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Characterization of microbial NO production, N_2O production and CH_4 oxidation initiated by aeration of anoxic rice field soil

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Abstract. Intermittent drainage of rice fields is discussed as an option to mitigate emission of CH_4, an important greenhouse gas. However N_2O, a potentially more effective greenhouse gas, may be emitted during the aeration phase. Therefore, the metabolism of NO, N_2O, NH_4^+, NO_2^- and NO_3^- and the kinetics of CH_4 oxidation were measured after aeration of methanogenic rice field soil. Before aeration, the soil contained NH_4^+ in relatively high concentrations (about 4 mM), while NO_2^- and NO_3^- were almost undetectable. Immediately after aeration both NO and N_2O were produced with rates of about 15 pmol h^{-1} gdw^{-1} and 5 pmol h^{-1} gdw^{-1}, respectively. Simultaneously, NH_4^+ decreased while NO_2^- accumulated. Later on, NO_2^- was depleted while NO_3^- concentrations increased. Characteristic phases of nitrogen turnover were associated with the activities of ammonium oxidizers, nitrite oxidizers and denitrifiers. Oxidation of NH_4^+ and production of NO and N_2O were inhibited by 10 Pa acetylene demonstrating that nitrification was obligatory for the initiation of nitrogen turnover and production of NO and N_2O. Ammonium oxidation was not limited by the available NH_4^+ and thus, concomittant production of NO and N_2O was not stimulated by addition of NH_4^+. However, addition of NO_3^- stimulated production of NO and N_2O in both anoxic and aerated rice soil slurries. In this case, 10 Pa acetylene did not inhibit the production of NO and N_2O demonstrating that it was due to denitrification which was obviously limited by the availability of NO_3^-. In the aerated soil slurries CH_4 was only oxidized if present at elevated concentrations (>50 ppmv CH_4). At atmospheric CH_4 concentrations (∼1.7 ppmv) CH_4 was not consumed, but was even slightly produced. CH_4 oxidation activity increased after preincubation at 20% CH_4, and then CH_4 was also oxidized at atmospheric concentrations. CH_4 oxidation kinetics exhibited sigmoid characteristics at low CH_4 concentrations presumably because of inhibition of CH_4 oxidation by NH_4^+.

1. Introduction

Rice fields are estimated to contribute 100 ± 50 Tg a^{-1} (Schütz et al. 1989) of the greenhouse gas CH_4 to a total global production of about 540 Tg a^{-1} (Prinn 1994; Cicerone & Oremland 1988). With a growing demand for rice, strategies to reduce CH_4 emission from paddy fields are needed. Intermittent drainage of flooded rice field soils is discussed as an option to mitigate CH_4 emission

(Kimura et al. 1991; Sass et al. 1992; Yagi et al. 1996). Methanogenesis in Italian rice soil slurries and vegetated microcosms stopped immediately following a brief aeration or drainage, respectively (Ratering & Conrad 1997). CH_4 production in paddy soil is inhibited by O_2 (Fetzer et al. 1993) and by electron acceptors such as Fe^{3+} and SO_4^{2-} (Achtnich et al. 1995) which are produced upon aeration or drainage (Ratering & Conrad 1997). Methane emission is also influenced and regulated by CH_4 oxidation. Oxic upland soils are a major sink of atmospheric CH_4 (Conrad 1995). Anoxic wetland soils, on the other hand, are usually sources for atmospheric CH_4. It is unclear whether they may act as a temporary sink for atmospheric CH_4 after drainage (Thurlow et al. 1995). Up to 80% of the CH_4 produced in anoxic flooded soil is oxidized by methanotrophs in the upper 2 mm layer, where O_2 is present (Conrad & Rothfuss 1991). However, this CH_4 oxidation takes place at micromolar concentrations, i.e. much higher than atmospheric concentrations. Bender & Conrad (1992) showed that oxidation of atmospheric CH_4 is possible in rice field soil if the soil is not water-saturated. Therefore, oxidation of atmospheric CH_4 during the brief drained periods of a rice field seems possible and would further reduce the total amount of CH_4 emitted by these fields.

However, during the drainage N_2O, an even more severe greenhouse gas than CH_4, is emitted (Freney & Denmead 1992; Byrnes et al. 1993; Ratering & Conrad 1997). The microbial processes responsible for N_2O emission from aerated paddy soil have not been identified. Frequent changes between oxic and anoxic conditions in soils are favorable for nitrogen loss. Reddy & Patrick (1975, 1976) attributed this loss of nitrogen in rice field soil to alternate nitrification and denitrification, i.e. to NO_3^- production by nitrification during the aerobic phase and denitrification of NO_3^- to gaseous nitrogen compounds during the anaerobic phases.

Basically all microbial processes that involve oxidation or reduction of N-compounds through the +1 or +2 state yield N_2O and NO at least in trace amounts (Conrad 1996a; Williams et al. 1992). Processes like denitrification, dissimilatory nitrate reduction to ammonia, autotrophic and heterotrophic nitrification were shown to produce NO and N_2O (Conrad 1996a). It is generally accepted that denitrification and nitrification are the main microbial sources for NO and N_2O. Chemodenitrification, a major source of NO in acidic soils, is thought to play only a minor role in neutral agricultural soils (Chalk & Smith 1983; Van Cleemput et al. 1976).

Denitrification is an anaerobic and heterotrophic process. It is dependent on anoxic conditions, the availability of NO_3^- as electron acceptor and organic carbon as electron donor. NO and N_2O are intermediates during the reduction of NO_3^- to N_2, and can be both produced and consumed.

Nitrification, on the other hand, is a strictly aerobic and chemolithotrophic process catalyzed by two different bacterial groups. The ammonia oxidizers oxidize NH_4^+ in a two step reaction via hydroxylamine to NO_2^-, while the nitrite oxidizers oxidize NO_2^- to NO_3^-. NO and N_2O result from a process called nitrifier-denitrification, in which under O_2-limiting conditions NO_2^- instead of O_2 is reduced to NO, N_2O and even N_2 (Poth & Foch 1985; Poth 1986; Remde & Conrad 1989). Nitrification and denitrification can take place simultaneously in microbial communities in soil and water, in bacterial co-cultures and even in bacterial pure cultures (Kuenen & Robertson 1994; Conrad 1996a).

To characterize the microbial sources of NO and N_2O and the potential for CH_4 oxidation in rice field soil, we measured the turnover of NO and N_2O, NH_4^+, NO_2^- and NO_3^- as well as the oxidation of different CH_4 concentrations in aerated rice soil slurries that had previously been methanogenic.

