_2\text{SO}_4$  had no significant effects ( $P > 0.05$ ) on  $\text{CH}_4$  oxidation or soil acidity (data not shown).

#### 3.2. Soil core experiments

Both concentrations of added  $\text{HNO}_3$  caused a significant reduction ( $P < 0.05$ ) in net  $\text{CH}_4$  oxidation and net  $\text{CO}_2$  production after six additions and a marked but non-signifi-

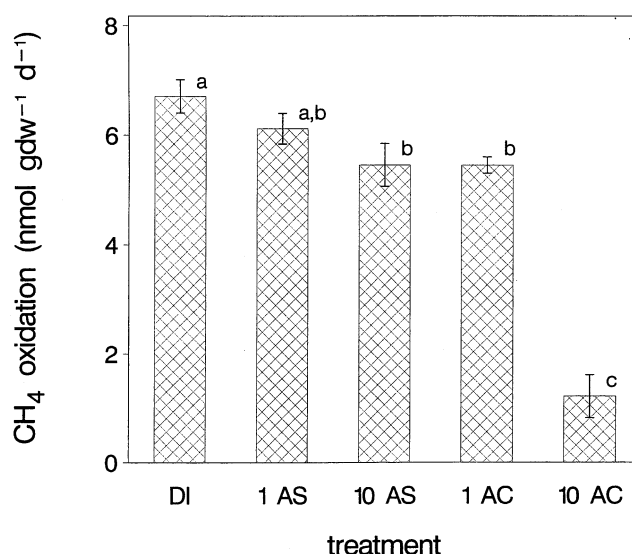


Fig. 1. Soil  $\text{CH}_4$  oxidation 3 days after a single addition of deionised water (DI),  $(\text{NH}_4)_2\text{SO}_4$  (AS) or  $\text{NH}_4\text{Cl}$  (AC), at  $1$  or  $10 \mu\text{mol NH}_4^+ \text{ gfw soil}^{-1}$ . Different letters denote significant differences ( $P < 0.05$ ) between treatments. Flux rates are means  $\pm 1$  SEM ( $n = 4$ ).

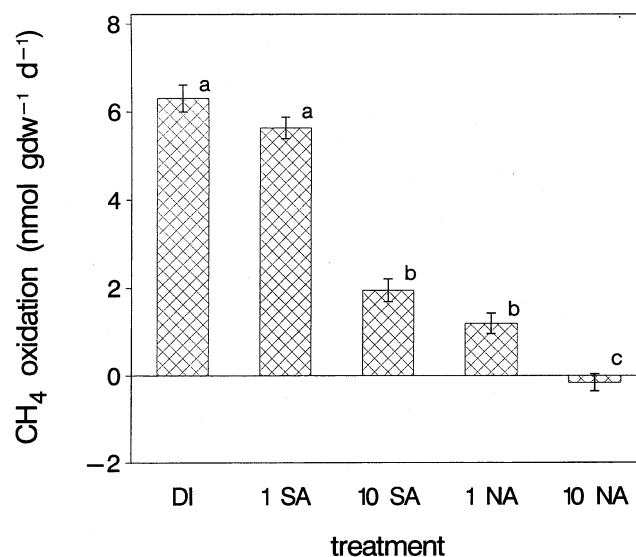


Fig. 2. Soil  $\text{CH}_4$  oxidation 3 days after a single addition of deionised water (DI),  $\text{H}_2\text{SO}_4$  (SA) or  $\text{HNO}_3$  (NA), at  $1$  or  $10 \mu\text{mol H}^+ \text{ gfw soil}^{-1}$ . Different letters denote significant differences ( $P < 0.05$ ) between treatments. Flux rates are means  $\pm 1$  SEM ( $n = 4$ ).

Table 2

pH of sieved A horizon soil after four repeated treatments with deionised water (DI) and aqueous  $\text{HNO}_3$ ,  $\text{NaNO}_3$  and  $\text{NH}_4\text{NO}_3$ . One addition was made every 7 days and provided  $0.64 \mu\text{mol NO}_3^- \text{ gfw soil}^{-1}$ . Mean pH values are shown ( $n = 4$ )

Treatment	DI	$\text{HNO}_3$	$\text{NaNO}_3$	$\text{NH}_4\text{NO}_3$
pH <sup>a</sup>	3.4a	2.9b	3.2b	3.2b

<sup>a</sup> 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<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O of soil cores after repeated additions of 1289  $\mu$ M (low N) and 3226  $\mu$ M (high N) HNO<sub>3</sub> solution. Controls received deionised water. Treatment additions (six in total) were made every 7 days. Flux rates are means  $\pm$  1 SEM ( $n$  = 3)

