

Wetlands and Aquatic Processes

Methane Production and Oxidation in an Anoxic Rice Soil as Influenced by Inorganic Redox Species

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ABSTRACT

The effects of addition of inorganic redox substances (species of NO_3^- , Mn^{4+} , Fe^{3+} , and SO_4^{2-}) on methane production and oxidation in anoxic rice (*Oryza sativa* L.) soil samples were examined. Sulfate was the most inhibitory for methane production followed by Fe^{3+} , NO_3^- , and Mn^{4+} , in that order. Addition of rice straw at a rate of 1% (w/w) as a carbon source to increase the electron donor to the electron acceptor ratio did not completely alleviate the inhibitory effects of redox species on methane production. Interestingly, laboratory incubation studies showed that addition of MnO_2 and K_2SO_4 enhanced aerobic methane oxidation in soil samples held at 60% water holding capacity. The suspensions of pretreated soil samples with different redox species, when tested for their ability to oxidize methane in soil solution equivalent medium supplemented with respective redox species under aerobic and anaerobic conditions showed differential effects of redox species. Nitrate and Fe^{3+} stimulated methane oxidation under anaerobic conditions and retarded it under aerobic conditions. Manganese(IV) ion retarded methane oxidation under anaerobic conditions, but enhanced it under aerobic conditions. However, SO_4^{2-} stimulated methane oxidation in soil solution equivalent medium under both aerobic and anaerobic conditions.

RICE soils are often flooded as a management practice to enhance their agronomic productivity. In the reduced environments of flooded rice soils, anaerobic mineralization of organic matter occurs, resulting in the gaseous production of N_2 , N_2O , H_2 , NH_3 , H_2S , CH_4 , mercaptans, and dimethyl sulfide (Ponnamperuma, 1972). Because of the biogenic methane production, the predominantly anoxic flooded rice soils are considered to be one of the major anthropogenic sources of atmospheric methane (Minami and Neue, 1994). Flooding of a soil does not necessarily result in the development of a uniformly reduced profile. A thin, oxidized surface horizon overlying a deep, reduced horizon is formed due to the dissolved oxygen from the overlying floodwater diffusing across the surface water-soil interface and, in soils planted with rice, the rhizosphere is oxidized because of the delivery of O_2 into roots. Thus, these flooded soils can also support the activities of methane-oxidizing bacteria in oxic zones (De Bont et al., 1978; Bosse and Frenzel, 1997; Bodelier and Frenzel, 1999).

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Methanogenic bacteria are active only under anoxic, reduced soil conditions where there is a high input of labile organic material. Wang et al. (1993) reported that CH_4 formation in flooded soils occurred when the redox potential (Eh) fell below -150 mV. Because most redox reactions are biologically mediated, a sequential ecological succession of microorganisms is expected. However, the different redox reactions in the anoxic environments are not mutually exclusive (Lovely, 1991) or cannot be explained only by the competition for common electron donors by those microorganisms that use the electron acceptor with the highest redox potential (Conrad et al., 1987). The oxidized inorganic species are reported to inhibit CH_4 production (Bollag and Czlonkowski, 1973) and exhibit a toxic effect on CH_4 formation (Roy and Conrad, 1999). Microbially mediated CH_4 consumption in soils may also be regulated by the reduction characteristics. The methane-consuming activity of soil incubated under aerobic conditions decreased with low concentration of O_2 (Schnell and King, 1995). Murase and Kimura (1994) demonstrated that CH_4 was anaerobically oxidized at less reduced sites in the plow layer than at the sites where CH_4 was produced. An association of methane oxidation with sulfate reduction under the anoxic conditions was suggested (Murase and Kimura, 1994). Anaerobic methane oxidation is a poorly understood process because the microorganisms capable of performing this process have not been isolated from flooded rice soils or marine sediments (Kumaraswamy et al., 2000; Valentine and Reeburgh, 2000). Depending on the availability of electron donors and acceptors, the different functional groups of microorganisms can be active during the sequential reduction processes in rice soils. In the present study, we investigated the effects of different inorganic redox species on methane production and oxidation in anoxic rice soil.

MATERIALS AND METHODS

Soil Samples

Soil samples were collected from flooded fields (planted to rice) in the experimental farm of the Central Rice Research Institute, Cuttack, Orissa, India (20°N , 86°E ; 23 m above mean sea level) during wet season, 1997. The soil is a deltaic alluvium (Typic Haplaquept), with a sandy, clay loam texture (25.9% clay, 21.6% silt, 52.5% sand), pH 6.2, maximum water holding capacity (WHC) 43.7%, cation exchange capacity (CEC) 18 $\text{cmol}_c \text{ kg}^{-1}$, organic carbon 1.12%, total N 0.07%, free Fe_2O_3

Abbreviations: sMMO, soluble methane monooxygenase.

3.96%, easily reducible MnO_2 0.05%, and electrical conductivity 0.7 dS m^{-1} . Larger dry lumps were broken manually by slight impounding before passing the samples through a sieve of 2-mm mesh size. Samples were stored in plastic containers.