2. Methods

The paddy soil originated from a rice field in Vercelli, Italy, of which the location and soil characteristics have been described by Holzapfel-Pschorn and Seiler (1986). The soil was collected from the drained and ploughed field during the winter season, was air-dried and stored at room temperature as described by Conrad et al. (1987). Subsequently, the soil was used to grow rice plants in flooded vats in a greenhouse. After harvest, the plants were removed and the soil was drained, air-dried, homogenized and passed through a screen (mesh $\leq$ 2 mm). NH_4^+, NO_2^-, NO_3^- were extracted from the soil with 1 M KCl and analyzed colorimetrically according to the methods described by Kandeler and Gerber (1988), Keeney and Nelson (1982) and Schlichting and Blume (1966), respectively. Soil organic carbon was analyzed by wet combustion (Schlichting & Blume 1966). The dry rice field soil contained per gram dry weight (gdw): 18.35 mg organic C, 1.02 μmol NH_4^+ and 0.55 μmol NO_3^-. NO_2^- was not detectable. To determine the soil dry weight, soil and slurry samples were dried at 105 °C (Schlichting & Blume 1966).

Soil-water-slurries 1:1 (w/v) were incubated (25 °C or 30 °C, dark) anaerobically in glass flasks (1.1 l) under N_2. After 30 days, when CH_4 was produced vigorously, subsamples were taken from the anoxic slurry and used for the experiments. In general, subsamples were transferred into stoppered flasks and flushed with synthetic air (20.5% O_2, 79.5% N_2). Gas samples (0.2–1 ml) were repeatedly taken over time with gas-tight pressure-lock syringes (Dynatech A-2 Series, Baton Rouge, Louisiana, USA) and analyzed by gas chromatography (GC). The O_2 content was daily analyzed by GC with a thermal conductivity detector (Shimadzu GC 8A, Kyoto, Japan). The O_2

concentration was kept constant at 20.5% by injections of pure O_2. N_2O was analyzed by a Carlo Erba GC 8000 with ^{63}Ni electron capture detector (Fisons Instruments, Mainz-Kastel, Germany) and a stainless steel column (4 m; ⌀ = 1/8") filled with HayeSep-N (80/100 mesh). A stainless steel precolumn (30 cm; ⌀ = 1/8") filled with Natron-asbestos was used to absorb interfering CO_2. NO was analyzed with a Thermo Electron chemoluminescent NO analyzer (14 BE, Hopkinton, MA, USA) (Remde & Conrad 1991). CH_4 was analyzed with a Carlo Erba GC (GC 6000 Vegaseries 2, Carlo-Erba-Instruments, Milano, Italy) equipped with a flame ionization detector and a stainless steel column (3 m, ⌀ = 3 mm) filled with Poropak Q (mesh 80/110).

NO and N_2O production were measured in 120 ml serum bottles with 5 or 14 ml slurry or in glass flasks (1.1 l volume) containing 50 ml slurry. NH_4^+, NO_2^- and NO_3^- concentrations were measured in the pore water of slurry subsamples (5 ml) taken through an outlet of 1.1 l glass flasks with 80 ml slurry. The slurry subsamples were centrifuged, the pore water was filtered through 0.2 μm membrane filters (regenerated cellulose; Sartorius, Göttingen, Germany) and stored frozen (-20 °C) until analysis. NO_2^- and NO_3^- were analyzed by ion chromatography (Bak et al. 1991). NH_4 was analyzed colorimetrically (Kandeler & Gerber 1988).

To distinguish between nitrification and denitrification as source of NO and N_2O, the acetylene inhibition method was applied (Klemedtsson et al. 1988) using soil slurries incubated in serum bottles. Experiments showed that 10 Pa acetylene completely inhibited the activity of the ammonia oxidizers, but did not inhibit the N_2O reductase of the denitrifiers. Thus, 10 Pa acetylene were added to the serum bottles 1–2 h prior to aeration to inhibit nitrification. After aeration, 10 Pa acetylene were again added to the serum bottles.

To determine a possibly stimulating effect on the nitrifying and denitrifying community, ammonium (NH_4Cl) or nitrate (KNO_3) were added to obtain concentrations of 50 μg N gdw^{-1}. This amount of nitrogen corresponds approximately to the amount of fertilizer (90 kg N ha^{-1}) applied to rice fields (Byrnes et al. 1993).

The NO compensation point was determined by NO uptake measurements using 120 ml serum bottles at 25 °C and 2 ml (1.4 gdw soil) slurry in 9 parallels. Elevated NO concentrations of 1 ppmv were adjusted in the bottles and the NO consumption was measured until no further decrease in NO concentration occurred. The NO consumption rate constant and NO compensation concentrations were determined by non-linear curve fitting to an exponential consumption model (Seiler et al. 1977; Bollmann et al. 1995) using Origin 4.1 (Microcal).

Methane oxidation was measured in soil slurries using 120 ml serum bottles. After aeration CH_4 was added to the bottles to give concentrations ranging between 1.7 ppmv (atmospheric) and 50,000 ppmv CH_4. Oxidation rates were calculated according to Bender & Conrad (1992). CH_4 oxidation in soil slurries was induced with 20% CH_4 in air for $>$100 h (Bender & Conrad 1992). Since the oxidation rates were possibly limited by gas diffusion, oxidation rates were measured in different volumina and dilutions of the soil slurry at 500 ppmv CH_4. The oxidation rates were directly proportional up to 0.25 gdw soil used, independently of the volumina of the soil slurries that were varied between 2 and 5 ml. Routinely, CH_4 oxidation rates were measured using 4 ml slurry, diluted 1:30 (equivalent to 0.17 ± 0.03 gdw soil). All bottles were incubated in triplicate at 25 °C or 30 °C in the dark on a horizontal shaker. The kinetic parameters of the CH_4 oxidation were determined by non-linear curve fitting of the Hill function with Origin 4.1 (Microcal).

3. Results

3.1 *Metabolism of NO and N_2O in anoxic rice soil slurry*

In freshly prepared rice soil slurries that were incubated under anoxic conditions CH_4 production started after 2–3 days. However, a vigorous and linear CH_4 production did not start before day 15, but was then maintained at a constant rate over 100 days. Subsequently CH_4 production slightly decreased, but CH_4 was still produced after more than 240 days.

During 100 d of anoxic incubation the NO_3^- concentration decreased in the soil slurries from 551 ± 268 μm to 4.5 ± 1.7 μm. The final NO_3^- concentration was apparently below the threshold of the nitrate-utilizing microorganisms. While NO_3^- was almost completely consumed, NH_4^+ increased from an initial concentration of approximately 1.1 mM to a final concentration of 4 mM, measured after extraction with KCl solution.

Subsamples of the methanogenic slurry were taken after day 30 and further incubated under anoxic conditions. The slurries were completely reduced and produced CH_4 at a maximum rate of about 47 nmol h^{-1} gdw^{-1} CH_4 (Figure 1). This methanogenic rice soil slurry did not show any nitrification or denitrification under anoxic conditions (Figure 1). The NO concentration fluctuated around the detection limit ($\sim$25 ppbv) and N_2O was not detectable at all ($<$20 ppbv). During the incubation time of 11 days there was also no change in the NH_4^+ and NO_3^- concentrations which stayed at about 1.3 mM and 5 μM, respectively, measured in pore water without extraction.