Trace gas flux	Treatment <sup>a</sup>		
	Deionised water	Low N	High N
CH <sub>4</sub> oxidation ( $\mu$ g m <sup>-2</sup> h <sup>-1</sup> )	33.0 $\pm$ 2.0a	11.2 $\pm$ 1.0b	5.1 $\pm$ 0.0b
CO <sub>2</sub> production (mg m <sup>-2</sup> h <sup>-1</sup> )	781.9 $\pm$ 196.6a	340.0 $\pm$ 63.8b	287.7 $\pm$ 22.7b
N <sub>2</sub> O production ( $\mu$ g m <sup>-2</sup> h <sup>-1</sup> )	93.1 $\pm$ 6.6a	375.9 $\pm$ 118.1a	203.0 $\pm$ 37.0a

<sup>a</sup> 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  $\mu$ M (low N) and 3226  $\mu$ M (high N) HNO<sub>3</sub> solution. Controls received deionised water. Treatment additions (six in total) were made every 7 days. Mean pH values are shown ( $n$  = 3)

Soil layer <sup>a</sup>	Treatment <sup>b</sup>		
	Deionised water	Low N	High N
H <sub>t</sub>	4.1a	4.0a	3.4b
H <sub>b</sub>	4.0a	4.0a	3.4b
A <sub>t</sub>	3.9a	3.8a	3.4b

<sup>a</sup> H<sub>t</sub>, top 4 cm of H horizon; H<sub>b</sub>, remaining 6 cm of H horizon; A<sub>t</sub>, top 4 cm of A horizon.

<sup>b</sup> 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.

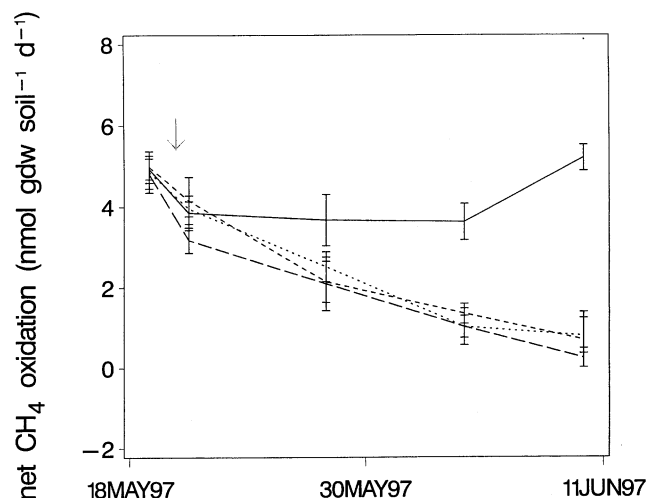


Fig. 3. Effect of repeated deionised water (—), NaNO<sub>3</sub> (---), HNO<sub>3</sub> (·····) and NH<sub>4</sub>NO<sub>3</sub> (-.-.-) additions on net soil CH<sub>4</sub> oxidation (↓ = 1st treatment addition). One addition was made every 7 days and provided 0.64  $\mu$ mol NO<sub>3</sub><sup>-</sup> gfw soil<sup>-1</sup>. All three NO<sub>3</sub><sup>-</sup> salts caused significant inhibition of net CH<sub>4</sub> oxidation relative to the deionised water control ( $P$  < 0.001). Flux rates are means  $\pm$  1 SEM ( $n$  = 4).

Table 5

Flux of CH<sub>4</sub> and CO<sub>2</sub> of soil cores after repeated additions of 564  $\mu$ M (low S) and 1408  $\mu$ M (high S) H<sub>2</sub>SO<sub>4</sub> solution. Controls received deionised water. Treatment additions (30 in total) were made every 7 days. Flux rates are means  $\pm$  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 <sub>4</sub> oxidation ( $\mu$ g m <sup>-2</sup> h <sup>-1</sup> )	20.3 $\pm$ 4.2	16.0 $\pm$ 3.5	17.4 $\pm$ 3.5
CO <sub>2</sub> production (mg m <sup>-2</sup> h <sup>-1</sup> )	717.7 $\pm$ 15.8	613.6 $\pm$ 34.1	684.2 $\pm$ 12.5

Table 6

pH of soil layers after repeated additions of 564  $\mu$ M (low S) and 1408  $\mu$ M (high S) H<sub>2</sub>SO<sub>4</sub> solution. Controls received deionised water. Treatment additions (30 in total) were made every 7 days. Mean pH values are shown ( $n$  = 3)

Soil layer <sup>a</sup>	Treatment <sup>b</sup>		
	DI	Low S	High S
H <sub>t</sub>	4.5	4.4	4.4
H <sub>b</sub>	4.5	4.3	4.3
A <sub>t</sub>	4.4a	4.3a	4.1b

<sup>a</sup> H<sub>t</sub>, top 4 cm of H horizon; H<sub>b</sub>, remaining 6 cm of H horizon; A<sub>t</sub>, top 4 cm of A horizon.

