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SOIL WATER CONTROLS ON AEROBIC SOIL EMISSION OF GASEOUS NITROGEN OXIDES

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Summary—Soil water content has multiple effects on the emission of gaseous N oxides. To separate and characterize these effects, we monitored rates of CO_2, NO, and N_2O evolution and changes in inorganic N concentrations of soil under a factorial combination of three N treatments and three water treatments during a 10-day laboratory incubation study. Because the emission of NO from control and NH_4NO_3-amended soil varied with the rate of chemoautotrophic NH_4^+ oxidation and was virtually eliminated by a specific inhibitor of that process [nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine)], we concluded that nitrification was the principal NO source over the entire tested range of soil water potentials (-10 to less than -1000 kPa). Denitrification made no significant contribution to N oxide emissions from even the wettest soil, so nitrifiers were probably also responsible for the much smaller emission of N_2O under all treatments. Slower gas diffusion in soil with highest, compared to lowest, water content caused a 3-fold reduction in the mean $NO:NO_3^-$ product ratio of nitrification, suggesting that the NO produced by NH_4^+-oxidizers is further oxidized unless conditions permit its rapid escape to the atmosphere. Nitrapyrin also eliminated the brief burst of N oxide emissions that typically follows wetting of dry soil, indicating that chemoautotrophic NH_4^+ oxidation is also involved in this phenomenon despite the poor correlation of the burst's magnitude with soil NH_4^+ or NO_2^- concentrations. A second burst of N oxide emissions from control or NH_4NO_3-amended soil with no inhibitor occurred only where desiccation reduced both NO and CO_2 evolution to near zero prior to rewetting the soil after a 7-day drying cycle.

INTRODUCTION

Gaseous N oxides, N_2O and NO_x ($NO + NO_2$), are trace atmospheric constituents with potentially important functions in various global climate change models (Williams *et al.*, 1992). Both also participate in the production or consumption of atmospheric oxidants (e.g. ozone, hydroxyl), and NO_x is removed from the atmosphere in a series of photochemical reactions that yield nitric acid, the fastest-growing component of acidic deposition (Logan, 1983). In addition to these important effects on the chemistry and physics of the atmosphere, it has been suggested that (1) NO_x emissions (usually >90% NO) comprise a significant fraction of the unaccounted N losses typically observed in soil N balance studies, and (2) emission, transport and subsequent redeposition of NO_x results in substantial N redistribution both within and among natural and agricultural ecosystems (Williams *et al.*, 1992). Because biochemical processes in soil are included among the principal sources of atmospheric N oxides, it becomes important to characterize these processes and to identify important controllers of their rates.

Both biotic and abiotic processes are involved in the production of gaseous N oxides in soil. The bacterial processes of nitrification and denitrification are generally accepted to be the dominant biotic sources of both NO and N_2O, but most microbial processes that result in oxidation or reduction of N through the +1 or +2 oxidation state may yield trace amounts of either species (Firestone and Davidson, 1989). Abiotic production of N_2O, and particularly NO, occurs primarily through a set of reactions collectively termed chemodenitrification. Most of these reactions have been demonstrated only in acid soils in the laboratory. However, their occurrence in neutral or alkaline soils in the natural environment cannot be discounted because of the possible existence in undisturbed soils of microsites where the required accumulation of NO_2^- and low pH can occur as a result of solute concentration in thin water films during freezing or drying, or because of proximity to a colony of NH_4^+ oxidizers etc. (Firestone and Davidson, 1989).

Because of its multiple effects on both biotic and abiotic processes, soil water content is one of the most influential, but least well-defined, controllers of soil NO and N_2O emission rates. Except for its universal requirement by all life processes, water's most important effect on gaseous N oxide production in soil results from its strong influence on the rate of O_2 supply. For example, denitrification occurs only when O_2 supply is limited, most commonly by high

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soil water content, while the activity of nitrifying microorganisms is dependent on a plentiful supply of O_2, which usually exists only at low or moderate soil water contents. Differences in O_2 supply across microsite scales may permit nitrification and denitrification to occur in close proximity, sometimes even in the same soil aggregate, so characterizing these relationships is not straightforward. Moreover, soil water content also controls the diffusive transport of other reactants and products of these two processes, thereby influencing not only their rates, but also their product ratios. For example, the fractional NO yields of nitrification (Remde *et al.*, 1989) and denitrification (Zafiriou *et al.*, 1989) have been reported to increase with the ease of escape of this gas from its site of production.

