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Nitrification and denitrification as sources of atmospheric nitrous oxide – role of oxidizable carbon and applied nitrogen

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Abstract Laboratory incubation experiments were conducted to study the influence of easily oxidizable C (glucose) and mineral N (NH_4^+ and NO_3^-) on N_2O emission, evolution of CO_2 and consumption of O_2. A flush of N_2O was always observed during the first few hours after the start of soil incubation, which was significantly higher with NH_4^+ compared to NO_3^- applications. The increase in N_2O emission was attributed mainly to enhanced soil respiration and subsequent O_2 limitation at the microsite level. Application of NH_4^+ helped to develop denitrifying populations since subsequent additions of NO_3^- and a C source significantly enhanced N_2O emissions. In soils treated with NH_4^+, N_2O emissions declined rapidly, which was related to decreasing concentrations of easily oxidizable C. Addition of glucose in different amounts and pre-incubation of soil for different lengths of time (to create variation in the amount of easily oxidizable C) changed the pattern of N_2O emissions, which was ascribed to changes in soil respiration.

Keywords Ammonium · Nitrate · Nitrous oxide · Oxidizable carbon · Anaerobic microsites

Introduction

Loss of N from the soil-plant system has not only economic implications but has raised environmental concern, especially when N gases are emitted. Of particular interest are the N oxides, especially N_2O, which acts as a greenhouse gas and contributes to the destruction of the ozone layer (Bouwman 1990; Crutzen 1981). Efforts have therefore been devoted to identifying the processes that contribute to N_2O emissions and possible mitigation strategies.

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On the global level, >65% of atmospheric N_2O comes from soil as a result of nitrification and denitrification (Bouwman 1990). Since the two processes occur simultaneously (aerobic and anaerobic microsites can develop within the same aggregate, supporting nitrification and denitrification, respectively), it is not easy to ascertain the real contribution of either process to the observed N_2O fluxes (Arah 1997). However, denitrification is considered to be the major source of N_2O under most situations, while nitrification is reported to make a substantial contribution to the N_2O emissions under aerobic conditions (Williams et al. 1998). Higher N_2O emissions are often reported from fertilized than unfertilized soils, rates of emission being greatest following application of NH_4^+ or NH_4^+-forming fertilizers (Breitenbeck et al 1980; Flessa et al. 1996; Smart et al. 1999). In several studies, using isotope methodology and nitrification inhibitors, this increase is attributed to losses of N_2O occurring during the process of nitrification (Abbasi and Adams 2000; Arah 1997; Bremner and Blackmer 1978; Flessa et al. 1996; McTaggart et al. 1997; Stevens and Laughlin 1997). Estimates of the amount of N_2O resulting from nitrification are variable but generally account for<1% of the fertilizer N applied (Breitenbeck et al. 1980). In the case of anhydrous NH_3, however, the losses may increase to 6–7% (Smith and Chalk 1980).

In most studies, the onset of N_2O emission is observed very early during the incubation, while nitrification continues for extended periods of time (Simarmata et al. 1993). Williams et al. (1998) reported active nitrification 7–12 days after application of NH_4NO_3, while a flush of N_2O emission from soil was observed around day 1, followed by a decline. These authors showed very low molar ratios of NO to N_2O and suggested that denitrification was the dominant process involved in N_2O emission. Mulvaney et al. (1997) found that NH_4^+ applied as $(NH_4)_2SO_4$ and NH_4NO_3 decreased to negligible levels within 7–14 days, suggesting rapid nitrification, but the N_2O emissions were not related to nitrification. Nitrification not only commences after a characteristic lag period following fertilizer application to initially dry soils, but it

continues for much longer than the reported N_2O emissions (Mulvaney et al. 1997; Williams et al. 1998). This observation, combined with a quick initiation of N_2O emission, would support the contention that NO_3^- produced through nitrification might be the principle process which could occur in adjacent anaerobic microsites. This is supported by studies reported by Wolf and Russow (2000), who found that accumulation of NO_3^- was not accompanied by a parallel increase in N_2O emissions. Employing ^{15}N, Poth and Focht (1985) observed that all NH_3-N is oxidized to NO_2^- before the formation of N_2O. Quantification of fractional contributions showed denitrification to be the dominant process initially (first 2 days) but then nitrification became the main contributor to N_2O formation for the rest of incubation period (Arah 1997).

In addition, NH_4^+ is preferentially assimilated by soil microorganisms compared to NO_3^- (Azam et al. 1993; Jansson et al. 1955), and is reported to increase the decomposition of organic matter and mineralization of N as compared to NO_3^- (Azam et al. 1995). Increased microbial activity in response to NH_4^+ additions can cause microsite anaerobiosis, especially at so-called "hot spots". In most studies, NH_4^+-driven microsite anaerobiosis has not been considered, although the role of easily oxidizable C (which may increase following incubation of air-dried soils or due to physical treatment of soil) is well documented (Burford and Bremner 1975). Additions of easily oxidizable organic matter have been shown to enhance N_2O emissions even under apparently aerobic conditions (Beauchamp et al. 1989). A substantial portion of increased N_2O emissions observed following the application of ammoniacal fertilizers may still be due to denitrification at anaerobic microsites rather than originate from nitrification.