However, addition of nitrate to the anoxic soil slurries resulted in immediate NO and N_2O production (Figure 2). NO was produced with a maximum

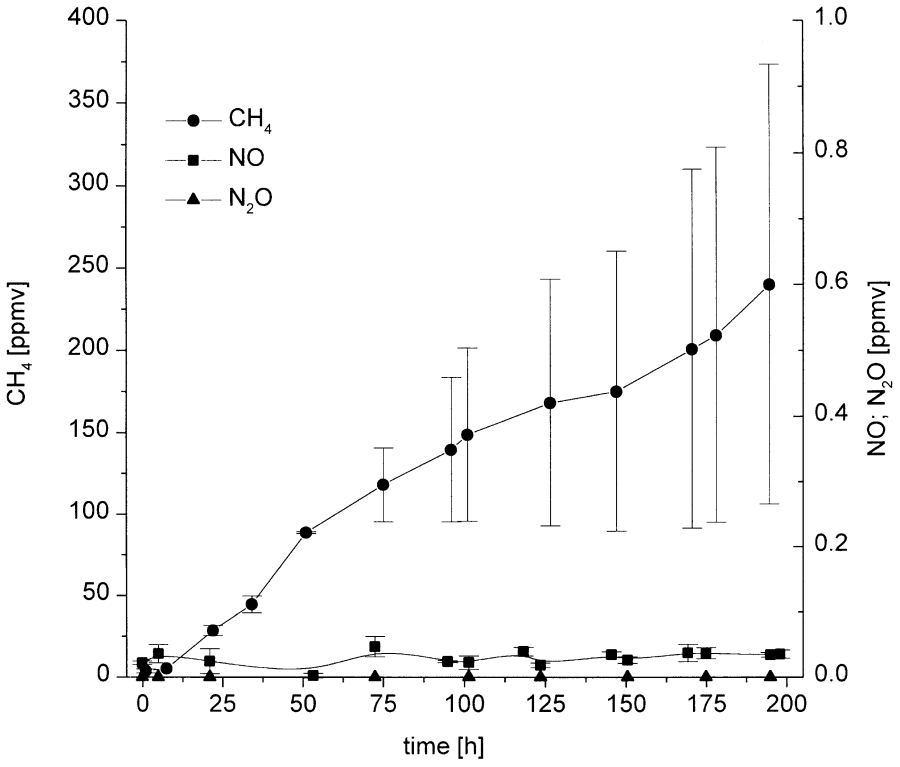

Figure 1. Production of CH_4, NO and N_2O in anoxic methanogenic slurries of Italian rice field soil incubated at 30 °C. Mean ± SD, $n = 3$.

rate of 130±10 pmol h^{-1} gdw^{-1} and N_2O with 1520±250 pmol h^{-1} gdw^{-1}. Production of both NO and N_2O started immediately, but NO production reached the maximum faster than N_2O production. After about 100 h the accumulated NO and N_2O decreased again, indicating consumption.

3.2 *Metabolism of NO and N_2O in aerated rice soil slurry*

Other subsamples of the methanogenic slurry were taken after day 30 and further incubated under oxic conditions. After aeration NO and N_2O were produced immediately (Figure 3). NO was released with a rate of 15±1 pmol h^{-1} gdw^{-1}. NO accumulated, reached a maximum at about 150 h and then decreased. The NO concentration reached a final constant value of 0.1 ppmv, indicating a possible NO compensation point. When aerated soil slurries were incubated with elevated NO concentrations (∼1 ppmv), NO decreased exponentially and again reached a final concentration of about 0.1 ppmv. Fitting of the NO decrease to an exponential consumption model

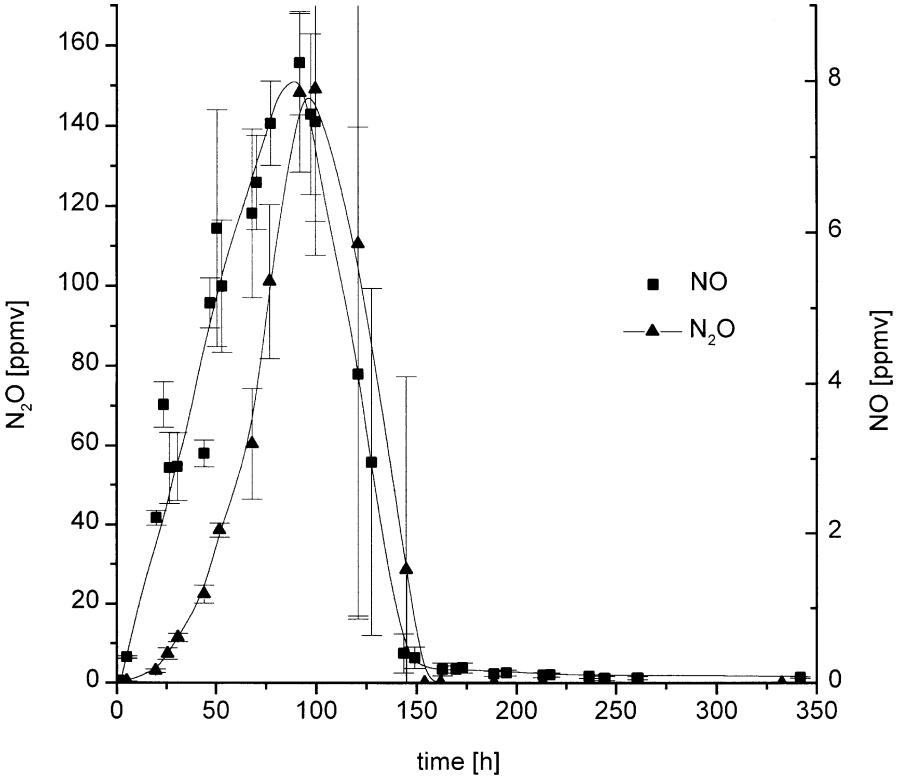

Figure 2. Production of NO and N_2O in anoxic methanogenic soil slurries (25 °C) after the addition of NO_3^- (50 μg N gdw^{-1}). Mean ± SD, n = 3.

(Bollmann et al. 1995; Seiler et al. 1977) resulted in NO consumption rate constants (average ± SD) of 7.9±0.3 cm^3 h^{-1} gdw^{-1} and NO compensation concentrations of 0.11±0.01 ppmv.

N_2O was produced after a lag of about 7 h with a rate of 4.8±0.2 pmol h^{-1} gdw^{-1} (Figure 3). After about 400 h the N_2O production stopped, resulting in a constant concentration. In contrast to the anoxic slurries N_2O was not consumed in the aerated slurries.

