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Microbial oxidation of methane, ammonium and carbon monoxide, and turnover of nitrous oxide and nitric oxide in soils

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Abstract. The effect of soil microbial processes on production and/or consumption of atmospheric trace gases was studied in four different soils which were preincubated in the presence of elevated concentrations of CH_4, NH_4^+ or CO, to simulate the growth of the resident populations of methanotrophic, nitrifying, or carboxydotrophic bacteria, respectively. Oxidation of CH_4, both at atmospheric (1.8 ppmv) and at elevated (3500 ppmv) CH_4 mixing ratios, was stimulated after preincubation with CH_4, but not with NH_4^+ or CO, indicating that CH_4 was oxidized by methanotrophic, but not by nitrifying or carboxydotrophic bacteria. However, the oxidation of CH_4 was partially inhibited by addition of NH_4^+ and CO. Analogously, oxidation of NH_4^+ was partially inhibited by addition of CH_4. Oxidation of CO at elevated mixing ratios (2300 ppmv) was stimulated after preincubation with CO, indicating oxidation by carboxydotrophs, but was also stimulated at a small extent after preincubation with CH_4, suggesting the involvement of methanotrophs. At atmospheric CO mixing ratios (0.13 ppmv), on the other hand, oxidation of CO was stimulated after preincubation with NH_4^+, indicating that the activity was due to nitrifiers. NO uptake was stimulated in soils preincubated with CH_4, indicating the involvement of methanotrophs. However, production of N_2O was only stimulated, if CH_4 was added as a substrate. The results indicate that especially the methanotrophic and nitrifying populations in soil not only oxidize their specific substrates, but are also involved in the metabolism of other compounds.

Introduction

Microbial processes in soils contribute significantly to the atmospheric budgets of methane, carbon monoxide, nitrous oxide and nitric oxide (Cicerone & Oremland 1988; Seiler & Conrad 1987; Conrad 1988, 1990; Davidson 1991; Khalil & Rasmussen 1992). All these trace gases play an important role in the chemistry of the atmosphere or the Earth's radiation balance. The soil microbial processes which are involved in production or consumption of these trace gases are not satisfactorily understood. It is also possible that the processes producing the different trace gases interact with each other and thus affect the fluxes of the trace gases between soil and atmosphere.

Field experiments indicate that the uptake of atmospheric methane (CH_4) by soils is inhibited by increased input of nitrogen (Steudler et al. 1989; Mosier et al. 1991). This observation may be interpreted as inhibition by ammonium (NH_4^+) of either catabolic CH_4 oxidation by methanotrophic bac-

teria (Whittenbury et al. 1970; Carlsen et al. 1991), or CH_4 co-oxidation (oxidation without support of growth) by the autochthonous ammonium-oxidizing nitrifiers (Jones & Morita 1983a; Hyman & Wood 1983). Both explanations are possible since the key enzymes of CH_4 and NH_4^+ oxidation share features which allow, to some extent, oxidation of the other substrate as well (Bedard & Knowles 1989). Both the methane monooxygenase (Anthony 1986) and the ammonium monooxygenase (Wood 1988) catalyze a reaction of the following type:

$$X + O_2 + 2e^- + 2H^+ \rightarrow XO + H_2O \quad (1)$$

where $X = CH_4$, NH_4^+ or CO. In general, the reaction rate of the methane monooxygenase with NH_4^+ and of the ammonium monooxygenase with CH_4 is about 3 orders of magnitude lower than with the preferred substrate (Bedard & Knowles 1989). In addition, both enzymes also share the same inhibitors. Both methanotrophs and nitrifiers are also able to co-oxidize CO (without supporting growth), probably due to the relatively low specificity of the two monooxygenase reactions (Bedard & Knowles 1989).

In general, it is not uncommon that more than one specific group of soil microorganism is involved in the metabolism of one particular trace gas, e.g. by fortuitous metabolism (i.e., metabolism not supporting growth). Thus, CO oxidation may not only be achieved by the carboxydotrophic bacteria (Meyer et al. 1993; Mörsdorf et al. 1992), but also by methanotrophic (Ferenci et al. 1975), nitrifying (Jones & Morita 1983b) and unknown oligotrophic bacteria (Conrad & Seiler 1982). Methanotrophic bacteria may not only oxidize CH_4, but may also produce N_2O (Yoshinari 1985; Knowles & Topp 1988) and consume NO (Krämer et al. 1990). Furthermore, methanotrophs may oxidize NH_4^+ (Hutton & ZoBell 1953; Dalton 1977) and thus, may be involved in heterotrophic nitrification in soil (Knowles & Topp 1988; Megraw & Knowles 1989a,b). However, very little is known about how methanotrophic, nitrifying and carboxydotrophic bacteria interact during metabolism of CH_4, NH_4^+, CO, N_2O and NO in soil.