From the forgoing it would appear that the contribution of nitrification to N_2O emissions could have been over-estimated in most studies. The purpose of the studies reported here was to evaluate the role of NH_4^+ and the concomitant increase in microbial activity (soil respiration) and its relationship to N_2O emission under variable conditions of C and N supply.

Materials and methods

Soil

A silty clay soil used in the studies was collected (0–15 cm) from Weilburger-Grenze, Gießen, Germany. Air-dried and sieved (<2 mm) soil had the following characteristics: organic C, 1.35%; total N, 0.15%; NH_4^+-N, 4.7 mg kg^{-1}; NO_3^--N, 49.3 mg kg^{-1}; NO_2^--N, 0.6 mg kg^{-1}; pH ($CaCl_2$), 6.95; maximum water-holding capacity, 45%; clay, 33%; silt, 62%; and sand, 5%.

Gaseous flux as affected by form and availability of N

Soil (40 g) was placed in 250-ml serum bottles and moistened to 20% (on a dry weight basis) with: (1) water, (2) glucose solution, (3) glucose+NO_3^-, (4) glucose+NO_3^-+NH_4^+, or (5) glucose+NH_4^+. The glucose concentration in soil was 1% (w/w), while N was applied at 50 mg N kg^{-1} as $(NH_4)_2SO_4$, KNO_3, or two sources together (50 mg N kg^{-1} from each source). The soil samples were incubated at 25 C. Headspace samples were analysed for N_2O, CO_2, and O_2 at 13, 20, 36, 44 and 60 h after incubation. Subsequently, the moisture level of the soil was raised to 40% with simultaneous additions of 0.5% glucose and KNO_3 at 50 mg N kg^{-1} soil to create conditions conducive for denitrification and to study the residual effect of various soil treatments. Headspace analyses were carried out at regular intervals during a second 45-h incubation, again at 25 C.

In a separate experiment, gaseous emissions were studied at increasing levels of NH_4^+ in the presence of NO_3^- and available C as glucose. The soil treatments were: 0.5% glucose; glucose+NO_3^- at 50 mg N kg^{-1}+NH_4^+ at 10, 25 and 50 mg N kg^{-1} soil. Two sets with three replicates for each treatment were prepared to allow incubations at 20% or 40% moisture. Headspace samples were analysed for N_2O, CO_2, and O_2 at 10, 14, 18 and 22 h of incubation at 25 C.

In order to study the effect of NO_3^- on gaseous flux, soil samples were pre-incubated at 25 C for 2 days at 20% moisture and 0.5% glucose to immobilize native NO_3^-. After air-drying, soil (25 g) in triplicate was moistened to 20% with solutions of glucose, $(NH_4)_2SO_4$, and KNO_3 to obtain glucose and NH_4^+-N concentrations of 0.5% and 50 mg kg^{-1} soil, respectively; the concentration of NO_3^- was 0, 25 or 50 mg N kg^{-1} soil. During 21 h of incubation at 25 C, headspace samples were analysed for CO_2, N_2O and O_2.

Gaseous flux at different levels of oxidizable C

Soil (40 g) placed in 250-ml serum bottles was moistened to 20% with a solution of NH_4NO_3 and glucose. The concentration of added N was 25 mg kg^{-1} soil, while that of glucose was 0, 0.1, 0.25 or 0.5%. The soil receiving 0.5% glucose was either incubated at natural packing or after mixing it thoroughly using a spatula. Triplicate soil samples were used for each treatment. Incubation was carried out as described before and headspace analyses performed at regular intervals.

In another experiment, 40 g soil was pre-incubated for 1, 25, 49 and 73 h at 20% moisture and 25 C to create variations in native oxidizable C. After pre-incubation, NH_4NO_3 was added at 75 mg N kg^{-1} soil in aqueous solution which increased the moisture content to 30%. Gaseous fluxes (N_2O, CO_2, and O_2) were monitored at different intervals up to 77 h.

Gas flux measurement

The incubations were carried out in 250-ml bottles, which were sealed with a perforated laboratory foil to allow gas exchange but avoid water loss. At the times of gas flux measurements the bottles were sealed with a lid that contained a silicon septum to allow headspace sampling. The lid was closed for 1–2 h and headspace samples were taken with 60-ml disposable syringes equipped with a three-way valve. Previous experience with the syringes indicated that they were gas-tight and kept the gas concentrations for at least 48 h. Gas samples were analysed immediately after sampling for N_2O, CO_2 and O_2 on a gas chromatograph (Perkin Elmer, Germany) equipped with a flame ionization and electron capture detectors. The system has been described by Mosier (1980).