When 10 Pa acetylene was applied to aerated slurries, no change in the initial concentrations of NH_4^+ and NO_3^- were observed and NO_2^- was not produced (data not shown). NO and N_2O were also not produced (Figure 3). A slight NO production after 24 h aeration was recognized in a few serum bottles, but repetition of the measurements showed a complete inhibition of NO production by 10 Pa acetylene. Hence, the initial NO and N_2O production in the inhibited control flasks must have resulted from nitrification. Nitrification seemed to be the obligatory process for nitrogen turnover in aerated soil slurries.

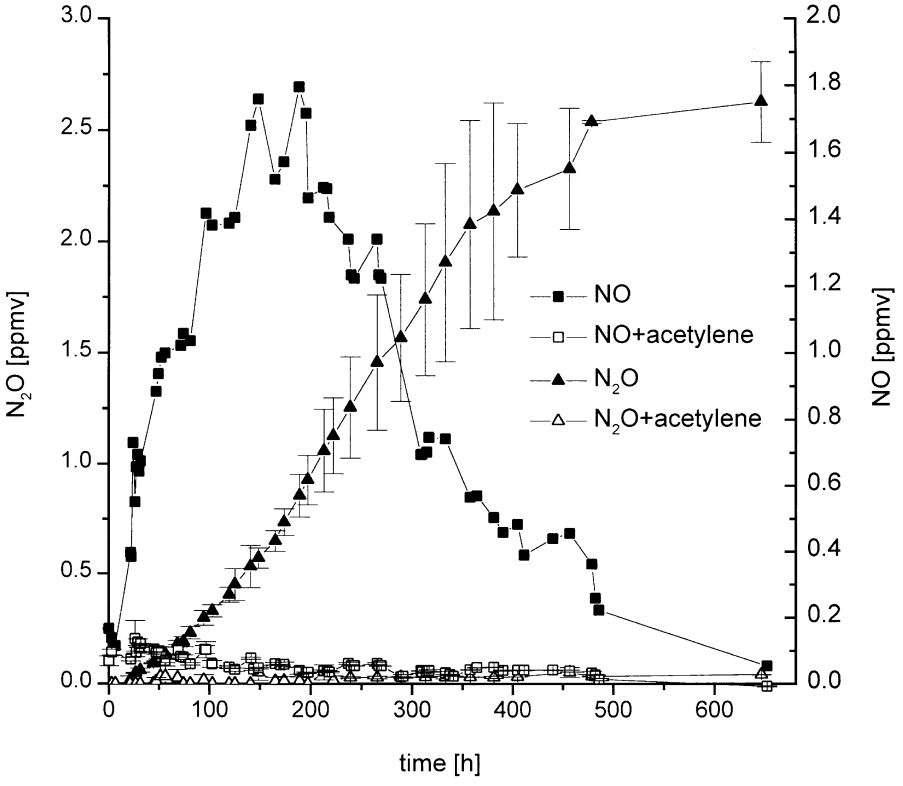

Figure 3. Production of NO and N_2O in aerated soil slurries (25 °C) in presence (open symbols) and absence of 10 Pa acetylene. Mean ± SD; SD for NO were about 0.4 ppmv and are not shown for clarity.

During the oxic incubation NH_4^+ in the soil slurries decreased steadily with a rate of 1.2±0.2 nmol h^{-1} gdw^{-1} (Figure 4), while NO and N_2O were released (Figure 3). After about 150 h a drastic increase of the oxidation rate of NH_4^+ to a value of 6.6±0.2 nmol h^{-1} gdw^{-1} occurred. NH_4^+ was subsequently consumed below the detection limit. NO_2^- was not detectable at the beginning of the aeration, but accumulated as NH_4^+ was oxidized. The rate of NO_2^- production was approximately 7 nmol h^{-1} gdw^{-1}. After about 200 h the NO_2^- concentration reached a maximum and was subsequently consumed with a rate of approximately 6 nmol h^{-1} gdw^{-1} to below the detection limit. Following aeration NO_3^- at first remained constant at about 5 μM for 70 h, although NH_4^+ was consumed. With the onset of NO_2^- oxidation NO_3^- accumulated rapidly with a rate of 42.1±4.5 nmol h^{-1} gdw^{-1}, indicating that NO_2^- consumption was predominantly due to nitrite oxidizers. Thus nitrate production coincided with the beginning of the complete nitrification process,

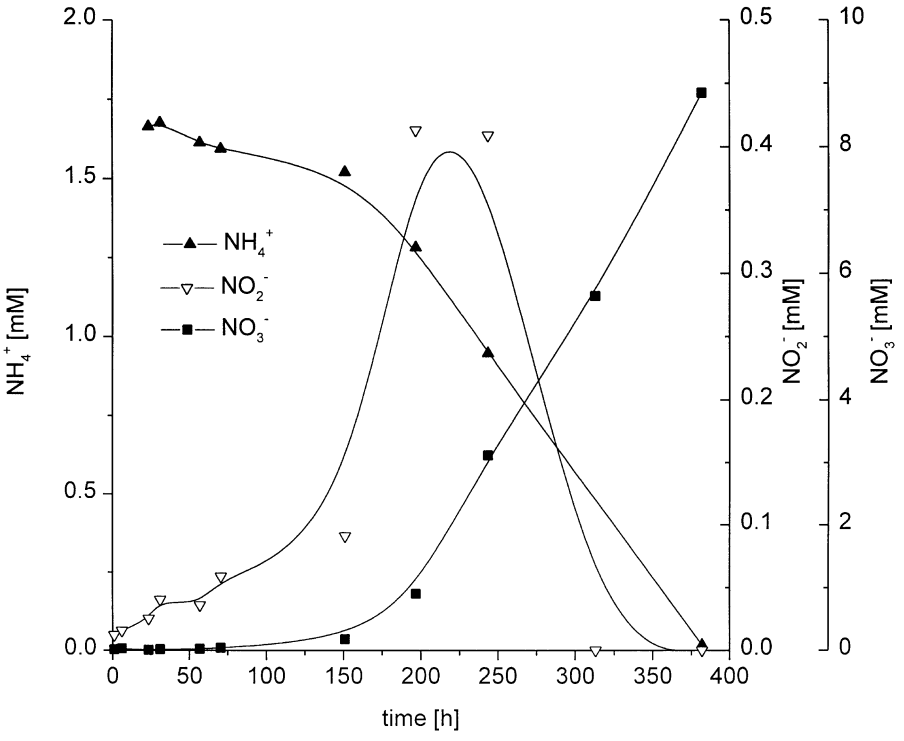

Figure 4. Turnover of NH_4^+, NO_2^- and NO_3^- in aerated soil slurries (25 °C). Concentrations were measured in the pore water of 5 ml subsamples. SD were usually <10% of the mean.

i.e. oxidation of NH_4^+ to NO_2^- to NO_3^-. The nitrate production rates exceeded by far the NO_2^- and NH_4^+ consumption rates.