Methane Production

For methane production studies, air-dried and sieved soil samples in 5-g portions were either unamended or amended with rice straw powder (aboveground parts of the rice plant after harvest, dried and pulverized to pass through a sieve of 0.2 mm, C to N ratio 64.03, at 1% level w/w), placed in presterilized 15-mL B-D vacutainer tubes ($125 \times 16 \text{ mm}$; Becton, Dickinson and Co., Rutherford, NJ), and flooded at a weight ratio of 1:1.25 in sterile distilled water. Aqueous solutions of different inorganic redox substances (KNO_3 , MnO_2 , Fe_2O_3 , and K_2SO_4 as NO_3^- , Mn^{4+} , Fe^{3+} , and SO_4^{2-} , respectively at 2500 mg kg^{-1} soil) were added separately, with minimum change to the total moisture content. Soil samples, unamended or amended with rice straw, were supplemented with equal amounts of sterile water to serve as controls. After closing with butyl rubber septa, the headspace of the vacutainer tubes was flushed with high-purity argon (99.98%) to create anoxic conditions rapidly. These soil tubes were then incubated in the dark at $32 \pm 2^\circ\text{C}$. At given time intervals, gas samples were withdrawn from the headspace after vigorously shaking the soil incubation tubes by hand to allow equilibration between the liquid and gas phases. The concentrations of CH_4 in the headspace gas samples were measured in a Varian (Palo Alto, CA) 3600 gas chromatograph as previously described (Ramakrishnan et al., 1998). For pH and redox potential measurements, bulk soil samples (50 g in 150-mL screw-capped containers, at a 1:1.25 soil–water ratio) were tested. The pH was measured with an Elico (Hyderabad, India) pH meter and the redox potential with a Barnant-20 digital ORP meter (Barnant Company, Barrington, IL). To estimate carbon dioxide concentrations, 10 mL of the headspace gas was injected into a 0.01 M NaOH trap solution in air-tight serum bottles. The amount of carbon dioxide trapped by NaOH solution was determined by titration (Stotzky et al., 1965) using 0.01 M HCl with phenolphthalein as an indicator. A control was used to determine the atmospheric carbon dioxide concentration, which was subtracted from the samples. The experiment was carried out by preparing in parallel numerous incubation tubes and containers, and all measurements were made on five replicates, sacrificed at each time of sampling.

Methane Oxidation

The incubation vessel for methane oxidation experiments was a 120-mL serum bottle sealed with butyl rubber septum. Soil samples in 10-g portions, placed in presterilized serum bottles, were held at 60% moisture holding capacity and then allowed to equilibrate with ambient air for 24 h in a dark incubator at $30 \pm 2^\circ\text{C}$. As described earlier, the aqueous solutions of different inorganic redox species (as KNO_3 , MnO_2 , Fe_2O_3 , and K_2SO_4 at 2500 mg kg^{-1} soil) were added separately. Controls included the sealed, autoclaved (121°C for 2 h) soil samples and another with acetylene, an inhibitor of methane oxidation, added at a final headspace concentration of 1%. A time course study for methane consumption was initiated by sealing the serum bottles and injecting the headspace with 10 mL of methane (5%) in argon (approximately $2200 \mu\text{mol CH}_4 \text{ L}^{-1}$). The gases in the headspace were in the ratio of 73.04 (nitrogen):18.26 (oxygen):7.89 (argon):0.04 (methane). These incubation bottles were kept in the dark in an incubator at $30 \pm 2^\circ\text{C}$, with intermittent shaking on a rotary shaker for

a period of 8 h on each day. At 2-d intervals, the headspace gas (0.2 mL) of the serum bottles of all soil samples was analyzed in a Varian 3600 gas chromatograph. As there was no statistically significant difference in methane concentrations between autoclaved soil and acetylene-treated controls, the decrease in methane concentration in the headspace between two consecutive samplings under the CH_4 -amended atmosphere was used to estimate methane oxidation (Kumaraswamy et al., 1997). On each sampling day, all determinations were made in a minimum of five incubation vessels for each treatment and the mean values were presented.

Soil Solution Equivalent Medium Experiment

In a follow-up experiment, soil samples in 10-g portions, placed in presterilized serum bottles (120 mL), were held at 60% moisture holding capacity and then treated with the aqueous solutions of different inorganic redox species (as KNO_3 , MnO_2 , Fe_2O_3 , and K_2SO_4 at 2500 mg kg^{-1} soil) separately. After closing with butyl rubber stoppers, a set of incubation vessels was flushed with nitrogen for 30 min and another set was left with ambient air to create anoxic and aerobic conditions in the headspace, respectively. The headspace of all the serum bottles was injected with 10 mL of methane (5%) in argon. Then, the incubation bottles were kept in the dark in an incubator, with intermittent shaking on a rotary shaker for a period of 8 h on each day, at $30 \pm 2^\circ\text{C}$ for 10 d. After 10 d of incubation, 1 g of soil slurry from the respective treatment was inoculated to a 10-mL portion of sterile soil solution equivalent medium (Angle et al., 1991), supplemented with KNO_3 , MnO_2 , Fe_2O_3 , and K_2SO_4 separately to give final concentration of 5 mM in serum bottles. The transfers were made under aseptic conditions using a sterile syringe. The headspace of the culture medium containing incubation vessels was then replaced with a mixture of nitrogen and oxygen (80:20) and pure argon (99.98%) to create aerobic and anoxic conditions, respectively. Incubation bottles were injected with methane (5%) in argon to provide $2500 \mu\text{mol CH}_4 \text{ L}^{-1}$ and incubated in the dark with intermittent shaking on a rotary shaker for a period of 8 h on each incubation day. Methane concentration in the headspace of the serum bottles was analyzed on alternate days until 10 to 12 d, in a Varian 3600 gas chromatograph. This experiment was performed twice and the mean values from a minimum of six determinations for each treatment at every sampling period were presented.