Separate from the effects of soil water content on both solution phase and gas phase transport in soil, several authors have reported a large burst of NO or N_2O evolution concurrent with the flush of CO_2 that typically follows wetting of dry soil (e.g. Anderson and Levine, 1987; Williams *et al.*, 1987; Hao *et al.*, 1988; Davidson, 1992). Gaseous N oxide emission rates during one of these bursts may be up to a 1000-fold higher than rates preceding or following the burst. As a result, the quantity of soil N lost during the brief duration of such an event may approach or exceed the total amount emitted during the much longer periods between times that the soil dries enough to support another emissions burst in response to the next addition of water. Reasons for the unusually large response of N oxide emissions to wetting of dry soil remain unclear. Davidson (1992) suggested that the wetting response may be due to chemodenitrification of NO_2^- produced by autotrophic NH_4^+ oxidizers, but this conclusion is not entirely consistent with data of other authors (e.g. Tortoso and Hutchinson, 1990).

To overcome inadequacies and inconsistencies in existing knowledge regarding the effects of a soil's water content on its emission of gaseous N oxides, we monitored rates of CO_2, NO, and N_2O evolution and changes in inorganic N concentrations of soil under several N and water treatments in a laboratory soil incubation experiment. Specific objectives of our research were (1) to determine the effect of soil water content on the relative importances of nitrification and denitrification as sources of NO and N_2O in the experimental soil, (2) to establish the contributions of the same two processes to the burst of N oxide emissions that follows wetting of dry soil, and (3) to characterize the effect of water-induced changes in diffusive transport rates in soil on the velocities and product ratios of N transformation processes involved in NO and N_2O production.

MATERIALS AND METHODS

The 10-day aerobic soil incubation study combined three N treatments with three soil water treatments in a repeated measures factorial design with two replicates per treatment. Initial soil water contents were 4, 7 or 10% by weight, equivalent to water potentials of *ca* -1000, -30 and -10 kPa, respectively (Table 1). The N treatments included a control and two with a N amendment, one of which also received nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine]. The source of N was NH_4NO_3 (50 mg N kg^{-1}), chosen because it provided substrate to both nitrifiers and denitrifiers. Nitrapyrin was used to inhibit chemoautotrophic oxidation of NH_4^+ to NO_2^- without influencing the activity of non-target microbial groups, thereby permitting evaluation of the relative contributions of nitrifying vs denitrifying microorganisms to soil NO and N_2O emissions from the experimental soil under each water treatment. Minimum effective concentration of the inhibitor (30 mg kg^{-1}) was selected on the basis of NO and CO_2 evolution rates and changes in NH_4^+ and NO_3^- concentrations of soil with water content near field capacity in an exploratory experiment where nitrapyrin concentration was the only variable (data not shown).

Soil used in the experiment was collected in midwinter from an experimental ungrazed Bermuda grass (*Cynodon dactylon*) pasture on well-drained, very uniform Kenney sandy loam (a member of the loamy, siliceous, thermic, Grossarenic Paleudalfs) in a humid, subtropical region of southern Texas. Each spring grass on the site was harvested and then fertilized with maintenance amounts of N and P. Selected soil properties are listed in Table 1; additional site information was given by Hutchinson *et al.* (1992). Several samples taken from 0 to 10 cm depth were combined, partially air-dried to permit sieving (< 2 mm) and stored at 2.8% water content for about 1 month at 4°C. All identifiable pieces of fresh plant residue that passed through the sieve were removed by hand-picking.

We used the automated flow-through soil incubation system described by Hutchinson and Andre (1989), except the air flow rate through each incubation jar was reduced to 60 ml min^{-1}, and we eliminated the water reservoir they used for final humidification of incoming air. As a result, air entering the jars was only about 75% saturated and extracted *ca* 0.5 g water day^{-1} from each jar. To compensate for this loss and to measure the soil's

Table 1. Selected properties of the experimental soil

Property		Value (%)
Sand		77
Silt		13
Clay		9
Organic C		1.00
Total N		0.088
pH		7.5
Water retention at:	3.3 kPa suction	20.2
	10 kPa suction	9.9
	33 kPa suction	6.1
	100 kPa suction	4.3
	1000 kPa suction	4.0

response to rewetting, we added distilled water (dropwise with a pipet) after day 7 of incubation to return each sample to its initial water content. Incoming air was blended from NO_x-free bottled gas mixtures to contain ambient atmospheric concentrations of O_2, CO_2 and N_2O.