Statistical analysis

For the ANOVA and correlations, the statistical package SigmaStat (SPSS) was used. All other calculations were carried out in the spreadsheet software Excel (Microsoft).

Results

Application of NH_4^+ and NO_3^- significantly increased N_2O emissions with each source having a similar effect (Fig. 1). When NH_4^+ and NO_3^- were applied together, the N_2O emissions were several-fold higher compared to

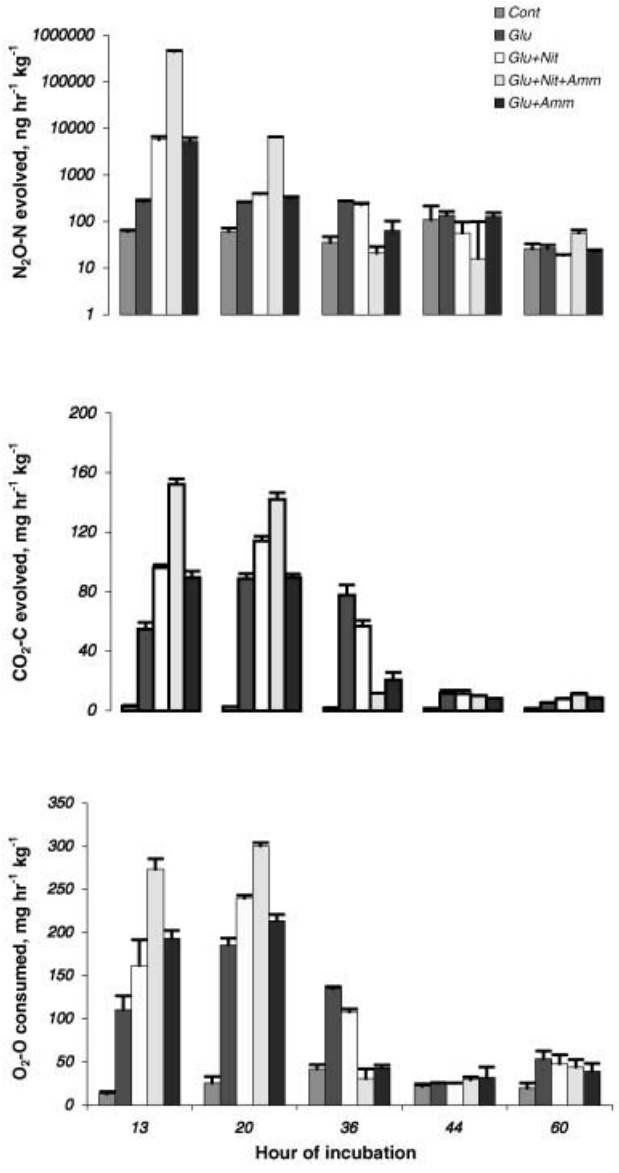

Fig. 1 Effect of glucose (*Glu*), NO_3^- (*Nit*) and NH_4^+ (*Amm*) on N_2O and CO_2 emissions and O_2 consumption. *Cont* Control