Enumeration of Microbial Population

Total aerobic heterotrophic bacterial population of soil samples was estimated by dilution plate technique (Rand et al., 1975). Most probable number (MPN) estimate of total methane oxidizers (TMO) was determined according to Arif et al. (1996). Methane oxidizers with soluble methane monooxygenase (sMMO) activity were enumerated as described by Graham et al. (1992). Dilution plates were incubated under the atmosphere of methane (5%)–air mixture in vacuum desiccators for 30 d at $30 \pm 2^\circ\text{C}$. The headspace was evacuated and replenished with methane–air mixture at 5-d intervals during the incubation period. The colonies that developed a colored complex with naphthalene and *O*-dianisidine (tetrazotized) were counted positive for methane oxidizers. Methanogens (H_2 and CO_2 utilizers) were counted by most probable number technique at 10-fold dilution using tubes prepared under N_2 and pressurized with a mixture of H_2 and CO_2 (Kaspar and Tiedje, 1982). The MPN culture tubes, incubated for 30 d at $28 \pm 2^\circ\text{C}$, were examined for the presence of methanogens by detection of methane in the headspace to make rapid observations on population densities.

Statistical Analysis

Data were analyzed by the standard statistical methods using IRRISTAT Version 3/93 (International Rice Research Institute, 1993). The significance of the differences between treatments was assessed by analysis of variance (ANOVA) and subsequently by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

In the experiment to examine the influence of different inorganic redox species (NO_3^- , Mn^{4+} , Fe^{3+} , and SO_4^{2-}) on methane production from flooded rice soil, different inorganic redox substances added at 2500 mg kg^{-1} soil distinctly suppressed the production of methane as compared with that in the untreated soil samples (Table 1). The inhibitory effects of all the tested inorganic redox species were more pronounced at 30 d of incubation and the effect of SO_4^{2-} was higher compared with those of other redox species, even at 10 d of incubation. Thus, the net methane production in soil samples under different treatments during the experimental period showed that SO_4^{2-} was the most inhibitory followed by Fe^{3+} , NO_3^- , and Mn^{4+} , in that order. Generally, methane is produced in soils following submergence after reduction of NO_3^- , Mn^{4+} , Fe^{3+} , and SO_4^{2-} , as predicted by the thermodynamic theory (i.e., electron acceptors with a higher redox potential will be reduced first) (Ponnamperuma, 1972). Methanogens are strictly anaerobic microorganisms and are inhibited by high redox. Macgregor and Keeney (1973) reported that addition of NO_3^- and SO_4^{2-} to a flooded soil maintained the soil in a more oxidized state and thereby inhibited methane production. Methanogenesis was found to be inhibited by the addition of NO_3^- , Fe^{3+} , and SO_4^{2-} in anoxic paddy soil (Achnich et al., 1995). The repression of CH_4 production may be mediated by redox active compounds, such as Fe^{3+} to Fe^{2+} ratios, acting as a signal (Peters and Conrad, 1996). Alternatively, the competition for acetate and H_2 , the important methanogenic substrates, with nitrate-, iron-, and sulfate-reducing bacteria is one of the important factors for the inhibition of methanogenesis (Achnich et al., 1995). Roy and Conrad (1999) showed that the inhibition of methanogenesis due to the addition of nitrate was caused by the toxic effects of the denitrification intermediates nitrite, nitric oxide (NO), and N_2O , rather than by competition for acetate. Thus, the inhibitory effects of different inorganic redox species are probably due to high redox conditions, the toxic effects, and the competition among different microorganisms for the methanogenic substrates. There is also evidence that methanogenesis is restricted in flooded soils with significant numbers of electron acceptors (Neue et al., 1995).

Addition of rice straw to soil samples at a rate of 1% w/w effected a several-fold increase in CH_4 production at all treatments relative to that of the respective unamended soil samples (Table 1). Decomposition of rice straw in predominantly anaerobic flooded soil can lead to the accumulation of acetate as a major but transitory intermediate (Rao and Mikkelsen, 1977). Acetate is one of the important substrates for methanogens in flooded soils and this would explain the substantial accumulation

Table 1. Methane production ($\mu\text{mol CH}_4$ produced kg^{-1} soil) in flooded rice soil samples, unamended or amended with rice straw (1% w/w), as influenced by different inorganic redox species (added at 2500 mg kg^{-1} soil) under laboratory incubation. In a column, means followed by the same letter are not significantly different by Duncan's multiple range test.