In this study, we measured the consumption of CH_4, NH_4^+, CO, and NO, the production of N_2O and NO, and the inhibitory effects of either CH_4, NH_4^+ or CO, and determined the population size of methanotrophic, nitrifying, and carboxydotrophic bacteria in different soils. In order to stimulate the growth of the resident populations of methanotrophic, nitrifying, or carboxydotrophic bacteria, the soils were also preincubated under elevated concentrations of CH_4, NH_4^+ or CO, respectively.

Table 1. Soil characteristics from 10 cm of mineral soil (Bender & Conrad 1992).

Soil	Cultivated loamy clay	Meadow sandy silty loam	Forest sandy clay loam	Paddy sandy clay loam
pH(H_2O)	8.0	7.5	4.6	6.8
Org. matter (%)	4.2	8.0	5.7	3.9
WHC[a] (%)	57.0	75.0	66.0	56.0
Total N (%)	0.16	0.42	0.1	0.19
C/N	15.0	11.0	33.0	12.0
NH_4^+ (μg-N g^{-1} d.w.)	1.8	12.1	10.7	1.0

[a] WHC = water holding capacity (g H_2O/100 g d.w. soil)

Materials and methods

Soil collection

Soil samples were taken from the A_h horizons (10 cm deep) of 4 different sites: cultivated cambisol (CC), meadow cambisol (MC), and forest luvisol (FL) were sampled near Konstanz (Germany), and rice paddy soil (PS) was sampled in Vercelli (Italy). Both the cultivated cambisol and paddy soil received regular additions of nitrogen fertilizers. The meadow and forest soils, on the other hand, were not managed. Collection, storage, processing and analysis of the soils have been described in detail by Bender & Conrad (1992). The main soil characteristics are summarized in Table 1.

Pretreatments

The experimental protocol is schematically shown in Fig. 1. Sieved (< 2 mm mesh) soil was sprayed with water solution and mixed to reach an average moisture content of 0.32 g H_2O g^{-1} d.w. The moist soils (a sample of about 500 g of each soil) were placed in polypropylene breakers and incubated at 25 °C in closed vessels under an atmosphere saturated with water vapour to prevent desiccation of the soils. The soils were conditioned for 9 weeks by incubation under 4 different conditions: (1) the untreated control (air-control) was incubated under 20% O_2 and 80% N_2; (2) the soil treated with NH_4Cl (1.25 mg N g^{-1} dw) was incubated under 20% and 80% N_2; (3) the soil treated with elevated CH_4 concentrations was incubated under 20% CH_4, 20% O_2 and 60% N_2; (4) the soil treated with elevated CO concentrations was incubated under 20% CO, 20% O_2 and 60% N_2. The gas phase was

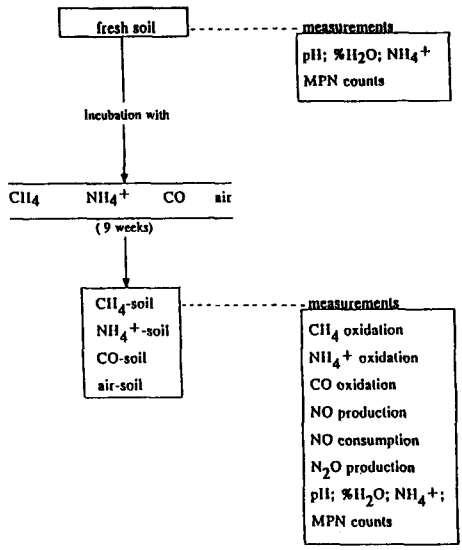

Fig. 1. Scheme of the experimental protocol.

refreshed every second day. At the beginning and the end of the incubation, soil moisture, pH, and the concentration of NH_4^+ were determined. All the variables stayed fairly constant during the incubation, except for NH_4^+ which decreased in the NH_4^+-pretreated soil to the background levels shown Table 1. Only in the acidic forest soil (FL) was the residual NH_4^+ still about half (600 μ g N g^{-1} d.w.) of the amount added.