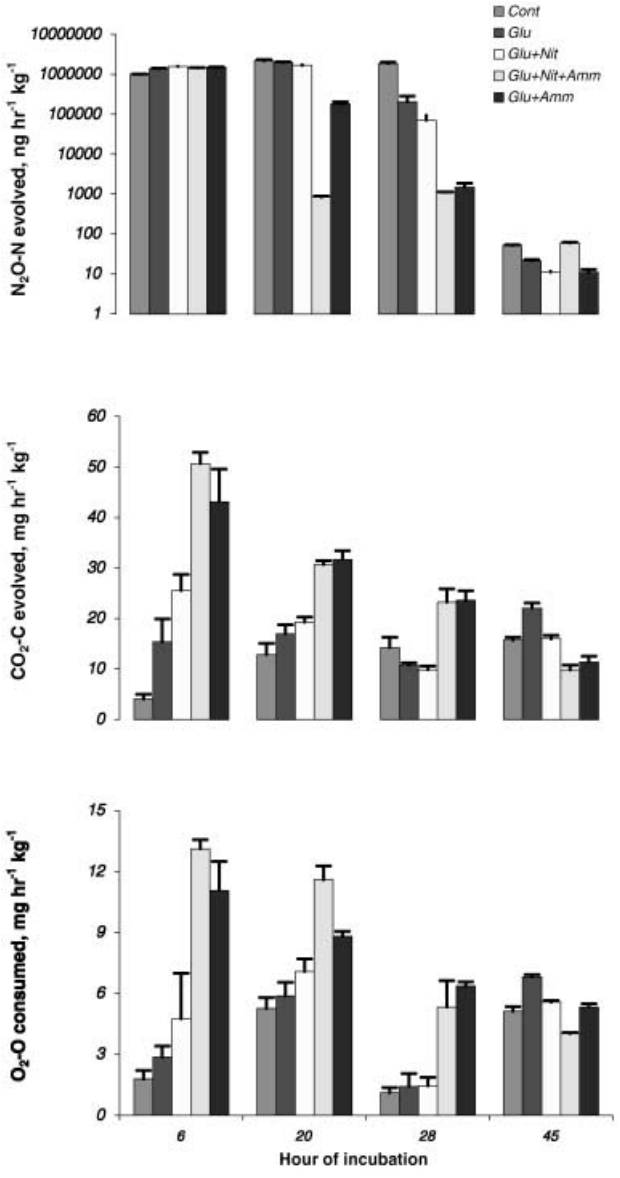

Fig. 2 Gaseous fluxes (N_2O, CO_2, O_2) from soil (pre-incubated at 20% moisture, see Fig. 1) incubated at 40% moisture following the addition of 0.5% glucose (*Glu*) and 50 mg NO_3^--N kg^{-1} soil (*Nit*). For other abbreviations, see Fig. 1

when only one source of N was applied. N_2O emissions decreased rapidly in all treatments but declined at the fastest rate when NH_4^+ and NO_3^- had been applied together. After 44 h of incubation, N_2O emissions were statistically similar in all treatments. Evolution of CO_2 and O_2 consumption followed the trend of the N_2O emissions (Fig. 1) and were highly significantly correlated (r=0.99); correlation between N_2O emitted and CO_2 evolved or O_2 consumed was also significant (r=0.64 and 0.65, respectively).

In the second incubation of the soil samples after raising the moisture content to 40% with the simultaneous addition of glucose (0.5%) and KNO_3 (50 mg N kg^{-1} soil), a flush of N_2O emission was observed after 6 h (Fig. 2). Treatment effects could not be differentiated clearly at 6 h incubation since the amounts of N_2O were above measurable limits. However, flux measurements at 20 h incubation revealed significant NH_4^++NO_3^- effects (NH_4^+ was applied separately or with NO_3^- before the first incubation). Emissions of N_2O from soil samples previously treated with both NH_4^+ and NO_3^- declined much faster than in other treatments. The decrease was relatively low when only NH_4^+ had been applied. After 28 h, a decrease in N_2O emission was also observed in all treatments except for the control (Fig. 2). The two NH_4^+ treatments showed a similar N_2O flux pattern with respect to time of incubation. In all treatments, negligible N_2O emissions were observed after 45 h of incubation.

The previous soil treatment had a significant positive effect on CO_2 evolution and O_2 consumption during the