Treatment	Unamended			Amended with rice straw		
	Days of incubation			Days of incubation		
	10	20	30	10	20	30
	— $\mu\text{mol CH}_4$ produced kg^{-1} soil —					
Control	6.1 ^a	38.4 ^a	1 179.0 ^a	36.6 ^a	970.5 ^a	25 492.9 ^a
+ NO_3^-	4.0 ^b	19.8 ^b	63.0 ^b	14.5 ^b	607.6 ^b	764.8 ^b
+ Mn^{4+}	4.6 ^b	22.0 ^b	53.4 ^c	10.4 ^c	146.0 ^c	475.6 ^c
+ Fe^{3+}	3.5 ^c	13.1 ^c	39.3 ^d	12.7 ^b	127.5 ^c	313.2 ^d
+ SO_4^{2-}	1.7 ^d	7.0 ^d	18.7 ^c	8.2 ^c	54.0 ^d	144.0 ^e

of methane in rice straw-amended soil under flooded conditions. Abundant production of methane during anaerobic decomposition of rice straw in flooded soils was reported before (Acharya, 1935). Rice straw as a carbon source can increase the electron donor to electron acceptor ratio, thereby reducing the time period for the reduction of oxidants and intensity of the competition for the electron donors among the various groups of microorganisms. In the present study, the net CH_4 production in rice straw-amended soil samples treated with different inorganic redox species, as in unamended soil, was significantly inhibited. Particularly interesting was the increased percentage of inhibition by addition of different inorganic redox species in soil samples amended with rice straw, relative to that of rice straw-amended soil samples without any addition of redox species at 10 d of incubation (Table 1). This strongly suggests that the intensity of competition for electron donors among these microorganisms was severe, despite the fact that addition of rice straw prevented the suppression of CH_4 production to some extent, compared with the corresponding unamended soil samples. A similar observation was made before by Kluber and Conrad (1998) that addition of rice straw to reduce competition for electron donors decreased the inhibition period only and did not prevent the inhibition of methanogenesis after addition of nitrate.

In anaerobic mineralization, organic carbon is transformed into methane or carbon dioxide, depending on the availability of electron acceptors. The anaerobic respiration by the nitrate, ferric iron, and sulfate reducers can channel the flow of electrons toward CO_2 production. In the present study, the rate of increase in CO_2 evolution from the unamended soil sample, without any addition of redox species, was lesser during the incubation period. On the contrary, the evolution of CO_2 from soil samples added with different redox species and rice straw-amended soil samples, with or without addition of different inorganic redox species, invariably increased in time (data not provided). Irrespective of the presence or absence of rice straw, soil samples amended with different redox species registered higher redox potentials (-81 to -172 mV) than soil samples not amended with inorganic redox species ($-175/-115$ and -260 mV in unamended and rice straw-amended samples, respectively; Table 2). According to Wang et al. (1993), CH_4

Table 2. Changes on the redox potential (Eh) and methanogenic (H_2 and CO_2 utilizers) population in soil samples, unamended or amended with rice straw (1% w/w), as influenced by different inorganic redox species (added at 2500 mg kg^{-1} soil). Redox potential measurements and methanogenic population density enumeration were done with samples after 30 d of incubation. Most probable number (MPN) estimates of methanogens were obtained from MPN culture tubes after 30 d of incubation.

Treatment	Redox potential		Methanogenic population	
	Without rice straw	With rice straw	Without rice straw	With rice straw
	mV		$\times 10^5$ MPN g^{-1} dry soil	
Control	-115	-261	24	61
+ NO_3^-	-102	-145	11	43
+ Mn^{4+}	-81	-110	16	18
+ Fe^{3+}	-116	-132	16	25
+ SO_4^{2-}	-97	-172	3	9

formation in flooded soils can occur when the redox potential (Eh) falls below -150 mV. However, the production of CH_4 in *Methanosarcina barkeri* starts as soon as the redox potential of the medium drops below $+50$ mV (Fetzer and Conrad, 1993). There are suppositions that only biogeochemical reactions whose oxidation potentials match the redox potential of a given environmental niche can operate. But, the environmental redox potential is only a symptom, as well as a measure of the dominant chemical oxidizing and reducing species present that can undergo the redox reactions (Ehrlich, 1993). Methanogenic population, enumerated by incubating the most probable number (MPN) culture tubes for a brief period of 30 d, was also suppressed by the inorganic redox species in rice straw-amended soil (Table 2). Evidently, under oxidized conditions in soil samples amended with the inorganic redox species, the toxic and/or the competitive effects were not congenial for the activities and proliferation of methanogens.