Assays of microbial populations and activities

The numbers of methanotrophic, ammonium-oxidizing nitrifying and carboxydotrophic bacteria in the differently pretreated soils were determined by the most probable number (MPN) technique using ELISA microtiter plates (Rowe et al. 1977). All the assays were done at 25 °C in the dark. Methanotrophic bacteria were counted as described by Bender & Conrad (1992). The microtiter plates were incubated under 20% CH_4 in air for 3 weeks and then tested for bacterial growth against a control under CH_4-free air. Ammonium-oxidizing bacteria were counted in the mineral medium described by Baumgärtner & Conrad (1992). The microtiter plates were incubated under air containing 1% CO_2 for 5 weeks and then tested for accumulation of NO_2^- and NO_3^- (Morgan 1930). Carboxydotrophic bacteria were counted in the mineral medium described by Schlegel et al. (1961). The microtiter plates were incubated under 20% CO in air for 6 weeks and then

tested for growth against a control under CO-free air. The MPN numbers with their standard errors (SE) were determined from the index tables (Rowe et al. 1977).

Rates of CH_4 oxidation were determined as described by Bender & Conrad (1992) using stoppered serum bottles (150 ml) which contained 10 g moist soil under air with either 2 ppmv or 3500 ppmv CH_4. The effect of CO (350 and 3500 ppmv) on CH_4 oxidation was tested in a short-term treatment, i.e. by addition of CO to a soil suspension (10 g moist soil plus 10 ml H_2O) incubated on a shaker, and immediately measuring the rate of CH_4 oxidation at an initial CH_4 mixing ratio of 350 ppmv. The effect of NH_4^+ (10 mM) on CH_4 oxidation was tested similarly at an initial CH_4 mixing ratio of 1400 ppmv.

Rates of CO oxidation were determined analogously to CH_4 oxidation rates using initial CO mixing ratios of either 0.13 or 2300 ppmv CO. The effect of CH_4 (3500 ppmv) and NH_4^+ (150 μM) on CO oxidation was tested in a short-term treatment, i.e. by addition of either CH_4 or NH_4^+ to a soil suspension (10 g moist soil plus 10 ml H_2O) incubated on a shaker, and immediately measuring the rate of CO oxidation at an initial CO mixing ratio of 10 ppmv. CO was analyzed in a gas chromatograph with a HgO-Hg-conversion detector (RGD2, Trace Analytical, Techmation, Düsseldorf, Germany) using a stainless steel column (1.4 m length, 1/4 inch diameter) filled with molecular sieve 5A (80–100 mesh). The detector temperature was 280 °C.

Potential rates of NH_4^+ oxidation were determined according to Berg & Rosswall (1985) as described by Saad & Conrad (1993). The effect of CH_4 (10%) on NH_4^+ oxidation was tested in a short-term treatment, i.e. by addition of CH_4 to a soil suspension (5 g moist soil plus 25 ml H_2O) incubated on a shaker, and immediately measuring the rate of NH_4^+ oxidation.

N_2O production was measured analogously to CH_4 oxidation as described by Schuster & Conrad (1992). The effect of CH_4 on N_2O production was tested in a short-term treatment, i.e. by addition of 100 ppmv CH_4. N_2O was analyzed by gas chromatography with an electron capture detector (Conrad & Seiler 1980).

Rates of NO production and NO uptake rate constants were measured as described by Remde et al. (1989) using 70 g moist soil gassed with synthetic air (80% N_2, 20% O_2) at a flow rate of 968 ml min^{-1} containing NO at mixing ratios between 0.2 and 1.6 ppmv. NO was analyzed in a Thermo Electron NO_x analyzer as described by Remde et al. (1989).

All consumption and production assays were done on duplicate subsamples from each pretreated soil. The data points from the time-linear range

of the duplicate experiments were pooled (n = 6–8) and analyzed by linear regression to determine the rate of production or consumption.

Statistical analysis

The statistical analyses of the effects of the different pretreatments on the results of the MPN and the production/consumption assays utilized the four different soils as replicates. The effect of each pretreatment was compared with the control (air-soil) and among each other using the Lord (Lord 1947) and Dixon test (Dixon 1953), respectively. The Lord test is especially suitable for a small number of replicates using the range instead of the standard deviation of the mean. The Dixon test is used for detecting outliers among a set of mean values.

Results

We used four (sometimes three) different soils (cultivated, meadow, forest, paddy) preincubated under conditions which resulted in increased populations of either methanotrophic, nitrifying or carboxydotrophic bacteria (i.e. incubated with CH_4, NH_4^+ or CO, respectively). These preincubated soils were then studied with respect to different soil metabolic capacities and compared to the corresponding control in which the microbial populations were not specifically enriched (i.e. pretreated under synthetic air). The individual soil values are presented in Figs. 2–5 to provide information about variability among soils. The effects of the different pretreatments on microbial numbers and activities are summarized in Table 2, giving the means and the ranges of the values obtained with the four (sometimes three) different soils as replicates.