<sup>b</sup> 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<sub>2</sub>O production (Table 3). The inability to detect a significant effect on N<sub>2</sub>O production was probably the result of the high spatial variability in N<sub>2</sub>O flux (Sitaula and Bakken, 1993) and the low replicate number of cores used. In contrast to the Duran bottle experiment, the inhibition of CH<sub>4</sub> consumption by the lower concentration HNO<sub>3</sub> treatment did not correspond to a significant acidification of the soil ( $P$  > 0.05). However, the higher HNO<sub>3</sub> concentration caused significant ( $P$  < 0.05) acidification throughout the soil profile (Table 4).

Thirty-one repeated additions of H<sub>2</sub>SO<sub>4</sub> had no significant effect ( $P$  > 0.05) on net CH<sub>4</sub> oxidation or CO<sub>2</sub> production (Table 5). The most concentrated H<sub>2</sub>SO<sub>4</sub> treatment caused significant acidification of the soil ( $P$  < 0.05) but this was restricted to the A<sub>t</sub> layer soil (Table 6).

#### 4. Discussion

The potential for (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to cause inhibition of CH<sub>4</sub> oxidation in the study soil, at concentrations that might be experienced in polluted throughfall, is low at least in the short term. Similarly, Gullledge et al. (1997) reported no short-term impact of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> on CH<sub>4</sub> 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  $(\text{NH}_4)_2\text{SO}_4$ . Considering the widely reported inhibitory effect of  $\text{NH}_4^+$  on soil  $\text{CH}_4$  oxidation (e.g. Steudler et al., 1989), these findings were unexpected.

In contrast to  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NH}_4\text{Cl}$  was a potent inhibitor of  $\text{CH}_4$  oxidation and at equimolar  $\text{NH}_4^+$  concentrations,  $\text{NH}_4\text{Cl}$  caused significantly greater inhibition of oxidation than  $(\text{NH}_4)_2\text{SO}_4$ . This suggests that the sensitivity of oxidation in our study soil to  $\text{NH}_4^+$  salts is regulated by the associated anion. King and Schnell (1998) hypothesised that  $\text{NH}_4^+$  was responsible for the actual inhibition but that the anion would modify this inhibition depending on its ability to desorb or adsorb  $\text{NH}_4^+$  from, or onto, cation exchange sites. They attributed the greater inhibition of  $\text{CH}_4$  oxidation by  $\text{NH}_4\text{Cl}$  than  $(\text{NH}_4)_2\text{SO}_4$  to increased  $\text{NH}_4^+$  adsorption by  $\text{SO}_4^{2-}$ , although  $\text{Cl}^-$  can also promote  $\text{NH}_4^+$  desorption (King and Schnell, 1998). The possibility of a  $\text{Cl}^-$  effect to explain the different inhibition patterns between  $\text{NH}_4\text{Cl}$  and  $(\text{NH}_4)_2\text{SO}_4$  was not dismissed and substituting  $\text{Na}^+$  for  $\text{NH}_4^+$  in the salts may have helped to explain these differences. However, there is always the complication that  $\text{NH}_4^+$  may be released from clays because of  $\text{Na}^+$  exchange (see Adamsen and King, 1993).

A single addition of  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  at equivalent  $\text{H}^+$  concentrations (assuming full dissociation of the acids) had different impacts on net  $\text{CH}_4$  oxidation, with  $\text{HNO}_3$  acting as a potent inhibitor of the process at both treatment levels (equivalent to pH 1 and 2). The reduction in  $\text{CH}_4$  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  $\text{CH}_4$  oxidation to  $\text{H}_2\text{SO}_4$  at pH 2 is surprising because this pH is far below the range of pH optima reported for  $\text{CH}_4$  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  $\text{H}^+$  treatment would have been counterbalanced by an inhibition of methanogenesis by acidity (Topp and Hanson, 1991; Dunfield et al., 1993) or  $\text{SO}_4^{2-}$  (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),  $\text{SO}_4^{2-}$  may have increased  $\text{NH}_4^+$  adsorption within the soil and this may have negated any inhibition of  $\text{CH}_4$  uptake by elevated acidity. Also, the mineral soil showed a marked buffering capacity to repeated additions of  $\text{H}_2\text{SO}_4$  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  $\text{H}_2\text{SO}_4$  additions.

If the  $\text{SO}_4^{2-}$  did not counter any inhibition of methanotro-

phy caused by elevated  $\text{H}^+$ , then the strong inhibition by  $\text{HNO}_3$  at pH 2 suggests that  $\text{CH}_4$  oxidation in our study soil is not particularly sensitive to elevated  $\text{H}^+$  concentrations and that  $\text{NO}_3^-$  is a potent inhibitor of the process. Inhibition by  $\text{NO}_3^-$  (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  $\text{CH}_4$  oxidation 14 days after the first  $\text{NO}_3^-$  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  $\text{CH}_4$  oxidation and, thus, our work suggests that  $\text{NO}_3^-$  or elevated acidity directly or indirectly inhibits methanotrophy.