Addition of NH_4^+ (50 μg N gdw^{-1}) resulted in no significant change of the production rates of NO and N_2O (Figure 5). Obviously, nitrification was not rate-limited by the availability of NH_4^+. However, when NO_3^- (50 μg N gdw^{-1}) was added to the aerated slurry a significant increase ($P < 0.05$) of NO and N_2O production resulted. The NO and N_2O production rates increased 3-fold and 4.5-fold, respectively (Figure 5).

Addition of 10 Pa acetylene to NO_3^--amended, aerated soil slurries (50 μg N gdw^{-1}) did not result in a significant ($P < 0.05$) decrease of NO and N_2O production rates, indicating that NO and N_2O production in NO_3^--amended slurries was mainly due to denitrification rather than nitrification. However, in the NO_3^--amended slurries without acetylene NH_4^+ was oxidized, NO_2^- accumulated and was then oxidized, indicating that nitrification was also active (Figure 6). Therefore, nitrification may also have contributed to some extent to the NO and N_2O production in NO_3^--amended soil slurries.

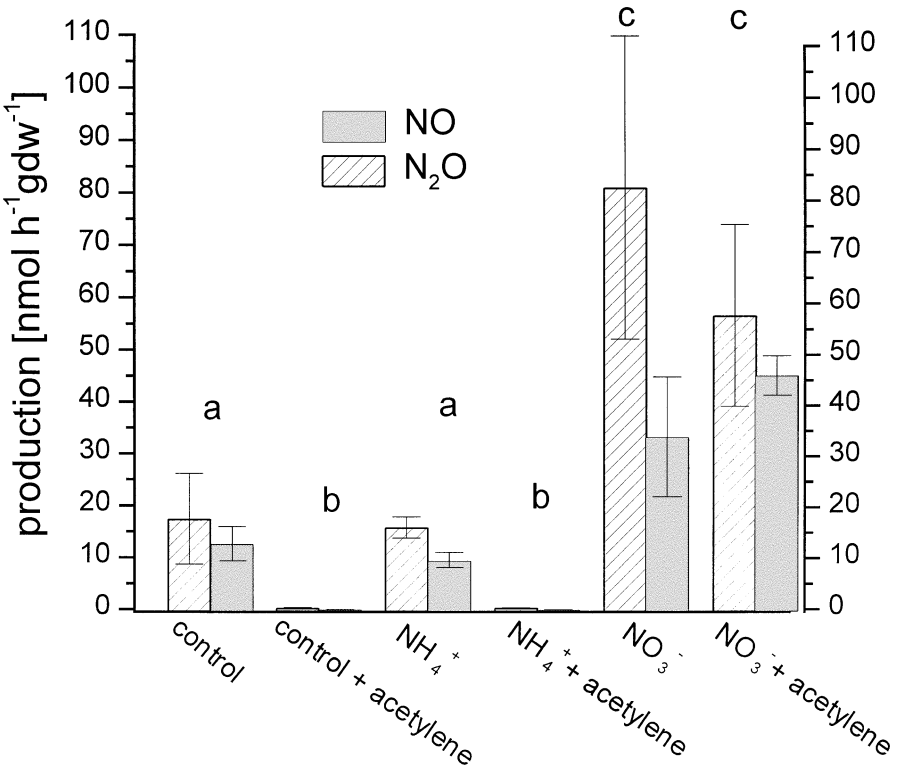

Figure 5. Comparison of NO and N_2O production rates in the aerated soil slurries (30 °C) that were treated with NH_4^+, NO_3^- (50 μg N gdw^{-1}) and/or 10 Pa acetylene. Different letters symbolize significant differences between the treatments. Mean ± SD, n = 3.

Although denitrification seemed to be the major cause of NO and N_2O production in NO_3^--amended slurries, there was no detectable consumption of NO_3^- (Figure 6). Either the consumption of NO_3^- was within the limits of detection, or NO_3^- production by nitrification exceeded consumption. NO_3^- even accumulated after onset of NO_2^- consumption with a rate of 40.25±5.58 nmol h^{-1} gdw^{-1}, suggesting that NO_2^- consumption again was predominantly due to nitrite oxidizers. Although denitrification was stimulated by NO_3^-, the NO_3^- production rates in NO_3^--amended slurries (40.2±5.6 nmol h^{-1} gdw^{-1}) and the control slurries (42.1±4.5 nmol h^{-1} gdw^{-1}) were almost identical, further indicating a very active nitrification.

3.3 *CH_4 oxidation in aerated rice soil slurries*

Methane was oxidized in aerated soil slurries (Figure 7). Occasionally, short lags of about 7 h were observed (not shown). CH_4 decreased linearly with

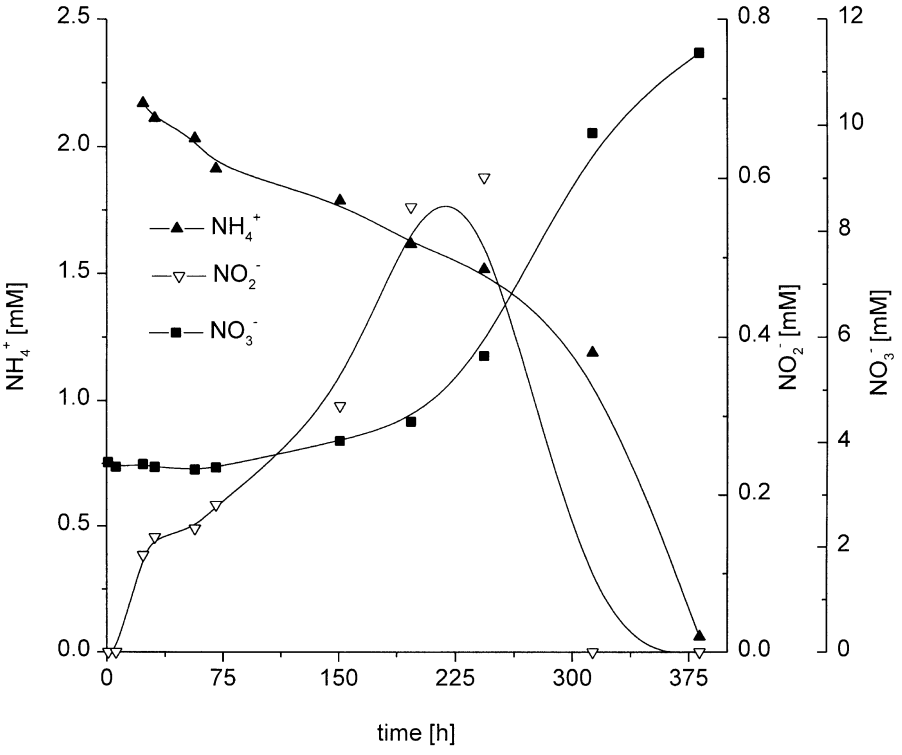

Figure 6. Turnover of NH_4^+, NO_2^- and NO_3^- in aerated soil slurries (25 °C) after the addition of NO_3^- (50 μg N gdw^{-1}). Concentrations were measured in the pore water of 5 ml subsamples. SD were usually <10% of the mean.