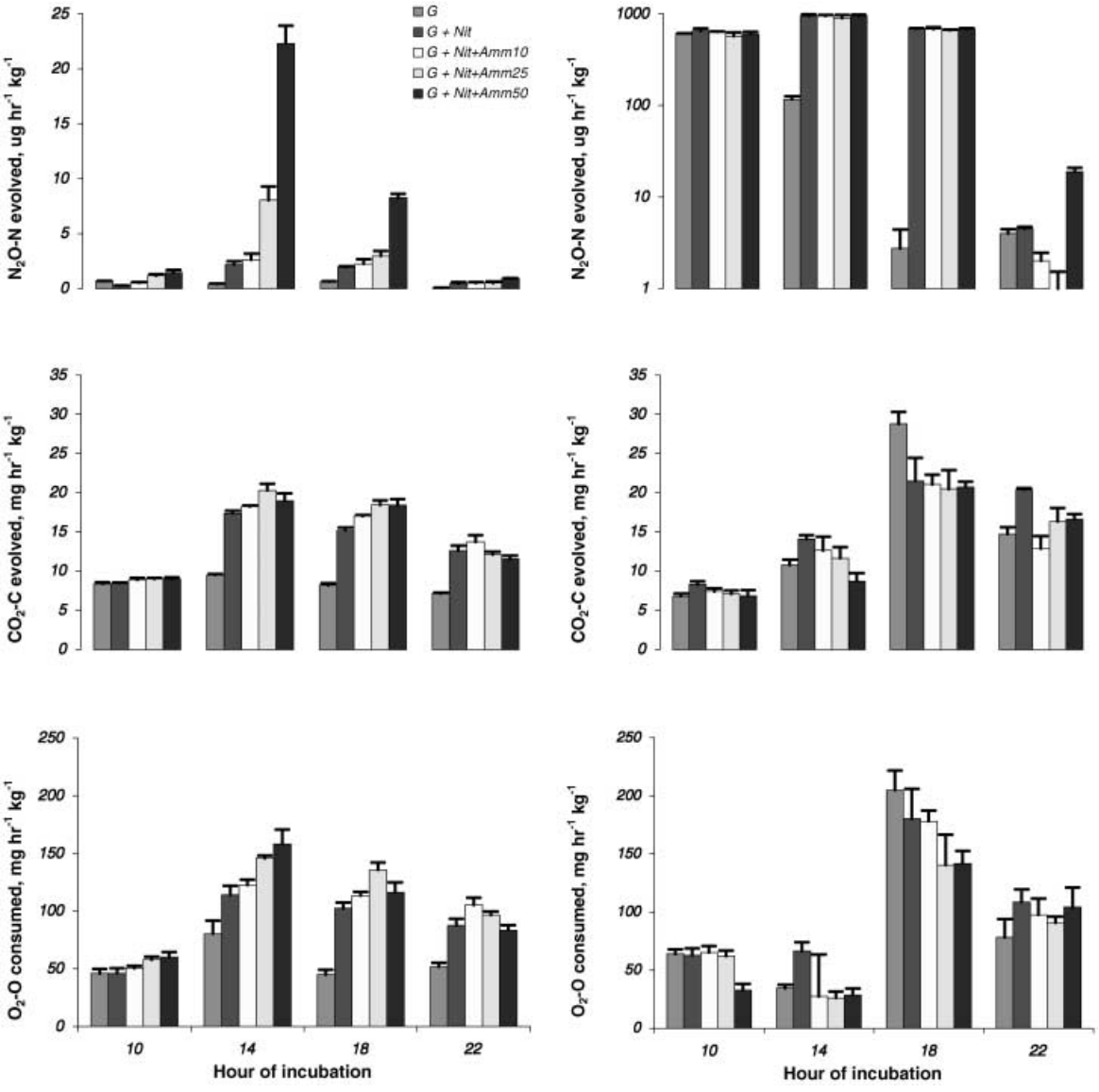

Fig. 3 Effect of constant NO_3^--N levels (*Nit*, 50 mg kg^{-1} soil) and variable NH_4^+-N levels [10 mg kg^{-1} soil (*Amm10*), 25 mg kg^{-1} soil (*Amm25*) and 50 mg kg^{-1} soil (*Amm50*)] on gaseous N fluxes from soil incubated at 20% (*left*) and 40% (*right*) moisture. *G* Glucose

second incubation at 40% moisture (Fig. 2). The effect was more pronounced in the two NH_4^+ treatments compared to the rest during the first 28 h of incubation. In contrast to N_2O emission, CO_2 evolution and O_2 consumption were still high. Amounts of CO_2 evolved and O_2 consumed were highly significantly correlated (r=0.95; n=20) while the correlation between N_2O and CO_2 or O_2 was not significant (r=0.49 and 0.55, respectively; n=20).

The results of experiments where different levels of NH_4^+ were applied along with a constant level of NO_3^- and glucose are presented in Fig. 3. At 20% moisture, N_2O emission increased substantially with the rate of applied NH_4^+. Peaks of N_2O emission were observed after 14 h of incubation followed by a decrease to negligible amounts after 22 h. Evolution of CO_2 and consumption of O_2 increased significantly over time but were not significantly different among treatments. A highly significant correlation was again observed between CO_2 evolution and O_2 consumption (r=0.98; n=20) and both gases showed significant correlations with N_2O (r=0.77 and 0.81, respectively; n=20). The respiratory quotient (RQ) (ratio of CO_2-C evolved to O_2-O consumed) averaged 0.47±0.06 in different treatments.

The increase in moisture from 20% to 40% led to several-fold higher N_2O emissions (Fig. 3). Up to 18 h the N_2O flux was beyond the machine limit and therefore treatment effects could not be differentiated until 22 h of incubation when N_2O emission at 40% moisture was only slightly higher than at 20% moisture. In treated soil, CO_2 evolution and O_2 consumption increased with time and were significantly correlated (r=0.95). No significant treatment effects were observed. The RQ was 0.61±0.32 and, therefore, higher than the one observed at 20% moisture.

