



SHORT COMMUNICATION

CH₄ OXIDATION IN SOILS FERTILIZED WITH ORGANIC
AND INORGANIC-N; DIFFERENTIAL EFFECTSTOBY W. WILLISON,¹* RACHEL COOK,¹ ANNETTE MÜLLER² and
DAVID S. POWLSON¹¹IACR-Rothamsted, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, England and²Centre for Environmental Research, Leipzig-Halle Ltd, Bad Lauchstädt, Germany

(Accepted 14 July 1995)

The significance of soils as a sink for CH₄ has only recently been recognized, and the environmental variables that regulate the strength of the sink are poorly documented. The use of N fertilizer has been shown to reduce rates of CH₄ uptake in forest, pasture and arable soils (Stuedler *et al.*, 1989; Keller *et al.*, 1990; Mosier *et al.*, 1991; Adamsen and King, 1993; Hütsch *et al.*, 1993). Work at Rothamsted has shown that the form of N fertilizer is also important: in a pasture soil, NO₃-N fertilizer had no effect on CH₄ uptake rates, whereas NH₄-N fertilizer completely inhibited net CH₄ oxidation (Hütsch *et al.*, 1994; Willison *et al.*, 1994). It has been reported from studies in pure culture that NH₄⁺ ions will competitively inhibit the oxidation of CH₄ by methanotrophs (Whittenbury *et al.*, 1970), and this could be an explanation for the reduction of CH₄ oxidation in soils following the long-term application of NH₄-containing fertilizer (Hütsch *et al.*, 1993). However, our work at the Broadbalk long-term experiment on plots receiving large amounts of farmyard manure (FYM) (Hütsch *et al.*, 1994; Willison *et al.*, 1994), which supplies 240 kg N ha⁻¹, much of which is released as NH₄-N, does not show an inhibitory effect on CH₄ oxidation. Measurements of microbial biomass on the plots receiving FYM show that it has risen to over twice that on the plots receiving solely inorganic-N fertilizer (Jenkinson and Powlson, 1976). This elevated total biomass may buffer the FYM plots against any inhibitory effect on CH₄ oxidation of NH₄ derived from the mineralization of FYM. We describe an experiment to further investigate these interactions, using soil from the long-term experiments at Bad Lauchstädt, Germany.

The Static Fertilization Experiment, began in 1902 at Bad Lauchstädt, Germany (51°24'N, 11°53'E.), is on a haplic chernozem overlying loess over boulder clay. There is a four-course rotation (sugar beet, spring barley, potato, winter wheat) and the experiment has been subdivided into plots receiving different rates and combinations of fertilizers. Soil samples were taken from the following plots: plot 18, which receives no N fertilizer (Control) in any form; plot 13, which receives inorganic-N but no organic manure (N); plot 12, which receives 20 t ha⁻¹ FYM but no inorganic fertilizer (FYM); and plot 7, which receives both 20 t ha⁻¹ FYM and inorganic fertilizer (FYM + N). Inorganic fertilizer is added annually in the spring as a top dressing according to calculated crop requirements usually between 40–130 kg N ha⁻¹ y⁻¹ as NH₄NO₃ (M. Körschens, pers. commun.).

CH₄ oxidation rates were measured on undisturbed soil cores (Hütsch *et al.*, 1993), cores were collected on 21 April 1994 (8 per plot), before the current year's inorganic-N fertilizer was applied. Additional soil was collected for measurement of soil water content, pH and mineral-N content. Incubation experiments were carried out using a modification of the procedure described by Hütsch *et al.* (1993). Cores were conditioned at 25°C for 24 h. The samples were then incubated at 13°C in jars fitted with an injection septum. At the start of the incubation the jars were flushed with ambient air and sealed. Headspace concentrations of CH₄ were measured on a GC fitted with a f.i.d. after 0, 6, 24, 48 and 72 h. The concentration of CH₄ at 0 h was slightly higher than that normally quoted for ambient (1.72 µl l⁻¹), we believe the reason for this is the proximity of buildings. The decrease in the CH₄ concentration in the headspace followed first-order kinetics and could be described by an exponential function ($y = ae^{bt}$) (Hütsch *et al.*, 1993). A log-transformation, $\ln y = a + bt$ resulted in straight lines where the slope of the lines can be interpreted as CH₄ oxidation rates (b -values).