To begin the experiment, 156.1 g (dry wt) of soil in each incubation jar was wetted to 4, 7 or 10% (w/w) water by misting with distilled water from a hand-operated pump spray bottle while mixing on a large platform balance covered with waxed brown paper. Finely-ground dry NH_4NO_3 and nitrapyrin were then added using 0.5 g talcum as a carrier as described by Anderson and Domsch (1973). After the talcum–chemical mixtures were thoroughly incorporated by vigorous stirring and shaking, 10 g (dry wt) of treated soil was weighed into each of five small sample vials, and the remaining soil was spread evenly across the bottom of each jar. Soil in the jar bottom served as a continuous, undisturbed source of the measured gases while that in the small vials provided convenient, pre-weighed subsamples for periodic chemical analyses. Soil depth was about 1 cm in both cases.

After four of the five vials were positioned as described by Hutchinson and Andre (1989), the jars were sealed and gas analyses initiated. Soil NO and CO_2 emission rates were determined by monitoring the concentrations of these gases in the air that flushed each jar—NO by a Model 1600 chemiluminescent NO_x analyzer (Columbia Scientific Industries, Austin, Tex.) and CO_2 by a Model NDIR-743 non-dispersive infrared gas analyzer (Esterline Angus Instrument Corporation, Indianapolis, Ind.). Exhaust air was also analyzed for N_2O by gas chromatography using electron capture detection (Mosier and Mack, 1980) after 1.5, 3, 6, 9, 12, 24, 48, 72, 168, 171 and 240 h of incubation. Periodic analysis of the exhaust air for NO_2 never revealed a detectable concentration of this gas. Minimum detectable NO and NO_2 fluxes were about 30–40 ng N kg^{-1} h^{-1} and for N_2O, about 0.2–0.5 μg N kg^{-1} h^{-1}.

Soil in the fifth vial from each jar was extracted immediately to determine initial inorganic soil N concentrations; the other vials were removed and extracted after 1, 2, 4 and 7 days of incubation. After 10 days, subsamples were removed from the bottom of each jar for final chemical analyses and pH measurement (McLean, 1982). Soil NH_4^+, NO_2^- and NO_3^- were extracted by shaking with 1 M KCl (1:5 soil to solution ratio) on a wrist action shaker for 1 h. The extracts were filtered through glass fiber filters (Sparrow and Masiak, 1987) and analyzed using modified Technicon Industrial Method No. 786-86T for NH_4^+ analysis and modified Technicon Industrial Method No. 818-87T for NO_2^- and NO_3^- analyses on a TRAACS 800 continuous flow analytical system (Technicon Industrial Systems, Bran + Luebbe Analyzing Technologies, Elmsford, N.Y.). Total N and organic C reported in Table 1 were determined using an automated Dumas combustion method.

Experimental data were analyzed using the repeated measures ANOVA available in SYSTAT, version 5.0 (SYSTAT Inc., Evanston, Ill.). To facilitate interpreting the multiple interactive effects of soil water content on the processes under study, CO_2 and N oxide emission data were divided into five independently analyzed periods that corresponded to the intervals between soil sampling times. These times had been selected to approximate the times of important transitions in the response of N oxide emissions to imposed N and water treatments. In addition, statistical analysis of emission data for day 8 was conducted separately from that for the last 2 days of the incubation to isolate the response of CO_2 and N oxide emissions to rewetting. Significance was assumed when $P < 0.05$.