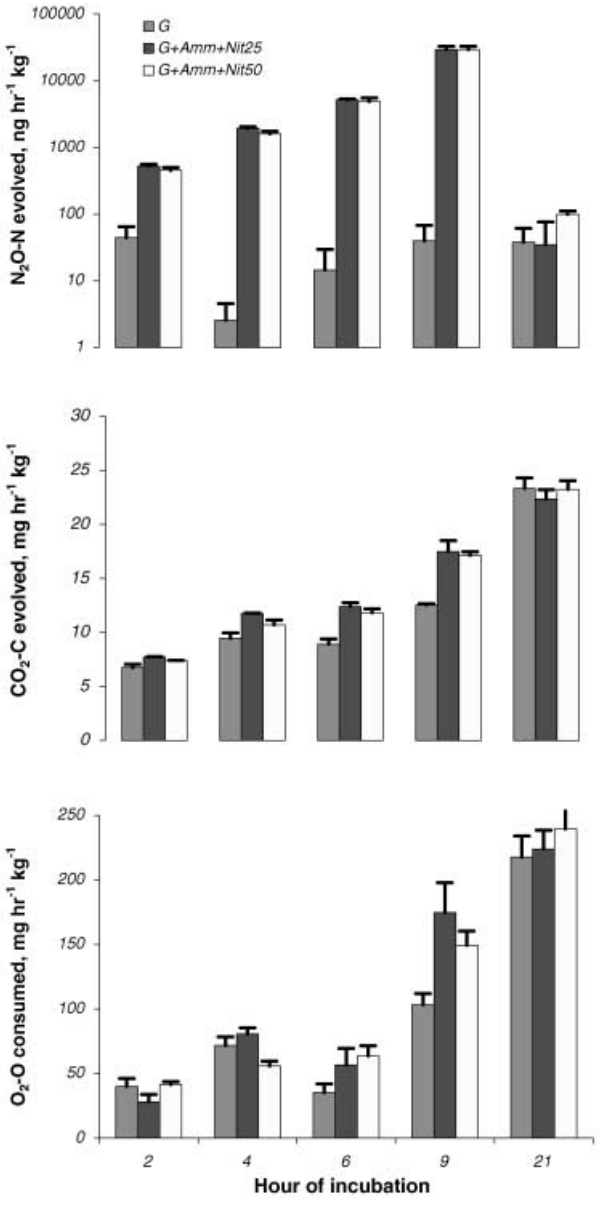

Fig. 4 Effect of NO_3^--N levels [25 mg kg^{-1} soil (*Nit25*) and 50 mg kg^{-1} soil (*Nit50*)] on gaseous fluxes from soil treated with 0.5% G and 50 mg NH_4^+ kg^{-1} soil (*Amm*)

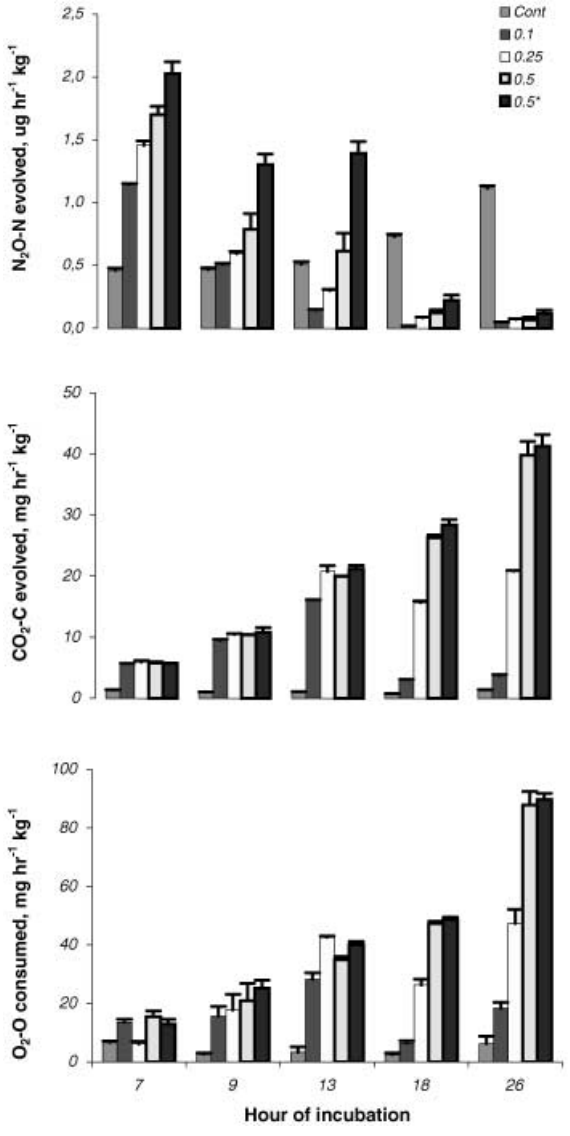

Fig. 5 Effect of G applied to soil (0.1% and 0.5%) on gaseous N flux. The incubation was carried out without mixing the soil after adding the solution, except for *G0.5** where soil was thoroughly mixed and incubated in a loose, well-aerated form

Soil samples incubated after immobilizing the native NO_3^- showed no N_2O emissions despite the fact that both glucose and NH_4^+ had been applied (Fig. 4). However, after the addition of NO_3, N_2O emissions increased with time. The amount of applied NO_3^- had a negligible effect on N_2O emissions. The release of N_2O started within 2 h of incubation and reached peak levels after 9 h with negligible amounts determined after 21 h. Evolution of CO_2 and consumption of O_2 (RQ, 0.58±0.21) were statistically the same for all treatments. CO_2 emissions and O_2 consumption were significantly correlated (P=0.05).