rates that increased as function of the initial CH_4 concentration and reached a maximum of about 0.14 μmol h^{-1} gdw^{-1} at 20,000 ppmv CH_4. After about 100 h a second phase of faster CH_4 oxidation followed. However, this induction of CH_4 oxidation activity occurred only if the initial CH_4 concentration was higher than 1000 ppmv CH_4 (Figure 7). By contrast, at initial CH_4 concentrations between 200 and 1000 ppmv no induction of the oxidation activity was observed and CH_4 continued to be consumed at the initial linear rate. Below 50 ppmv CH_4 no significant ($P < 0.05$) CH_4 oxidation was detected at all. Atmospheric CH_4 (1.7 ppmv) was never consumed, when using soil slurries that had not been induced by previous exposure to high CH_4 concentrations. Instead CH_4 was released from the aerated soil slurry if initial CH_4 concentrations were below 7 ppmv CH_4 (Figure 8), although oxic conditions were ensured by thoroughly flushing the incubation vessels with synthetic air and keeping the O_2 concentration constant at about 20%.

By contrast, aerated soil slurries in which CH_4 oxidation had been induced by preincubation under 20% CH_4 oxidized CH_4 even at atmospheric concen-

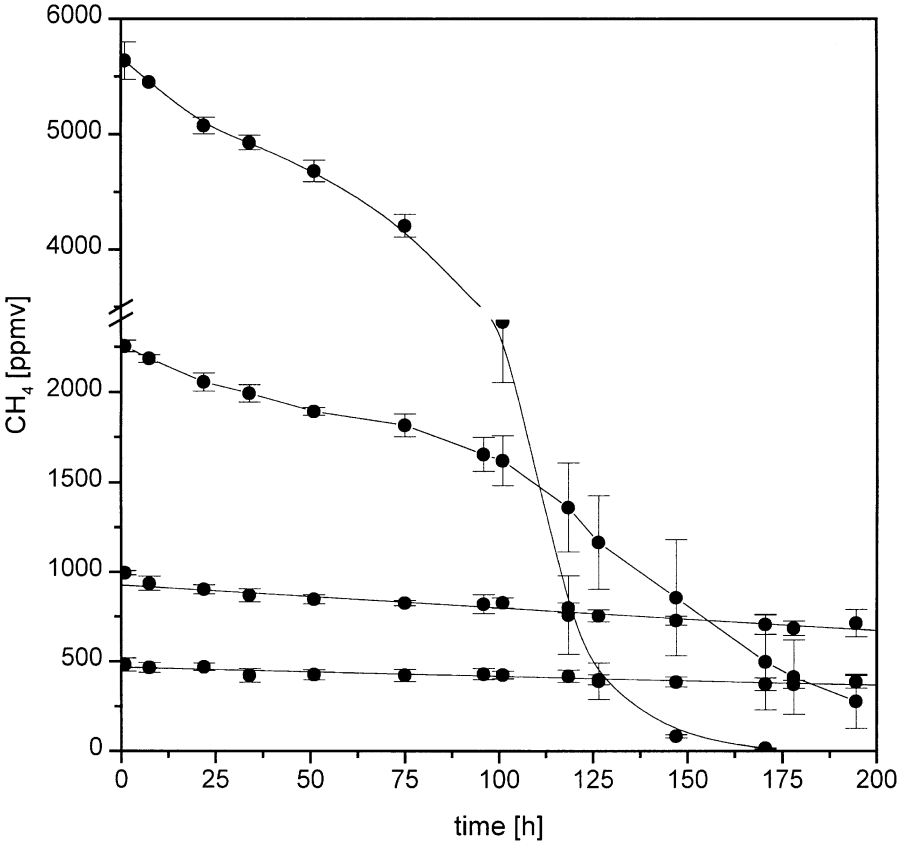

Figure 7. Oxidation of CH_4 in aerated soil slurries (25 °C) at different initial CH_4 mixing ratios. Mean ± SD, $n = 3$.

trations with significant rates ($P < 0.05$). No CH_4 release was observed at 1.7 ppmv CH_4. However, CH_4 was released with a very low but significant rate of 0.03 $\pm$0.01 nmol h^{-1} gdw^{-1} when the induced soil slurries were flushed with CH_4-free synthetic air (Figure 8). Incubation of induced soil slurries at concentrations $>$1000 ppmv CH_4 resulted in no further induction of the CH_4 oxidation rate.

To characterize methane oxidation in aerated rice soil slurries, we measured the CH_4 oxidation rates at different initial CH_4 concentrations and plotted them in a saturation plot (Figure 9). The CH_4 oxidation kinetics resulted in slightly sigmoid CH_4 saturation curves that were better fitted by the Hill than by the Michaelis-Menten equation. Non-linear curve fitting to the Hill function (Segel 1993) resulted in an apparent Hill coefficient n_{app} of 1.9 (Figure 9). The sigmoid character of the saturation curves suggests inhibition of CH_4 oxidation at low CH_4 concentrations.