RESULTS

Soil water contents at selected times during the 10-day incubation are shown in Fig. 1. Because there were no significant differences in water content among N treatments, only the means for all jars under the same water treatment are presented. Note that after 7 days incubation, soil water content in the medium and high water treatments had fallen below the initial water content of the next drier treatment, while that of the low water treatment declined to 1.4% (w/w). As a result, CO_2 and N oxide emissions were measured over the entire range of water contents from 10 to 1.4% at some time during the experiment (equivalent to water potentials of about -10 to much less than -1000 kPa). The rate of soil drying following rewetting after 7 days incubation was slightly higher than at the beginning of the experiment, because air flowing through each jar was

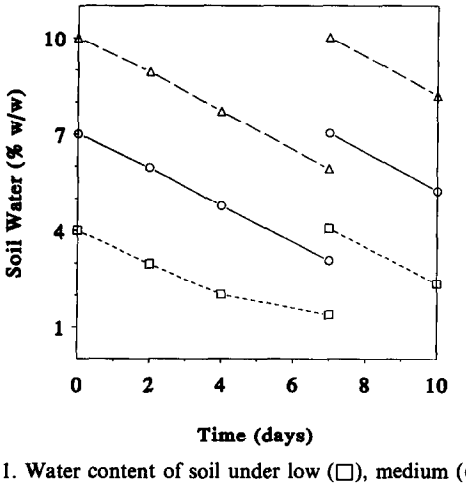

Fig. 1. Water content of soil under low (□), medium (○), and high (△) water treatments during 10 days of incubation. After the seventh day, each soil was returned to its initial water content of 4, 7 or 10% (w/w). Data points represent the means of both replicates of all N treatments (n = 6).

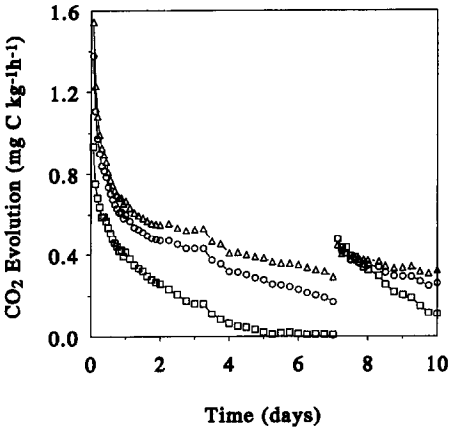

Fig. 2. Carbon dioxide evolution from soil under low (□), medium (○), and high (△) water treatments during 10 days of incubation. After the seventh day, sufficient water was added to return each soil to its initial water content of 4, 7 or 10% (w/w). Data points represent the means of both replicates of all N treatments (n = 6).

saturated by evaporation from a smaller soil area (all the small vials had been removed and their contents extracted for soil chemical analyses).

The flush of CO_2 observed following addition of water at the beginning of the experiment (Fig. 2) was typical of that observed by other authors (e.g. Birch, 1960). There were no significant differences among N treatments, so only mean CO_2 evolution rates for each water treatment are presented. Differences among the three water treatments were statistically significant throughout the first 7 days. Maximum rates of CO_2 evolution apparently occurred prior to the first set of measurements made 90 min after the addition of water. Evolution rates from soil under all water treatments declined rapidly during the first day of incubation, then much more slowly until day 7; the rate from the low water treatment became insignificantly different from zero during day 5. An additional flush of CO_2 was observed from all treatments following rewetting after 7 days incubation. During the first day after rewetting, CO_2 evolution rates from the three water treatments were not significantly different, but then began to diverge as observed after the initial addition of water. Maximum CO_2 evolution rates measured during the second flush were significantly smaller than during the first, particularly for the two higher water treatments.

The response of soil N transformations, and therefore gaseous N oxide evolution, to addition of water was more complex than the response of soil respiration described above. Figure 3 compares the NO emission rates and NH_4^+ and NO_3^- concentrations of soil under all three water treatments without added N or nitrapyrin. Soil emission of NO rose rapidly for the first 3–4 h of incubation, then more slowly to peaks after 30 h of 1.43, 1.30 and 1.21 $\mu g\ N\ kg^{-1}\ h^{-1}$ for the low, medium and high water treatments, respectively [Fig. 3(a)]. Differences among the three water treatments during this period were consistent, statistically significant, and probably reflect the effect of water content on soil gas diffusion rates; i.e. the NO yield of nitrification increases with its ease of escape to the atmosphere (Hutchinson and Andre, 1989; Remde *et al.*, 1989). After 30 h, rates of NO evolution declined rapidly until the end of day 4. Thereafter, NO emissions from the high water treatment remained relatively constant, averaging 0.18 $\mu g\ N\ kg^{-1}\ h^{-1}$. Emissions from the medium water treatment averaged 0.16 $\mu g\ N\ kg^{-1}\ h^{-1}$ except for the 12 h after rewetting, during which there was a second burst of NO evolution that was much smaller and shorter than the first. In contrast, NO emissions from the low water treatment continued declining after day 4, becoming insignificantly different from zero at about the same time that CO_2 evolution from this treatment also approached zero. When this dry soil was rewet to its initial water content (4%), a second large burst of NO emissions occurred; peak emissions were 35% lower, but durations of the two bursts were nearly identical.