The amount of easily available C had a significant, positive effect on N_2O emissions (Fig. 5). After 7 h of incubation, N_2O emissions had more than doubled in the presence of glucose (0.1%) than without glucose. During the subsequent incubation period, however, N_2O emissions gradually decreased in all treatments except for the control (no glucose) reaching negligible levels after 26 h. Loss of CO_2 and consumption of O_2 increased with the amount of glucose applied and with length of incubation; the two processes showed a significant positive correlation (r=0.99; n=25). Negligible CO_2 was evolved from untreated soil. The RQ varied from 0.74 to 3.56 (average 1.71±0.59) and consistently decreased with time (data not presented).

Figure 6 presents the results of an experiment where soil samples were pre-incubated for different incubation times to create variations in easily oxidizable C before studying gaseous fluxes. Soils pre-incubated for 49 h and

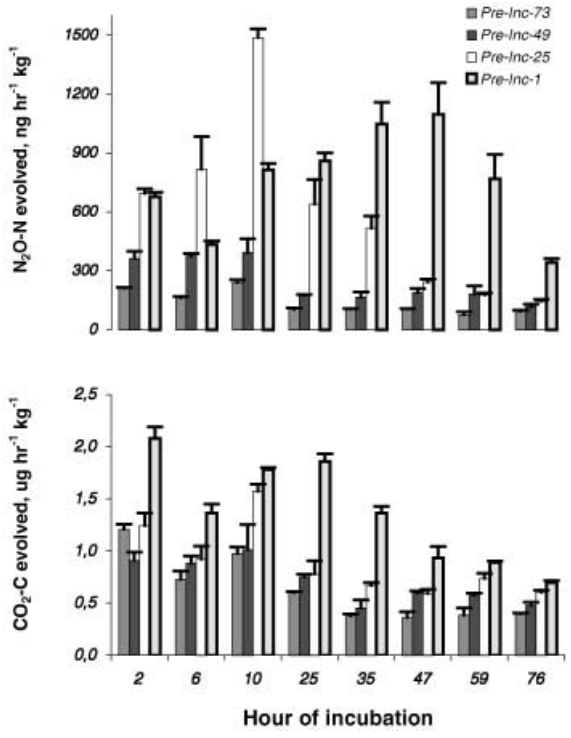

Fig. 6 Effect of pre-incubation (*Pre-Inc*) time [*-1* (1 h), *-25* (25 h), *-49* (49 h), *-73* (73 h)] on the emission of N_2O and CO_2 from soil (incubation of moist soil was carried out at 25% moisture and 25°C to achieve different levels of easily oxidizable C)

73 h showed a small increase in N_2O emissions during the initial 10 h of the second incubation. Pre-incubation for 25 h resulted in a consistent increase in N_2O emission up to 5 h of the second incubation followed by a steady decrease. For soil samples pre-incubated for 1 h, an increase in N_2O emission occurred up to 10 h of the second incubation followed by a steep decline. Highest CO_2 emissions were recorded shortly after the start of the second incubation with a steady decrease in all treatments. Higher emissions were observed from soils with short pre-incubation periods. Amounts of N_2O and CO_2 emitted were significantly and positively correlated (r=0.84).

Discussion

In the studies reported here, N_2O emission was found to increase from soils treated with glucose and mineral N, the increase being substantially higher when both NH_4^+ and NO_3^- were applied (Fig. 1) which would suggest that the N_2O producing process is either NO_3^--N or C limited. Considering that active nitrification starts with a time lag of 4–10 days following re-wetting of soils (Mulvaney et al. 1997; Williams et al. 1998), the process cannot be considered responsible for the observed N_2O emissions recorded shortly after the start of the experiment. In addition, if nitrification was the process responsible, the amounts of N_2O lost should have been essentially similar whether NH_4^+ was applied alone or along with NO_3^-. Instead, a several-fold higher N_2O loss occurred when NH_4^+ and NO_3^- had been applied together (Fig. 1). Moreover, the rate of NO_3^- application had a negligible effect on N_2O emissions (Fig. 4) and N_2O rates increased with soil moisture (Fig. 3). The results point to the fact that N_2O emissions may mainly be driven by reducing rather than oxidising processes in soil.

The loss of N_2O increased with the amount of applied NH_4^+, as did the consumption of O_2 and evolution of CO_2 (Fig. 1, 2, 3). After inducing conditions which stimulate denitrification in soil (increased water content, supply of NO_3^- and C), NH_4^+-pre-treated soil (Fig. 1) showed reduced N_2O emission with time (Fig. 2). The rate of decline in N_2O emissions was several-fold faster than observed in other treatments (Fig. 2). This observation suggests that NH_4^+ may have stimulated microbial activity and growth because of its preferential assimilation (compared to NO_3^-) by soil microorganisms (Azam et al. 1993; Jansson et al. 1955). An increase in microbial activity and concurrent high microbial consumption of O_2, particularly in the presence of NH_4^+, is certain to cause microsite anaerobiosis and encourage denitrification (Arah 1998; Williams et al. 1998). These phenomena are affected positively by the availability of organic C and the latter is often correlated positively with the rate of denitrification (Burford and Bremner 1975; Simek and Hopkins 1999). Availability of C not only supports the activity of denitrifiers per se but also has the indirect effect of causing microsite anaerobiosis due to increased respiratory demand for O_2. Under these conditions, NH_4^+-N may cause an increase in N_2O emissions simply by favouring these processes. Bauhus et al. (1996) attributed the increase in N_2O emission to: (1) the creation of anaerobic microsites and (2) increased nitrification.