The inhibition of  $\text{CH}_4$  oxidation by soil cores exposed to  $\text{HNO}_3$  may have been caused by a direct or indirect inhibition of methanotrophy. If indirect, a possible mechanism might be that the high  $\text{H}^+$  concentrations in the  $\text{HNO}_3$  treatment could have released  $\text{NH}_4^+$  from cation exchange sites due to  $\text{H}^+$  having a much greater affinity for cation exchange sites than  $\text{NH}_4^+$  (Aber and Melillo, 1991).

Aluminium toxicity may have been another possible cause of the inhibition of  $\text{CH}_4$  oxidation in  $\text{HNO}_3$  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  $\text{HNO}_3$  treatment caused soil acidification,  $\text{Al}^{3+}$  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  $\text{CH}_4$  sink strength of this soil.

For the lower concentration  $\text{HNO}_3$  treatment applied to the soil cores, where significant soil acidification was not observed, the mode of action might have been via  $\text{Al}^{3+}$  release within methanotrophic microsites, or may suggest a direct  $\text{NO}_3^-$  impact on  $\text{CH}_4$  oxidation within the Perridge soil. The reduction in  $\text{CO}_2$  release, in combination with a decrease in  $\text{CH}_4$  uptake, has also been observed by Ineson et al. (pers. comm.) for a forest soil subjected to repeated  $\text{NaNO}_3$  addition. As the methanotrophs are unlikely to be associated with a significant proportion of the soil respiration at Perridge, the  $\text{HNO}_3$  impact is probably unspecific and affects a large proportion of the soil microbial biomass. For this reason, we favour the  $\text{Al}^{3+}$  hypothesis, rather than  $\text{NO}_3^-$  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  $\text{HNO}_3$  inhibition mechanism, our study suggests that elevated atmospheric deposition of  $\text{HNO}_3$  could reduce the soil  $\text{CH}_4$  sink strength.

The high concentration  $\text{H}_2\text{SO}_4$  treatment added to soil

cores represented a S concentration of  $45 \text{ mg l}^{-1}$  and a pH of 2.6, with the low treatment having a S concentration of  $18 \text{ mg l}^{-1}$  and a pH of 2.9. Although the  $\text{H}^+$  concentration in the high  $\text{H}_2\text{SO}_4$  treatment approached that for the high  $\text{HNO}_3$  treatment, the S treatment only significantly acidified the mineral soil, whereas the higher  $\text{HNO}_3$  treatment also acidified the organic horizon. In addition, the acidification was slight in the  $\text{H}_2\text{SO}_4$  experiment relative to the acidification caused by  $\text{HNO}_3$  and additions were made over a time scale about three times longer in the  $\text{H}_2\text{SO}_4$  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  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  behave in soil. Sulphate will adsorb onto soil surfaces but  $\text{NO}_3^-$  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  $\text{HNO}_3$  is greater than its acid load, whereas for  $\text{H}_2\text{SO}_4$ , potential and actual acidification are roughly equivalent.

In the absence of soil acidification, it was expected that  $\text{CH}_4$  oxidation in soil cores would increase in response to the elevated  $\text{SO}_4^{2-}$  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  $\text{CH}_4$  oxidation increased in response to field additions of chronic  $\text{H}_2\text{SO}_4$ . The absence of a response in the laboratory may have been because the  $\text{H}_2\text{SO}_4$  additions were for too short a period, or because the current laboratory study may not have adequately mimicked in situ soil conditions necessary for  $\text{H}_2\text{SO}_4$  additions to stimulate  $\text{CH}_4$  uptake. If the latter was the case, this may suggest that the stimulation of field soil  $\text{CH}_4$  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  $\text{CH}_4$  oxidisers. Alternatively, the  $\text{SO}_4^{2-}$  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  $\text{CH}_4$  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  $\text{H}_2\text{SO}_4$  deposition on  $\text{CH}_4$  oxidation.

## 5. Conclusion

The effect of wet deposited N and S on net  $\text{CH}_4$  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  $\text{CH}_4$  uptake. The presence of  $\text{SO}_4^{2-}$  as the

associated anion might have counteracted any inhibitory impact of  $\text{NH}_4^+$  on  $\text{CH}_4$  oxidation. Additionally, deposition of  $\text{H}_2\text{SO}_4$  at concentrations likely to be experienced in heavily acid S polluted throughfall, is unlikely to have a significant direct impact on soil  $\text{CH}_4$  oxidation in systems similar to Perridge Forest, at least in the short term. In contrast,  $\text{CH}_4$  oxidation in the forest soil was strongly inhibited by elevated  $\text{HNO}_3$  deposition.

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