Methane oxidation

In all soils studied, the methanotrophic population density increased dramatically when the soils were preincubated under high (20%) CH_4 (Fig. 2A). Preincubation in the presence of NH_4^+, on the other hand, had no effect on the methanotrophic population size compared to the control (Table 2). Interestingly, preincubation in the presence of high (20%) CO also increased (although not significantly, Table 2) the methanotrophic population densities to some extent (Fig. 2A). However, the increased MPN counts in the CO-soils did not affect the CH_4 oxidation (Fig. 2B,C).

Only in the CH_4-pretreated soils were the increased methanotrophic populations paralleled by increased rates of CH_4 oxidation (Table 2). The increased CH_4 oxidation rates were obvious at both atmospheric (Fig. 2B) and elevated

Table 2. Effect of preincubation conditions on numbers of methanotrophic, ammonium-oxidizing and carboxydotrophic bacteria, and consumption and production of CH_4, NH_4^+, CO, N_2O and NO averaged for four different soils.

	Preincubation with			
	air	CH_4	NH_4^+	CO
Numbers of [counts/g d.w.]				
Methanotrophs	$2.2 \times 10^5(8.0 \times 10^5)$	$4.9 \times 10^8(6.4 \times 10^8)^{aabb}$	$1.1 \times 10^5(2.9 \times 10^5)$	$3.3 \times 10^7(5.3 \times 10^8)$
Nitrifiers	$1.9 \times 10^5(7.6 \times 10^5)$	$2.6 \times 10^5(6.9 \times 10^4)$	$1.9 \times 10^6(3.5 \times 10^6)^{abb}$	$5.7 \times 10^4(1.2 \times 10^5)$
Carboxydotrophs	$4.4 \times 10^6(5.3 \times 10^5)$	$2.4 \times 10^6(3.8 \times 10^6)$	$3.8 \times 10^6(1.1 \times 10^6)$	$1.6 \times 10^8(3.7 \times 10^8)^{abb}$
CH_4 oxidation [nmol/h/g d.w.]				
at 1.8 ppmv	0.035(0.02)	$0.3(0.27)^{aabb}$	0.035(0.04)	0.027(0.02)*
at 3,500 ppmv	4.0(4.6)	$162.5(90.0)^{aabb}$	4.3(2.0)	4.0(4.0)*
Potential NH_4^+ oxidation [μg N/h/g d.w.]	0.61(1.9)	0.68(2.4)	0.84(2.6)	0.68(1.4)*
CO oxidation [nmol/h/g d.w.]				
at 0.13 ppmv	0.09(0.05)*	0.12(0.12)*	$0.36(0.68)^{aab}$*	$0.16(0.1)^{a}$*
at 2,300 ppmv	1.6(0.7)*	$2.4(0.6)^{a}$*	1.5(0.10)*	$3.5(3.1)^{a}$*
NO uptake rate constant [cm^3/h/g d.w.]	27.1(42.8)*	$72.0(13.1)^{a}$*	$55.7(4.3)^{a}$*	44.3(52.7)*
NO maximum production rate [nmol/h/g d.w.]	0.28(0.36)*	0.55(0.63)*	0.4(0.46)*	0.6(0.87)*
N_2O accumulation after 350 hours [nmol/g d.w.]	2.2(4.5)	1.6(2.5)	$9.4(13.0)^{abb}$	

Values are means of $n = 4$ different soils; (*) $n = 3$. The values in parenthesis give the range (maximum-minimum).

a) Values were significantly higher (Lord test) than the air-control at $P < 0.05$ (a) and $P < 0.01$ (aa).

b) Values were significantly different (Dixon text) from the other values in the row at $P < 0.1$ (b) and $P < 0.01$ (bb).