Results presented in Figs. 5 and 6 point to the importance of easily oxidizable C and its effect on N_2O emissions under apparently aerobic soil conditions. Even in well-aerated soil (incubated after thorough mixing and thus loosely packed soil), substantial loss of N_2O occurred. The persistence of N_2O emission from untreated soil for relatively long periods of time was probably due to the presence of available C. In treated soils the supply of easily oxidizable C appeared to have become exhausted rapidly, resulting in a sharp decline of N_2O emission (Figs. 1, 2, 3). The presence of large quantities of denitrifying enzymes in aerobic soils is evidence of microsite anaerobiosis that often occurs during the build-up of these enzymes. Tiedje et al. (1982) suggested that organic C is more important than O_2 as regards stimulating the growth of denitrifier populations.

A significant positive correlation between N_2O and respiratory gases (CO_2 and O_2) observed in the present study and those reported by others (Dendooven and Anderson 1994) support the contention that enhanced microbial activity is responsible, at least partially if not entirely, for the observed N_2O emissions following NH_4^+ addition. However, the increase in N_2O emission may

not be accompanied by a similar increase in CO_2 evolution (Williams et al. 1998). The levelling off of N_2O emission following an initial flush suggests that easily oxidizable C is exhausted while nitrification could still continue and contribute to N_2O emissions. However, even under conditions promoting rapid nitrification rates, the NO_3^- produced may be lost through denitrification (Wolf and Russow 2000). Rapid nitrification may stimulate the creation of anaerobic microsites which could support denitrifying activity. In situations where a rapid turnover of the applied NH_4^+ is promoted, ^{15}N-labelled NO_3^- originating from applied $^{15}NH_4^+$ may end up in the observed N_2O label, although it in fact originates from denitrification rather than nitrification processes. The NO_3^- produced can reach anaerobic zones by diffusion, while the site of NO_3^- production can become anoxic if easily oxidizable C is present, thereby stimulating denitrification. A generally reported decrease in N_2O emission in the presence of nitrification inhibitors could result from decreased availability of NO_3^- rather than the cessation of losses during nitrification. When nitrification is inhibited using specific inhibitors, a concomitant inhibition of denitrification may occur, due to the lack of NO_3^- that should have been produced and denitrified quickly. This argument is supported by the study presented here where oxidizable C was eliminated to various degrees during pre-incubation or where differences in easily oxidizable C were created by glucose addition (Figs. 5, 6). Further support for enhanced microbial activities being responsible for elevated N_2O emissions is provided by the higher RQ (ratio of CO_2 evolved to O_2 consumed) observed during the initial periods of the incubation that coincided with increased N_2O emissions. Higher RQs may reflect the onset of anaerobic metabolism as a result of O_2 limitation at higher respiration rates (Grant 1995). The most important habitats for denitrifiers are probably those with an aerobic-anaerobic interface for NO_3^- produced by nitrification (an aerobic process), and then NO_3^--N is removed by denitrification in an adjacent O_2-limited zone. The development of aerobic and anaerobic microsites within close proximity, indeed in the same soil aggregate, permits nitrification and denitrification to occur simultaneously. According to Reddy et al. (1982), a considerable portion of available C is used in normal oxidative respiration by denitrifiers and other microorganisms until the system becomes anoxic. A portion of the N_2O produced may also result from dissimilatory NO_3^- reduction under conditions of limited O_2 supply (Poth and Focht 1985). Ottow and Fabig (1985) suggested that in bulk soil or sediment, denitrification starts as soon as the trapped O_2 is utilized, and the subsequent O_2 diffusion is limited. According to these authors, respiration and denitrification may occur simultaneously even at a relatively high rate of O_2 supply if a sufficient amount of easily decomposable organic matter is made available through organic amendments (as practised in the present study) or physical treatment of the soil (wetting/drying, freezing/thawing or disturbance, etc.).

Understanding the interactions of oxidative and reductive processes at the microsite level are essential to understanding the mechanisms of N_2O production and emission in soil. From this study it may be inferred that denitrification is the dominant process for N_2O production, especially under conditions of high C availability. NH_4^+ not only provides NO_3^- through nitrification but also stimulates microbial activity which in turn could help create conditions conducive for denitrification. Further work is needed to elucidate these processes at the microsite level.

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