Mineral-N measurements were made on individual samples at the end of the incubation (Table 1). All samples were sieved (≤ 4 mm) and 50 g (fresh wt) extracted by shaking with 200 ml 2 M KCl for 1 h and filtered through Whatman No. 1 filter paper. The extracts were stored frozen until measurement of NO₃⁻ and NH₄⁺ with an ALPKEM rapid flow analyser. The concentrations are expressed as kg N ha⁻¹.

Figure 1 shows the rate of disappearance of CH₄ in the four treatments. When plot 18 (Control) and plot 13 (N) are compared the rate of oxidation was reduced from 4.60 to 1.34 nl CH₄ l⁻¹ h⁻¹ (Table 1). Figure 1 also shows the same application rate of inorganic-N, but to plots that have received 20 t y⁻¹ FYM. The rate of CH₄ oxidation in plot 07 (FYM + N) was 33% of that in plot 12 (FYM). When the comparison is made between plots that received different rates of FYM, the FYM plots oxidized CH₄ at approximately 2.5 times the rate of the control plots receiving no FYM (Table 1).

There is good evidence that the addition of inorganic-N fertilizer reduces the rate at which CH₄ is oxidized in forest, pasture and arable soils. However, there is still uncertainty regarding the relative importance of methanotrophs and NH₄ oxidizers in regulating this flux (Hütsch *et al.*, 1993). The reason for this uncertainty is largely due to the ability of the enzymes CH₄ monoxygenase and NH₄ monoxygenase to co-metabolize compounds which cannot be used as an energy source for growth (Bedard and Knowles, 1989). It

*Author for correspondence.

Table 1. CH₄ oxidation rates (*b*-values) and mineral-N content at the end of the incubation, pH and moisture content at the start of the incubation (*n* = 8)

Plot	Start of incubation		End of incubation		
	pH (in H ₂ O)	Moisture (%)	NO ₃ -N (kg ha ⁻¹)*	NH ₄ -N (kg ha ⁻¹)*	<i>b</i> -value (nl CH ₄ l ⁻¹ h ⁻¹)
Plot 18: Control	6.8	13.1	0.83	0.20	-4.60†
Plot 13: N	6.9	14.0	15.36	3.1	-1.34
Plot 12: FYM	7.0	14.6	1.98	0.22	-11.2†
Plot 7: FYM + N	7.2	15.3	5.01	0.71	-3.76

*Values are means, *n* = 5.

†Indicates where *b*-values are significantly different (*P* ≤ 0.05) between treatments receiving the same amount of organic-N with or without inorganic-N.

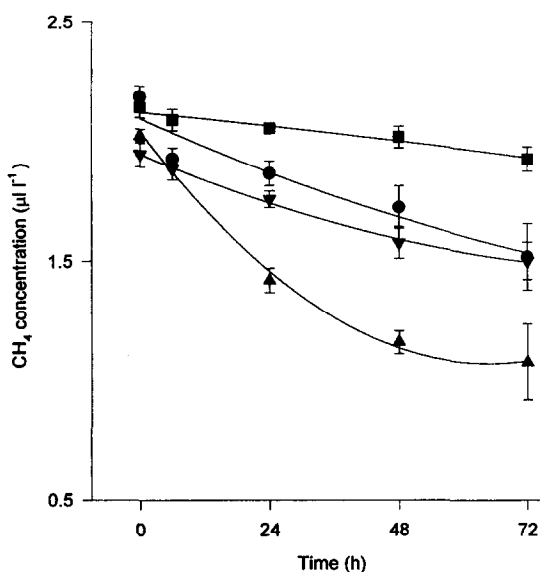


Fig. 1. Plots of CH₄ oxidation in soils from Bad Lauchstädt. Lines are means ± 1 SD. Control (●) received no N fertilizer in any form, N (■) received only inorganic-N fertilizer, FYM (▲) received only organic-N fertilizer and FYM + N (▼) received organic-N and inorganic-N fertilizer.