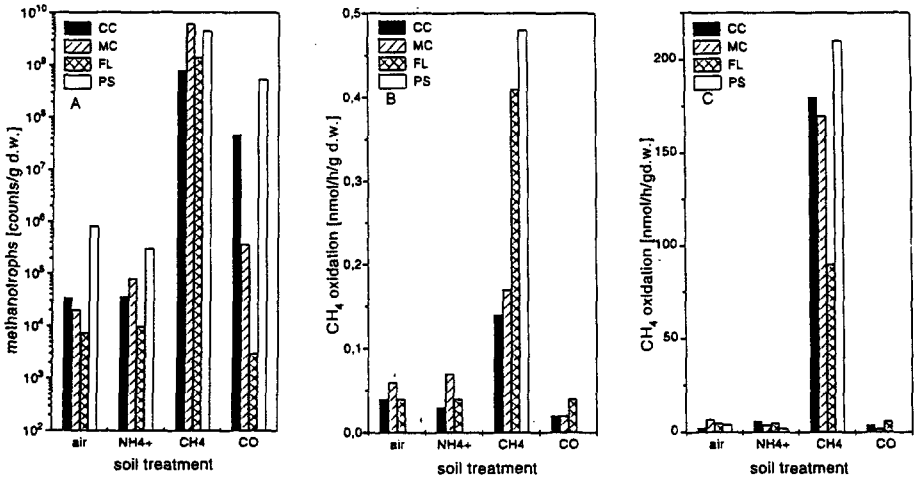

Fig. 2. Effect of preincubation conditions (see Fig. 1) on (A) numbers of methanotrophic bacteria, (B) rates of CH_4 oxidation at atmospheric (1.8 ppmv) CH_4 mixing ratios, and (C) rates of CH_4 oxidation at elevated (3500 ppmv) CH_4 mixing ratios in different soils, i.e. cultivated cambisol (CC), meadow cambisol (MC), forest luvisol (FL), and paddy soil (PS).

CH_4 (Fig. 2C) mixing ratios. Addition of NH_4^+ resulted in an instantaneous inhibition of CH_4 oxidation reducing the rates by more than 60% except in the NH_4^+-pretreated soils (Table 3). Addition of CO also resulted in an instantaneous inhibition of CH_4 oxidation (Table 3). The inhibitory effect increased with increasing CO mixing ratio and was completely reversible by removal of CO.

Ammonium oxidation

The nitrifying population density was highest in the NH_4^+-pretreated soils and was lowest in the air-controls (Fig. 3A). In some of the CH_4- and CO-pretreated soils the nitrifier population densities were slightly increased. The paddy soil (PS) was an exception, since the air-control also exhibited relatively high nitrifier population densities. However, only the NH_4^+-pretreated soils showed a consistently increased nitrifier population (Table 2). The NH_4^+ (1250 $\mu g\ N\ g^{-1}$ dw) which had initially been added to the NH_4^+-pretreated soils was depleted to background concentrations of 0.6–2.1 $\mu g\ N\ g^{-1}$ dw during the 9 week incubation period except in the acidic forest soil (FL), which showed a final concentration of 600 $\mu g\ N\ g^{-1}$ dw.

Differences in nitrifier population density had little effect on the potential rates of ammonium oxidation (Table 2), although there seemed to be a slight tendency to maximum rates in some of the NH_4^+-pretreated soils (Fig. 3B,C).

Table 3. Inhibitory effects of CH_4, NH_4^+ and CO addition in short-term treatments on microbial activities in differently preincubated soils.

	Preincubation with			
	air	CH_4	NH_4^+	CO
Inhibition of CH_4 oxidation [%]				
by NH_4^+ (10 mM) §	64.3(33)	90.7(15)	21.3(39)[a]	63.3(20)
by CO (350 ppmv) $		42.7(40)		
by CO (3,500 ppmv) $		95.3(3)		
Inhibition of potential NH_4^+ oxidation [%]				
by CH_4 (10%)	71.3(67)	9.0(24)[a]	76.3(49)	45.3(98)
Inhibition of CO oxidation [%]				
by CH_4 (3,700 ppmv) #	81.0(49)[b]	15.3(55)	35.7(31)	36.7(55)
by NH_4^+ (0.15 mM) #	−13.3(38)[a]	21.3(31)	16.7(50)	
Inhibition of N_2O accumulation [%]				
by CH_4 (100 ppmv)	10.5(8)*	−113(134)[aac]*	8.0(139)*	

Values are means of $n = 3$ different soils; (*) $n = 4$. The values in parenthesis give the range (maximum-minimum).
All data are expressed as percent of response in samples with inhibitor compared to samples without inhibitor in short-term treatments.
Negative values indicate stimulation instead of inhibition.
§) measured at 1,400 ppmv CH_4; $) measured at 350 ppmv CH_4; #) measured at 10 ppmv CO.
a) Values were significantly lower (Lord test) than the other values in the row at $P < 0.05$ (a) and $P < 0.01$ (aa).
b) Value is significantly higher (Lord test) than the other values in the row ($P < 0.05$).
c) Value is significantly different (Dixon test) from the other values in the row ($P < 0.01$).

The ammonium oxidation rates were inhibited as soon as the soils were exposed to increased CH_4 mixing ratios, except with the CH_4-pretreated soils (Table 3).

Carbon monoxide oxidation

The population density of carboxydotrophs was highest in the CO-pretreated soils (Fig. 4A; Table 2). The other soil conditions showed densities which were at least one order of magnitude lower.

The CO-pretreated soils also showed the highest rates of CO oxidation, especially when measured at high (2300 ppmv) CO mixing ratios (Fig. 4C).