Methane Oxidation

In another experiment, the effect of different inorganic redox species on methane oxidation, measured as the decrease in the headspace concentration of methane in soil samples held at 60% water holding capacity, was examined under aerobic conditions. The headspace concentration of methane decreased rapidly both in the control and in soil samples treated with inorganic redox species (Fig. 1). Addition of SO_4^{2-} and Mn^{4+} enhanced the oxidation of methane while NO_3^- was slightly inhibitory during the initial days. Jugsujinda et al. (1995) demonstrated that there was no possibility of NO_3^- -N as an oxygen source for CH_4 oxidation, by using methyl fluoride, a CH_4 oxidation inhibitor. We also monitored the effect of these redox substances on total aerobic heterotrophic bacteria, total methanotrophs, and methane oxidizing population with sMMO activity in soil samples. Total aerobic bacterial population (colony forming units [cfu] $\times 10^7 g^{-1}$ air-dried soil) decreased in soil samples treated with Fe_2O_3 and K_2SO_4 while it increased in soil samples with KNO_3 and MnO_2 (Table 3). These differences were probably due to the effects of different redox substances under the atmosphere of methane in the headspace. In another recent study, methane was found to be an electron donor for denitrification in oxygen-limited bioreactors, with concomitant increase in the population of denitrifying bacteria (Costa et al., 2000). Likewise, additions of MnO_2 and Fe_2O_3 to soil samples under the atmosphere of methane resulted in increase of total methanotrophic- and sMMO-bearing methanotrophic populations, respectively (Table 3). The sulfate species had repressing effects on the population of methane oxidizers with sMMO activity as well as total methanotrophs.

In a follow-up experiment, soil samples were treated

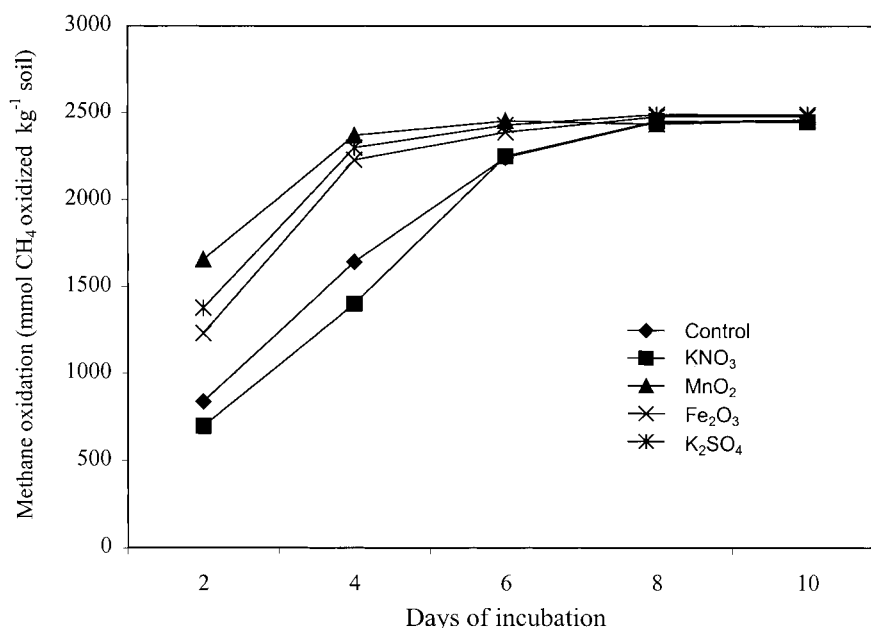


Fig. 1. Time course of methane oxidation (mmol of net CH_4 oxidized kg^{-1} air-dried soil) in soil samples treated with different inorganic redox substances and incubated under aerobic conditions. The data are means of triplicate measurements. For clarity, error bars are not shown for the treatments, but typically were in the order of CV = $<20\%$.

Table 3. Population of total heterotrophic bacteria, total methanotrophs, and soluble methane monooxygenase (sMMO)-bearing methanotrophs in soil samples treated with different inorganic redox species (added at 2500 mg kg⁻¹ soil). Data represent mean values of three replicate plates for each dilution tested or five most probable number (MPN) tubes for each dilution tested. Analyses were done using soil samples after 13 d of incubation.

Treatment	Total aerobic heterotrophic bacteria	Total methanotrophs	Methanotrophs with sMMO activity
	× 10 ⁷ colony forming unit g ⁻¹ dry soil	× 10 ⁶ MPN estimate g ⁻¹ dry soil	× 10 ⁵ colony forming unit g ⁻¹ dry soil
Control	18	13	20
+ NO ₃ ⁻	22	10	15
+ Mn ⁴⁺	26	34	18
+ Fe ³⁺	13	11	30
+ SO ₄ ²⁻	10	8	17