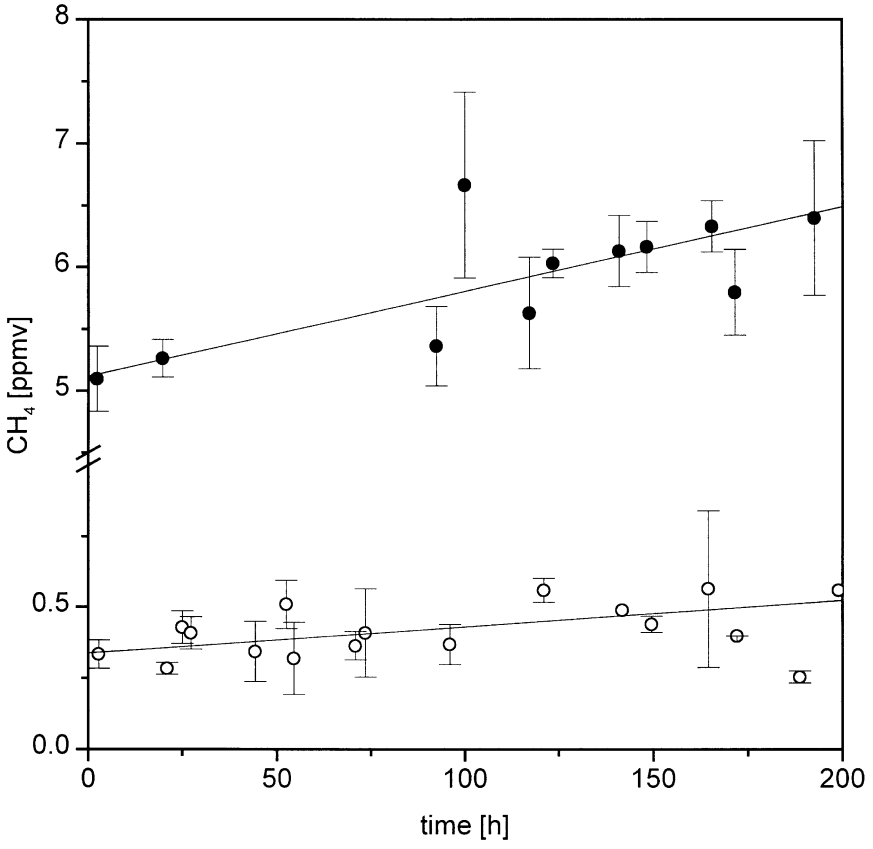

Figure 8. Production of CH_4 in aerated soil slurries (25 °C) at initial CH_4 mixing ratios below 6 ppmv (closed symbols) and without added CH_4 (open symbols). The latter experiment was done with induced soil slurries that had been preincubated in the presence of 20% CH_4 in air. Mean ± SD, $n = 3$.

4. Discussion

After anoxic preincubation Italian rice field soil produced vigorously CH_4. At this time, the slurry was characterized by a high NH_4^+ concentration (approx. 4 mM) and almost undetectable NO_3^- and NO_2^-. It is known that the form of available nitrogen compounds changes during anoxic incubation of rice field soil from mainly oxidized to reduced compounds (Ponnamperuma 1972). While NO_3^- is consumed below threshold concentrations, NH_4^+ accumulates from mineralisation of organic nitrogen. Beside reduction of NO_3^- other reduction processes (i.e. reduction of Mn^{4+}, Fe^{3+}, SO_4^{2-}) take place sequentially after the flooding of soil with CH_4 production being the

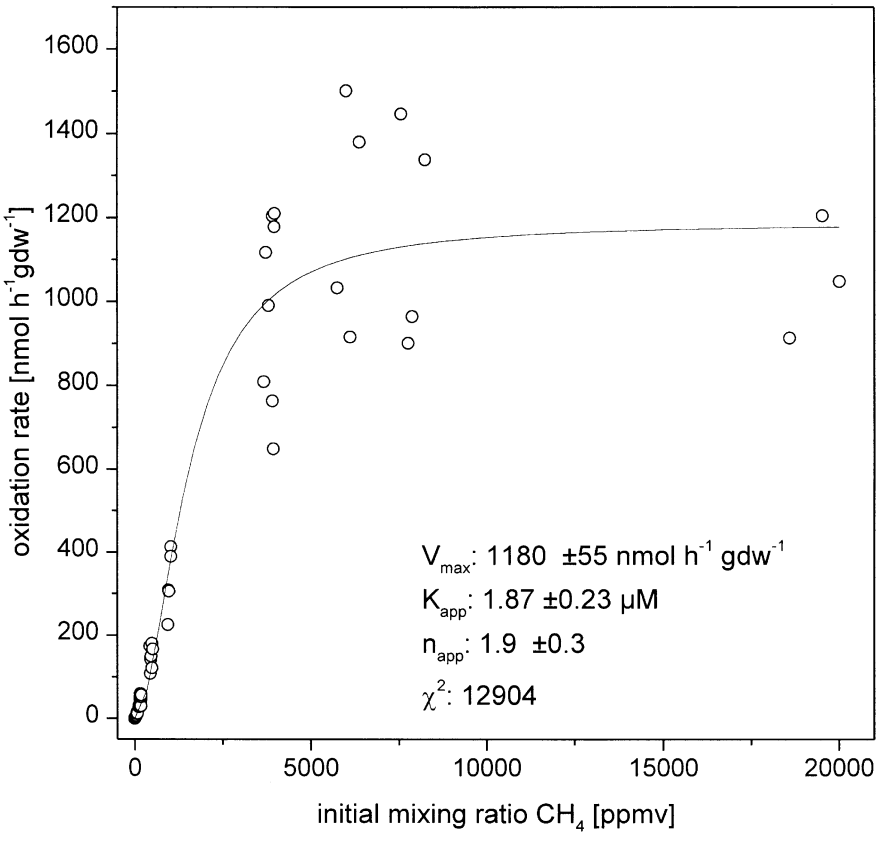

Figure 9. Saturation curve of the CH_4 oxidation activity in induced rice soil slurries (25 °C) that had been preincubated in the presence of 20% CH_4 in air for >100 h. The curve was fitted to the data points using the Hill equation.

final reduction process (Reddy & Patrick 1984; Achtnich et al. 1995). Consequently, methanogenic paddy soil did not show any production of NO and N_2O. However, when NO_3^- was added to this anoxic slurry the immediate production and consecutive consumption of NO and N_2O must have been due to denitrification of NO_3^-. The sudden switch from production to consumption of NO and N_2O probably resulted when the added NO_3^- had been consumed. The previously produced NO and N_2O were then probably used as electron acceptors to further maintain denitrification.

Aeration of the methanogenic slurry led to an almost immediate production of NO and N_2O. However, this production was due to nitrification. Denitrification was not involved in the immediate NO and N_2O production, since any condition leading to an inhibition of the ammonia oxidizers, e.g. anoxia

or inhibition with 10 Pa acetylene, prevented NO and N_2O production and any further nitrogen turnover. Thus, the oxidation of NH_4^+ was an obligatory process for nitrogen turnover. NO and N_2O production by ammonia oxidizers is probably due to nitrifier-denitrification. Under O_2 limitation and at high NO_2^- concentrations, electrons from the oxidation of hydroxylamine are alternatively transferred to the product NO_2^- itself, resulting in a production of NO, N_2O and N_2 (Poth & Foch 1985; Poth 1986; Remde & Conrad 1989).

The nitrogen turnover following the aeration of flooded rice field soil could be differentiated into different phases. The immediate NO and N_2O production resulted from ammonia oxidizers, since neither nitrite oxidizers nor denitrifiers were able to metabolize at this time due to the lack of NO_2^- and NO_3^-. However, NO_2^- accumulated transiently through the oxidation of NH_4^+. The following consumption of NO_2^- led to a production of NO_3^- probably due to nitrite oxidizers. Thus, with the accumulating NO_2^- the nitrite oxidizers became active. The rates of production and consumption of NH_4^+, NO_2^- and NO_3^- did not balance. The NO_3^- production rates were much higher than the NH_4^+ consumption rates. However NH_4^+ was measured in the pore water and was not extracted from the adsorbing clay minerals. Thus, the NH_4^+ consumption rates were probably underestimated. Some of the NH_4^+ may also have been freshly produced by mineralisation of organic nitrogen and instantaneously been nitrified to NO_3^-.