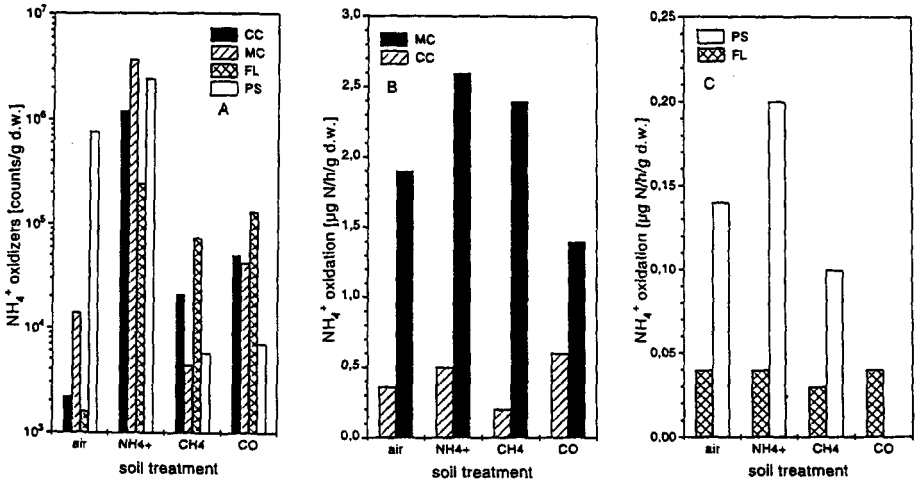

Fig. 3. Effect of preincubation conditions (see Fig. 1) on (A) numbers of NH_4^+-oxidizing bacteria, and (B,C) rates of potential NH_4^+ oxidation (using two different scales; B and C), in different soils (CC, MC, FL, PS).

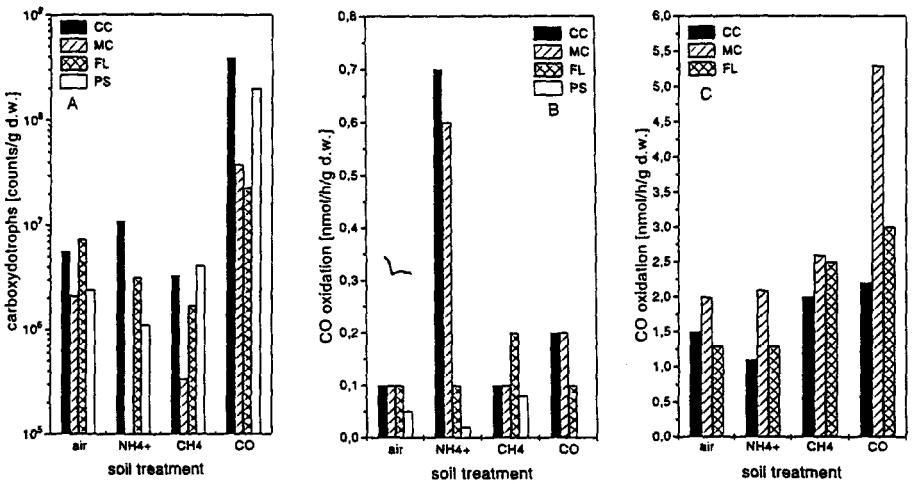

Fig. 4. Effect of preincubation conditions (see Fig. 1) on (A) numbers of carboxydotrophic bacteria, (B) rates of CO oxidation at atmospheric (0.13 ppmv) CO mixing ratios, and (C) rates of CO oxidation at elevated (2300 ppmv) CO mixing ratios in different soils (CC, MC, FL, PS).

The CH_4-pretreated soils also showed slightly elevated rates of CO oxidation at high CO mixing ratios (Fig. 4C; Table 2). At atmospheric CO mixing ratios (0.13 ppmv), on the other hand, the NH_4^+-pretreated soils showed by far the highest rates of CO oxidation (Fig. 4B). Although this effect was restricted