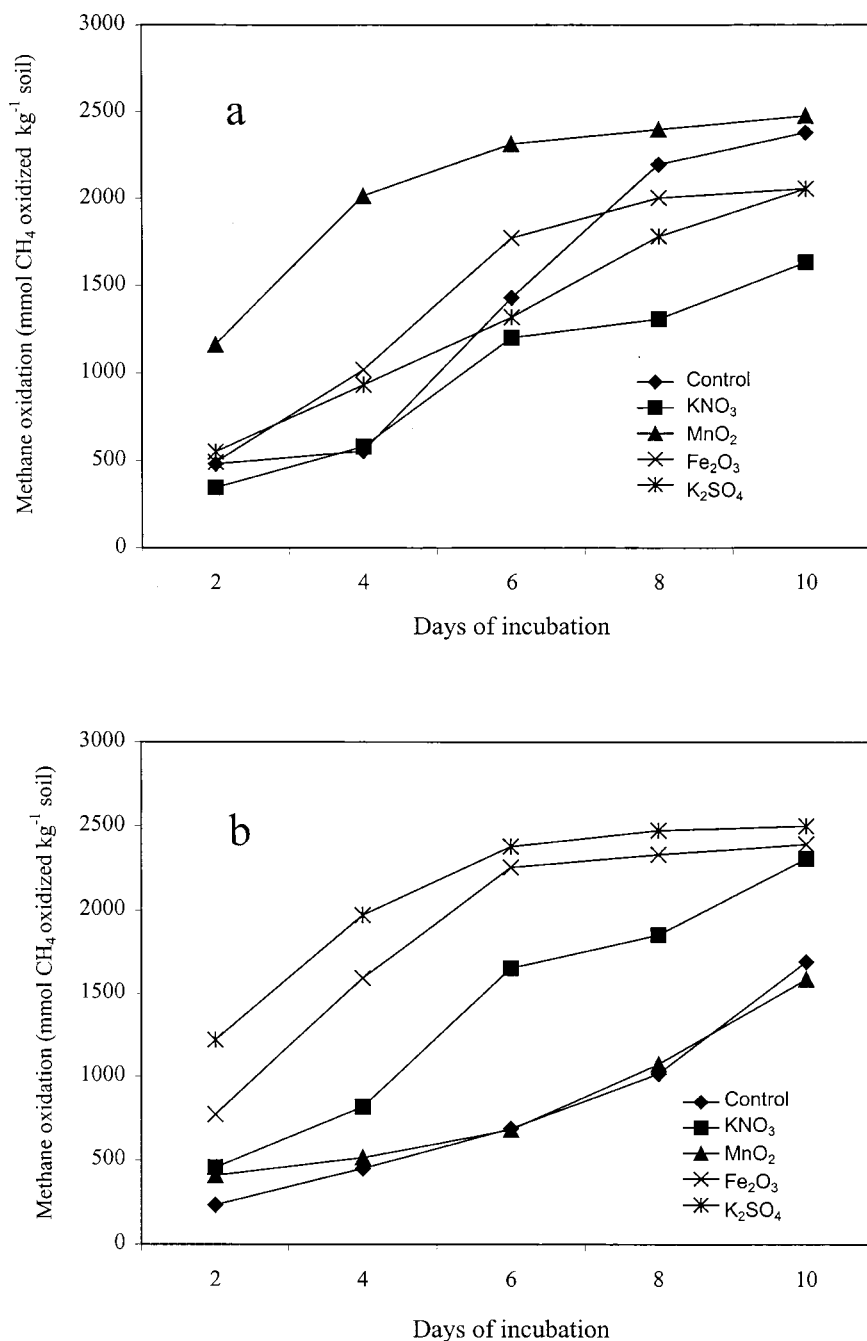


Fig. 2. Methane oxidation (mmol of net CH₄ oxidized kg⁻¹ air-dried soil) in a soil solution equivalent medium supplemented with different inorganic redox species separately, inoculated with samples pretreated with respective inorganic redox species and incubated under (a) aerobic and (b) anoxic conditions. The data are means of six replicate measurements. For clarity, error bars are not shown for the treatments, but typically were in the order of CV = <20%.

with different inorganic redox substances separately and provided with methane as substrate in the headspace for 10 d. Then, the aliquots of soil slurries from the control and those pretreated with different redox substances were tested for their ability to oxidize methane in a soil solution equivalent (culture) medium, in the presence of respective redox species under both aerobic (ambient) and anaerobic (argon atmosphere) conditions. As in the previous experiment, Mn^{4+} appeared to enhance methane oxidation in soil solution under aerobic conditions (Fig. 2a). On the other hand, addition of NO_3^- retarded the oxidation of methane in soil solution equivalent medium. Interestingly, methane oxidation proceeded rapidly also under anaerobic conditions (argon atmosphere). But, the effects of different redox species on methane oxidation under anaerobic conditions (Fig. 2b) distinctly differed from that of aerobic conditions (Fig. 2a). Nitrate stimulated methane oxidation under anaerobic conditions and retarded it under aerobic conditions. Manganese(IV) ion retarded methane oxidation under anaerobic conditions, but enhanced it under aerobic conditions. Iron(III) was stimulatory to methane oxidation under anaerobic, but not under aerobic conditions. Interestingly, SO_4^{2-} effected very rapid oxidation of methane in soil solution equivalent medium under both aerobic and anaerobic conditions. Presently, the reasons for differential effects of different redox substances on methane oxidation under aerobic and anaerobic conditions are not clear.