Addition of NH_4^+ did not increase the nitrogen oxide emission. The activity of nitrifiers in rice field soils therefore did not seem to be limited by NH_4^+, but only by O_2. In contrast, addition of NO_3^- immediately increased the NO and N_2O production dramatically. This increase was not inhibited by 10 Pa acetylene, so that the production of NO and N_2O in NO_3^--amended rice soil slurries was predominantly due to denitrification. In NO_3^--amended slurries, whether anoxic or aerated, denitrification was the major process of NO and N_2O production. It is therefore likely that denitrification contributed to the production of NO and N_2O in aerated rice soil slurries as soon as sufficient NO_3^- had been produced by nitrification. However, in the first few days of aeration denitrification only played a minor role.

N_2O was only consumed under anoxic conditions. The only known N_2O consumption process is denitrification. Since the N_2O reductase is the most O_2-sensitive enzyme of the denitrifiers, N_2O consumption is probably inhibited by aeration (Firestone et al. 1979, 1980; McKenney et al. 1994). NO, on the other hand, was consumed under both oxic and anoxic conditions. The pathway of NO consumption in rice field soil is unknown. Both reductive and oxidative consumption of NO have been described in soil (Rudolph et al. 1996; Baumgärtner et al. 1996).

Methane was oxidized in the aerated methanogenic soil slurry at concentrations >50 ppmv CH_4. However, atmospheric CH_4 was not oxidized. At CH_4 concentrations <10 ppmv even a slight CH_4 release was detected, although oxic conditions were ensured. This slight CH_4 production could possibly indicate a CH_4 compensation point for paddy soil (Conrad 1994). A low CH_4 production has sometimes also been observed in upland soils (e.g. Yavitt et al. 1995), but the responsible production processes have so far not been identified (Conrad 1996b).

Methane concentrations >200 ppmv were oxidized with maximum rates of about 0.14 μmol h^{-1} gdw^{-1}. Similar rates of CH_4 oxidation were observed in soil taken from planted rice microcosms (Bosse & Frenzel 1997). Above 1000 ppmv CH_4 an induction of the oxidation activities occurred. The maximum CH_4 oxidation rate was about 10-fold higher than before induction. Induction of CH_4 oxidation in rice field soil has been reported before (Le Mer et al. 1996; Bender & Conrad 1995; Bosse & Frenzel 1997). The induction is dependent on the physico-chemical parameters of the soil and was found to occur at CH_4 concentrations of 100 to 10,000 ppmv (Bender & Conrad 1995). The induction of CH_4 oxidation can be due to enzyme synthesis and/or increase of the methanotrophic population. Bender & Conrad (1995) detected an increase of the population when incubating the rice field soil at CH_4 concentrations >7000 ppmv. After induction CH_4 oxidation also occurred at atmospheric CH_4 concentrations.

The kinetics of CH_4 oxidation fitted with the Hill function suggested a saturation curve with a sigmoid shape. If this indeed was the case then it can possibly be interpreted as inhibition of CH_4 oxidation at low CH_4 concentrations (<500 ppmv). The methanogenic soil slurry was characterized by high concentrations of NH_4^+. NH_4^+ and its oxidation products hydroxylamine and NO_2^- were reported to inhibit CH_4 oxidation in soil and methanotrophic bacteria, the inhibition mechanism being partially competitive (Dalton 1977; Carlsen et al. 1992; King & Schnell 1994a,b). Sigmoid CH_4 oxidation kinetics have also been observed in littoral lake sediments (Bosse et al. 1993). Sigmoid kinetics were also reported by Ward (1987) for NH_4^+ oxidation by *Nitrosococcus oceanus* that was inhibited by CH_4, whereas hyperbolic kinetics resulted in the absence of CH_4. King & Schnell (1994a,b) showed that the extent of inhibition by NH_4^+ of CH_4 oxidation in soil and methanotrophic bacteria increased with increasing CH_4 concentrations up to about 100–500 ppmv and then decreased again. Therefore, we speculate that the sigmoid shape of the CH_4 saturation curves was due to the high NH_4^+ concentrations which inhibited CH_4 oxidation at low CH_4 concentrations, whereas at high concentrations (>1000 ppmv) the inhibitory effect of NH_4^+ was compensated by CH_4. The sigmoid shape of the saturation curves also prevented to

detect the "high-affinity" activity of CH_4 oxidation that had been observed by Bender & Conrad (1992) in rice field soil. Bender & Conrad (1992) used soil that was just moistened but not slurried and was incubated only under oxic conditions so that NH_4^+ had probably not accumulated to inhibitory levels.

The objective of our studies was to understand the processes involved in greenhouse gas emissions during drainage and aeration of rice field soil. Laboratory studies cannot replace appropriate field studies but may help to interpret them. Previous field studies have shown that a brief drainage of flooded rice field soils results in a decrease of CH_4 emissions (Kimura et al. 1991; Sass et al. 1992; Yagi et al. 1996). On the other hand, the positive effect of a reduction of the greenhouse gas CH_4 may partially be alleviated by the production of harmful NO and N_2O (Freney & Denmead 1992; Byrnes et al. 1993). Recently we measured emission rates of both CH_4 and N_2O in planted rice microcosms and in rice soil slurries that were intermittently drained and aerated, respectively (Ratering & Conrad 1997). In terms of CO_2 equivalents, the emitted N_2O was always less than 2% of the mitigated CH_4, both due to drainage and aeration. Thus, N_2O emission was only of minor importance. Our present experiments are in agreement with this observation. The rates of NO and N_2O production were relatively low, i.e. about 15 pmol h^{-1} gdw^{-1} NO and 5 pmol h^{-1} gdw^{-1} N_2O. Various oxic soils were found to release NO and N_2O with rates ranging between 1 and 1000 pmol h^{-1} gdw^{-1} (Bollmann & Conrad 1997; Baumgärtner & Conrad 1992; Vermoesen et al. 1996). The rates observed in the aerated rice soil slurries were at the lower end of this range. Since nitrogen turnover and concomittant NO and N_2O production in aerated soil slurries were not limited by NH_4^+, but by either NO_3^- or O_2, it is unlikely that increased application of ammonium fertilizer would result in higher NO and N_2O production. Increased application of nitrate fertilizer would probably stimulate NO and N_2O production, however, not specifically during aeration but also during the flooding period (Freney & Denmead 1992). Therefore, drainage of rice fields to mitigate CH_4 emissions will probably not be made obsolete by unreasonably high NO and N_2O emissions. However, field studies are required for confirmation. Supply of O_2 after drainage allows methanotrophic bacteria to become active. However, the potential CH_4 oxidation activity in aerated rice soil was relatively low, did not operate at atmospheric CH_4 concentrations and was possibly inhibited by the NH_4^+ present in the flooded soil. Therefore, our laboratory experiments suggest that it is unlikely that rice fields will become net sinks for atmospheric CH_4 during intermittent drainage. Again, field studies are necessary to prove this conclusion.

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