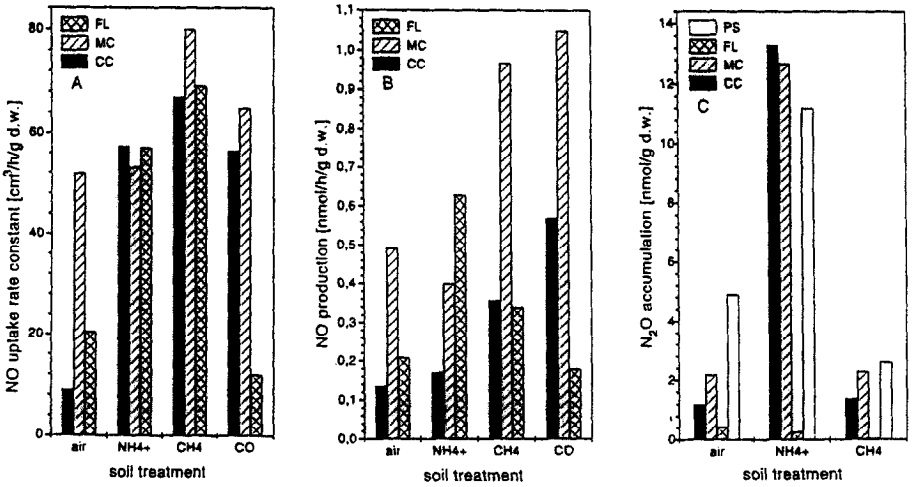

Fig. 5. Effect of preincubation conditions (see Fig. 1) on (A) NO uptake rate constants, and (B) maximum NO production rates, and (C) N_2O accumulation in different soils (CC, MC, FL, PS).

to the cultivated (CC) and meadow (MC) soils, and was not obvious in the FL and PS soils, the soils on the average, showed a more significant increase in oxidation of atmospheric CO after pretreatment with NH_4^+ than with CO (Table 2).

The CO oxidation activity was slightly inhibited by addition of CH_4, but the inhibition was only statistically significant in the air-controls (Table 3). The CO oxidation activity was also slightly inhibited by NH_4^+, but this inhibition was not significant (Table 3). In the air-controls, however, CO oxidation was stimulated by addition of NH_4^+, and this stimulation was significant (Table 3).

Metabolism of nitrogen oxides

Uptake of NO was observed in all soils, regardless of preincubation conditions. The highest uptake rate constants were generally found in the CH_4-preincubated soils (Fig. 5A, Table 2). Depending on the soil type, however, NO uptake could also be relatively high in other treatments, e.g. in the NH_4^+- and CO-pretreated soils of the CC, or in the NH_4^+-pretreated soil of the FL. (Fig. 5A, Table 2).

NO production showed a different behaviour than NO uptake (Fig. 5B). The CC and MC showed highest rates in the CO-pretreated soils followed by the CH_4-pretreated soils. The FL, on the other hand, showed highest rates in the NH_4^+-pretreated soil, followed by the CH_4-pretreated soil. On average,

however, the different soil pretreatments had no significant effect on NO production (Table 2).

N_2O production rates were significantly stimulated in the NH_4^+-pretreated soils (Table 2). However, in the acidic FL, N_2O production rates were not affected by any of the soil treatments (Fig. 5C). Production of N_2O was not instantaneously inhibited by increased CH_4 mixing ratios in the production experiments (Table 3). Quite in contrast, CH_4 stimulated N_2O production in the CH_4-pretreated soils significantly.

Discussion

Our results showed that increasing the population density of either methanotrophs, nitrifiers or carboxydotrophs not only stimulated the oxidation of CH_4, NH_4^+ or CO, respectively, but had more complex effects. Complex effects were not unexpected because of the capacity of these bacteria to fortuitously oxidize other compounds in addition to their natural substrate (see Introduction). We will discuss our results, therefore, in light of some of the more interesting interactions.

Methane oxidation and response to ammonium additions

Previous field studies have shown that inputs of nitrogen fertilizer apparently inhibits the uptake of atmospheric CH_4 by soil (Steudler et al. 1989; Mosier et al. 1991). Our experiments also revealed strong instantaneous inhibition of CH_4 oxidation with NH_4^+ additions (Table 3), but suggest that nitrogen-induced inhibition of CH_4 oxidation does not involve activities of the nitrifier population. Soils with high nitrifier populations due to the NH_4^+ pretreatment did not have elevated rates of CH_4 oxidation at either ambient or elevated CH_4 mixing ratios (Fig. 2). Given that the NH_4^+ added in the pretreatment was completely depleted at the end of the pretreatment incubation (except in the forest soil), the nitrifiers were most likely not saturated with NH_4^+ and thus should have been able to oxidize CH_4; we did not see such an effect. Likewise, in the NH_4^+-pretreated soils, further addition of NH_4^+ resulted in less (rather than more) inhibition of CH_4 oxidation as compared to the other pretreatments (Table 3). This suggests that nitrifier activity and nitrifier response to added NH_4^+ does not directly affect CH_4 oxidation. Our results rather indicate that the added NH_4^+ inhibits the soil methanotrophs. Similar conclusions were drawn in a recent review by King (1992).