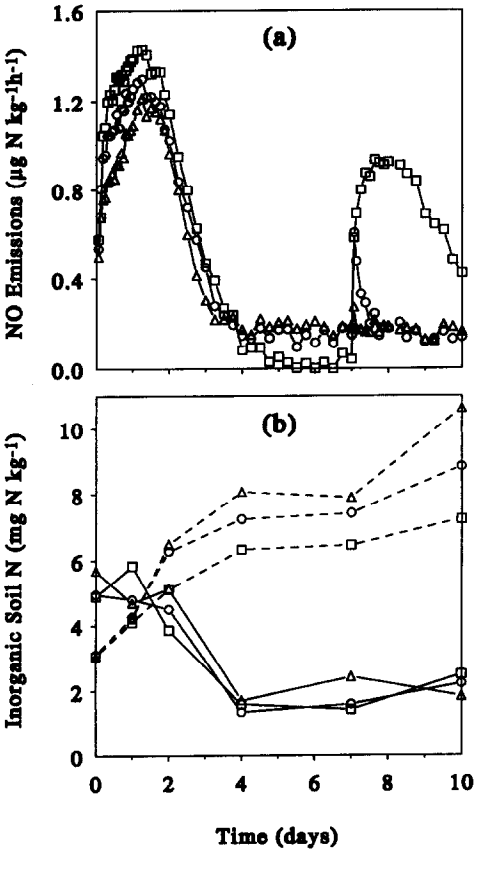

Fig. 3. Nitric oxide emission rates (a) and NH_4^+ and NO_3^- concentrations (b) of soil under low (□), medium (○), and high (△) water treatments during 10 days of incubation with no N amendment. In (b) symbols joined by solid lines represent NH_4^+ concentrations, while symbols joined by dashed lines represent NO_3^- concentrations. Data points represent the means of two replicates.

Inorganic N concentrations shown in Fig. 3(b) reflect the progress of microbially-mediated nitrification. Soil NH_4^+ concentrations decreased during the first 4 days of incubation before stabilizing at about 2 mg N kg^{-1}, while NO_3^- concentrations increased by corresponding amounts during the same periods. Rates of N_2O evolution from soil under these three treatments, as well as the remaining treatments, rarely exceeded our minimum detectable N_2O flux and are not presented; generally, they were about an order of magnitude smaller than NO evolution rates.

Figure 4 shows the effects of NH_4NO_3 and nitrapyrin on the NO evolution rates and NH_4^+ and NO_3^- concentrations of soil under all three water treatments. In the two wetter treatments the peak rate of NO emission was delayed about 30 h and elevated about 3-fold when NH_4NO_3 was added without nitrapyrin [Fig. 4(b), (c)]; thereafter, the emissions