has been suggested that the long-term addition of inorganic fertilizer increases the populations of nitrifiers at the expense of methanotrophs (Hütsch *et al.*, 1993). The validity of this explanation relies on the two populations competing for, and being limited by, ecological niches (Bedard and Knowles, 1989). Though both groups of microorganisms may favour the same aerobic-anaerobic interface this hypothesis requires further testing. It has been a weakness of this hypothesis that plots receiving FYM have oxidised CH₄ as strongly as plots receiving zero N, although having as great an NH₄ input as plots receiving large inorganic-N inputs (see Hütsch *et al.*, 1994; Willison *et al.*, 1994). The data in this experiment show the same reduction of CH₄ oxidation following the long-term application of inorganic-N that has been reported at Rothamsted (Hütsch *et al.*, 1993). A more rapid oxidation of CH₄ in the FYM plot receiving no inorganic-N compared to a plot receiving no N, shows the overall effect of FYM addition in increasing microbial biomass. However, and most significantly, for the first time it has been possible to show a reduction of CH₄ oxidation rate in a site receiving FYM by the addition of inorganic-N. This supports the contention of Hütsch *et al.*

(1993) that the NH₄ oxidizer population increases at the expense of methanotrophs.

Acknowledgements—This work was carried out with the aid of a travel grant from the International Science Interchange Scheme—Agricultural and Food Research Council to T. W. W. and through partial funding from the U.K. Ministry of Agriculture, Fisheries and Food and from the Commission of the European Union. The authors would like to thank W. S. Gregory for inorganic-N measurements. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

REFERENCES

- Adamsen A. P. S. and 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.
- Bedard C. and Knowles R. (1989) Physiology, biochemistry and specific inhibitors of CH₄, NH₄⁺ and CO oxidation by methanotrophs and nitrifiers. *Microbiological Reviews* **53**, 68–84.
- Hütsch B. W., Webster C. P. and Powlson D. S. (1993) Long-term effects of nitrogen fertilization on methane oxidation in soil of the Broadbalk wheat experiment. *Soil Biology & Biochemistry* **25**, 1307–1317.
- Hütsch B. W., Webster C. P. and Powlson D. S. (1994) Methane oxidation in soil as affected by land use, pH, and N fertilization. *Soil Biology & Biochemistry* **26**, 1613–1622.
- Jenkinson D. S. and Powlson D. S. (1976) The effects of biocidal treatments on metabolism in soil—V. A method for measuring soil biomass. *Soil Biology & Biochemistry* **8**, 209–213.
- Keller M., Mitre M. E. and Stallard R. F. (1990) Consumption of atmospheric methane in soils of Central Panama: effects of agricultural development. *Global Biogeochemical Cycles* **4**, 21–27.
- Mosier A., Schimel D., Valentine D., Bronson K. and Parton W. (1991) Methane and nitrous oxide fluxes in native and cultivated grasslands. *Nature* **350**, 330–332.
- Stuedler P. A., Bowden R. D., Melillo J. M. and Aber J. D. (1989) Influence of nitrogen fertilization on methane uptake in temperate forest soils. *Nature* **341**, 314–316.
- Whittenbury R., Phillips K. C. and Wilkinson J. F. (1970) Enrichment, isolation and some properties of methane utilizing bacteria. *Journal of General Microbiology* **61**, 205–218.
- Willison T. W., Webster C. P., Goulding K. W. T. and Powlson D. S. (1994) Methane oxidation in temperate soils; effects of land use and the chemical form of nitrogen fertilizer. *Chemosphere* **30**, 539–546.