The reason for the reduced inhibition in the NH_4^+-pretreated soils receiving additional NH_4^+ is not clear. It is probable that the methanotrophic populations had adapted to high NH_4^+ concentrations as a result of the pretreatment and thus were less affected by the short-term NH_4^+ additions than were the

populations subject to the other pretreatments. Alternatively, the high nitrifier population in the NH_4^+-pretreated soils may have rapidly consumed NH_4^+, thus removing the CH_4 oxidizers' inhibitory substrate.

Nitrification and response to CH_4 additions

Just as methane oxidation was apparently inhibited by ammonium additions, the NH_4^+ oxidation was also inhibited by CH_4. Inhibition of NH_4^+ oxidation by CH_4 has been reported for pure cultures of ammonium oxidizers (Hyman & Wood 1993; Ward 1987, 1990) and for soils (McCarty & Bremner 1991). Megraw & Knowles (1987) suggested that inhibition of NH_4^+ oxidation by CH_4 results when methanotrophs outcompete nitrifiers for available O_2 under high CH_4 mixing ratios. In our experiments, however, increased methanotrophic populations diminished rather than intensified the inhibition of NH_4^+ oxidation by CH_4, in analogy to the effects observed on CH_4 oxidation by NH_4^+ in NH_4^+-pretreated soils.

The increased methanotrophic populations also correlated with increased NO uptake in soils (Table 2). This observation agrees with earlier results (Krämer et al. 1990). It is likely that methanotrophs (Yoshinari 1985; Knowles & Topp 1988) or methanotrophic consortia (Megraw & Knowles 1989a,b) acted as heterotrophic nitrifiers and thus affected the turnover of NO. The soils with increased population densities of methanotrophs also showed a potential for increased rates of N_2O production (Table 3), provided that sufficient CH_4 was added as a substrate.

CO oxidation

Our results showed only a slight stimulation of CO oxidation at atmospheric CO mixing ratios in soils with elevated populations of carboxydotrophs (Table 2). Although carboxydotrophic bacteria may be involved in oxidation of atmospheric CO in soils (Conrad & Seiler 1982; Mörsdorf et al. 1992), they seem to be better adapted to the oxidation of elevated concentrations of CO (Fig. 4). Methanotrophic bacteria possibly also contribute to the oxidation of CH_4 at elevated CO mixing ratios (Table 2).

At atmospheric CO concentrations, however, soils with increased populations of carboxydotrophs exhibited only a slight stimulation of CO oxidation (Table 2). Instead, our results showed a higher and more significant stimulation of CO oxidation in soils with elevated nitrifier populations (Fig. 4). *Nitrosomonas* species were reported to oxidize CO efficiently (Jones & Morita 1983b; Jones & Morita 1984). Since the CO uptake by soils shows kinetic parameters (K_m values) which are closer to those observed in *Nitrosomonas*

than in carboxydotrophic bacteria, it is likely that the nitrifiers are the actual oxidizers of atmospheric CO in soils (Conrad 1988).

The soils with an increased population of carboxydotrophs showed neither increased rates of CH_4 oxidation nor increased rates of NH_4^+ oxidation. In fact, any fortuitous oxidative capacity of carboxydotrophs is questionable, since the first enzyme in the degradation of CO by the carboxydotrophs is not a monooxygenase, but a dehydrogenase (Meyer 1985):

$$CO + H_2O \rightarrow CO_2 + 2e^- + 2H^+ \quad (2)$$

The CO dehydrogenase of carboxydotrophs has a completely different reaction mechanism and thus, one should *a priori* not expect any similarity in its behaviour to the methane or ammonium monooxygenases.

Conclusion

Our results are based on the microbial reactions in soil which had been treated with substrates to stimulate the growth of the resident populations of methanotrophic, nitrifying, or carboxydotrophic bacteria. The assumption implicit in this study is that the populations which grew following addition of exogenous substrate were identical to those active in the untreated soil. This assumption is not necessarily true, since addition of high levels of a growth substrate may favour the growth of only a part of the original population. Thus, our conclusions must be considered with this potential artifact in mind. Our results indicate that methanotrophs were responsible for the oxidation of CH_4 in soils both at high and at atmospheric CH_4 mixing ratios. Nitrifiers apparently did not contribute to CH_4 oxidation in soils, but contributed significantly to oxidation of atmospheric CO. Methanotrophs, on the other hand, were at least partially involved in heterotrophic nitrification as seen by their potential contribution to N_2O production and turnover of NO, and were involved in the oxidation of CO at high mixing ratios. Together, our results suggest that production and/or consumption of a number of atmospheric trace gases involve a number of microbial processes, singly and in interaction with each other.

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