Rapid methane oxidation even under anaerobic conditions merits discussion. There is evidence, but not very conclusive, to suggest that anaerobic methane oxidation can be considerable in marine environments (Alperin and Reeburgh, 1985) and in rice soils, too (Murase and Kimura, 1994). We observed that methane oxidation in soil solution equivalent medium (with or without different redox species) was considerable even under anaerobic conditions (Fig. 2b). In addition, methane oxidation was found to be distinctly stimulated by SO_4^{2-} and Fe^{3+} in anaerobically incubated soil solution medium. Pure cultures of sulfate reducers could also oxidize methane under anaerobic conditions, but only in the presence of SO_4^{2-} as an additional electron acceptor (Panganiban et al., 1978). Likewise, addition of SO_4^{2-} stimulated anaerobic methane oxidation in enrichment cultures (Iversen and Joergensen, 1985), as noticed in soil solution equivalent medium in the present study. Geochemical evidence suggests that anaerobic methane oxidation is associated with SO_4^{2-} , Fe^{3+} , and NO_3^- reduction (Miura et al., 1992; Murase and Kimura, 1994). Anaerobic methane oxidation may involve the back reaction of methane to CO_2 and H_2 in methanogens (Zehnder and Brock, 1979). Lipid biomarker and phylogenetic studies using marine sediments show the involvement of archaea and sulfate-reducing bacteria in anaerobic methane oxidation (Valentine and Reeburgh, 2000). Recently, Hinrichs et al. (1999) and Orphan et al. (2001) provided evidence from studies of anoxic marine sediments that methane is being consumed by archaea that are phylogenetically distinct from known methanogens. Thus, the recognition of methanotrophy also as a mode of growth can provide

new perspectives on archaeal physiology and ecology. The results of the present study provide further evidence for the involvement of anaerobic methane oxidation, which can be considerable depending upon the dominant species of inorganic redox substances in flooded rice soils. In summary, we conclude that addition of different inorganic redox substances to soil samples suppressed methane production and had differential effects on aerobic and anaerobic methane oxidation.

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REFERENCES

- Acharya, C.N. 1935. Studies on the anaerobic decomposition of plant materials. I. The anaerobic decomposition of rice straw (*Oryza sativa*). *Biochem. J.* 29:528–541.
- Achtmich, C., F. Bak, and R. Conrad. 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. *Biol. Fertil. Soils* 19:65–72.
- Alperin, M.J., and W.S. Reeburgh. 1985. Inhibition experiments on anaerobic methane oxidation. *Appl. Environ. Microbiol.* 50:940–945.
- Angle, J.S., S.P. McGrath, and R.S. Chaney. 1991. New culture medium containing ionic concentration of nutrients similar to concentrations found in the soil solution. *Appl. Environ. Microbiol.* 57:3674–3676.
- Arif, S.M.A., F. Houwen, and W. Verstraete. 1996. Agricultural factors affecting methane oxidation in arable soil. *Biol. Fertil. Soils* 21:95–102.
- Bodelier, P.L.E., and P. Frenzel. 1999. Contribution of methanotrophic and nitrifying bacteria to CH_4 and NH_4^+ oxidation in the rhizosphere of rice plants as determined by new methods of discrimination. *Appl. Environ. Microbiol.* 65:1826–1833.
- Bollag, J.M., and S.T. Czlonkowski. 1973. Inhibition of methane formation in soil by various nitrogen-containing compounds. *Soil Biol. Biochem.* 5:673–678.
- Bosse, U., and P. Frenzel. 1997. Activity and distribution of methane-oxidizing bacteria in flooded rice soil microcosms and in rice plants (*Oryza sativa*). *Appl. Environ. Microbiol.* 63:1199–1207.
- Conrad, R., F.S. Lupton, and J.G. Zeikus. 1987. Hydrogen metabolism and sulfate-dependent inhibition of methanogenesis in a eutrophic lake sediment (Lake Mendota). *FEMS Microbiol. Ecol.* 45:107–115.
- Costa, C., C. Dijkema, M. Friedrich, P. Garcia-Encina, F. Fernandez-Polanco, and A.J.M. Stams. 2000. Denitrification with methane as electron donor in oxygen-limited bioreactors. *Appl. Microbiol. Biotechnol.* 53:754–762.
- De Bont, J.A.M., K.K. Lee, and D.F. Bouldin. 1978. Bacterial oxidation of methane in a rice paddy. *Ecol. Bull.* 26:91–96.
- Ehrlich, H.L. 1993. Bacterial mineralization of organic carbon under anaerobic conditions. p. 219–247. *In* J.M. Bollag and G. Stotzky (ed.) *Soil biochemistry*, Vol. 8. Marcel Dekker, New York.
- Fetzer, S., and R. Conrad. 1993. Effect of redox potential on methanogenesis by *Methanosarcina barkeri*. *Arch. Microbiol.* 160:108–113.
- Graham, D.W., D.G. Korich, R.P. LaBlanc, N.A. Sinclair, and R.G. Arnold. 1992. Applications of a colorimetric plate assay for soluble methane monooxygenase activity. *Appl. Environ. Microbiol.* 58:2231–2236.
- Hinrichs, K.-U., J.M. Hayes, S.P. Sylva, P.G. Brewer, and E.F. DeLong. 1999. Methane-consuming archaeobacteria in marine sediments. *Nature* 398:802–805.
- International Rice Research Institute. 1993. IRRISTAT Version 3/93. IIRI, Los Banos, Philippines.
- Iversen, N., and B.B. Joergensen. 1985. Anaerobic methane oxidation rates at the sulphate–methane transition in marine sediments

- from Kattegard and Skagerrack (Denmark). *Limnol. Oceanogr.* 30:944–955.
- Jugsujinda, A., R.D. DeLaune, and C.W. Lindau. 1995. Influence of nitrate on methane production and oxidation in flooded soil. *Commun. Soil Sci. Plant Anal.* 26:2449–2459.
- Kaspar, H.F., and J.M. Tiedje. 1982. Anaerobic bacteria and processes. p. 987–1009. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Kluber, H.D., and R. Conrad. 1998. Effects of nitrate, nitrite, NO and N₂O on methanogenesis and other redox processes in anoxic rice field soil. *FEMS Microbiol. Ecol.* 25:301–318.
- Kumaraswamy, S., B. Ramakrishnan, A.K. Rath, S.R. Misra, V.R. Rao, and N. Sethunathan. 1997. Spatial distribution of methane-oxidizing activity in flooded rice soil. *Plant Soil* 191:241–248.
- Kumaraswamy, S., A.K. Rath, B. Ramakrishnan, and N. Sethunathan. 2000. Wetland rice soils as sources and sinks: A review and prospects for research. *Biol. Fertil. Soils* 31:449–461.
- Lovely, D.R. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* 55:259–287.
- MacGregor, A.N., and D.R. Keeney. 1973. Methane formation by lake sediments during in vitro incubation. *Water Resour. Bull.* 9: 1153–1158.
- Minami, K., and H.U. Neue. 1994. Rice paddies as a methane source. *Climate Change* 27:13–26.
- Miura, Y., A. Watanabe, J. Murase, and M. Kimura. 1992. Methane production and its fate in paddy fields II. Oxidation of methane and its coupled ferric oxide reduction in subsoil. *Soil Sci. Plant Nutr.* 38:673–679.
- Murase, J., and M. Kimura. 1994. Methane production and its fate in paddy fields. IV. Sources of microorganisms and substrates responsible for anaerobic methane oxidation in subsoil. *Soil Sci. Plant Nutr.* 40:57–61.
- Neue, H.U., L.H. Ziska, R.B. Matthews, and Q. Dai. 1995. Reducing global warming—The role of rice. *GeoJournal* 35:351–362.
- Orphan, V.J., K.-U. Hinrichs, W. Ussler III, C.K. Paull, L.T. Taylor, S.P. Sylva, J.M. Hayes, and E.F. Delong. 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Appl. Environ. Microbiol.* 67:1922–1934.
- Panganiban, A.T., T.E. Patt, Jr., W. Hart, and R.S. Hanson. 1978. Oxidation of methane in the absence of oxygen in lake water samples. *Appl. Environ. Microbiol.* 37:303–309.
- Peters, V., and R. Conrad. 1996. Sequential reduction processes and inhibition of CH₄ production upon flooding of oxic upland soils. *Soil Biol. Biochem.* 28:371–382.
- Ponnamperuma, F.N. 1972. The chemistry of submerged soils. *Adv. Agron.* 24:29–96.
- Ramakrishnan, B., S. Kumaraswamy, K. Mallick, T.K. Adhya, V.R. Rao, and N. Sethunathan. 1998. Effect of various anionic species on net methane production in flooded rice soils. *World J. Microbiol. Biotechnol.* 14:743–749.
- Rand, M.C., A.E. Greenberg, M.J. Taras, and M.A. Franson. 1975. Standard methods for the examination of water and wastewater. Am. Public Health Assoc., Washington, DC.
- Rao, D.N., and D.S. Mikkelsen. 1977. Effect of rice straw addition on methane production and organic acids in a flooded soil. *Plant Soil* 47:303–311.
- Roy, R., and R. Conrad. 1999. Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiol. Ecol.* 49:49–61.
- Schnell, S., and G.M. King. 1995. Stability of methane oxidation capacity to variations in methane and nutrient concentrations. *FEMS Microbiol. Ecol.* 17:285–294.
- Stotzky, G. 1965. Microbial respiration. p. 1550–1572. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Valentine, D.L., and W.S. Reeburgh. 2000. New perspectives on anaerobic methane oxidation. *Environ. Microbiol.* 2:477–484.
- Wang, Z.P., C.W. Lindau, R.D. DeLaune, and W.H. Patrick, Jr. 1993. Methane emission and entrapment in flooded rice soils as affected by soil properties. *Biol. Fertil. Soils* 16:163–168.
- Zehnder, A.J.B., and T.D. Brock. 1979. Methane formation and methane oxidation by methanogenic bacteria. *J. Bacteriol.* 137:420–432.