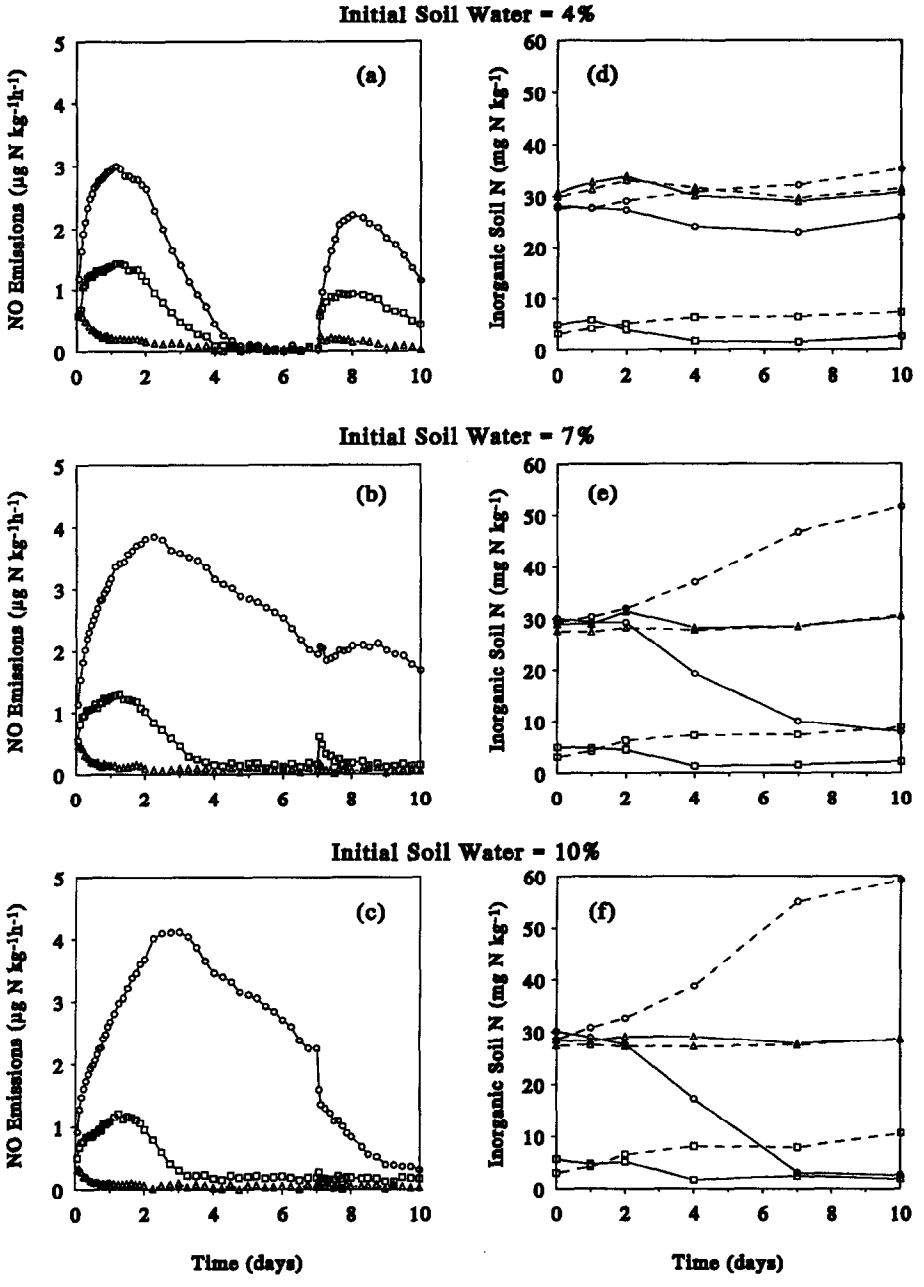

Fig. 4. Nitric oxide emission rates (a)–(c) and NH_4^+ and NO_3^- concentrations (d)–(f) of soil treated with no N amendment (□), NH_4NO_3 (○), or NH_4NO_3 + nitrapyrin (△) during 10 days of incubation. In (d)–(f) symbols joined by solid lines represent NH_4^+ concentrations, while symbols jointed by dashed lines represent NO_3^- concentrations. Data points represent the means of both replicates under the low (a) and (d), medium (b) and (e), and high (c) and (f) water treatments.

generally followed declining NH_4^+ availability. The small but sharp decrease in the rate of NO evolution from fertilized soil shown in Fig. 4(c) coincided with rewetting and probably resulted from the dependence of NO yield on soil gas diffusion rates described earlier. In Fig. 4(b) an analogous drop in NO emissions from the same N treatment was probably offset by a tenuous burst of emissions induced by wetting the moderately dry soil (similar to the small burst of NO from control soil under this water treatment). In contrast, the temporal dependence of NO emissions from NH_4NO_3-treated soil with 4% initial water content was nearly identical to that of the corresponding unfertilized soil, but amplitudes of the two emission peaks were about doubled [Fig. 4(a)]. Soil under this water treatment apparently became dry enough to affect the survival or metabolism of the organisms responsible for NO production.

When nitrapyrin was added with the NH_4NO_3, NO evolution from the medium and high water treatments [Fig. 4(b), (c)] was immediately reduced and continued to decline for about 16 h before becoming relatively constant for the remainder of the incubation. The delay in achieving maximum inhibition probably represents the time required for this sparingly-soluble chemical to dissolve and diffuse to every colony of NH_4^+ oxidizers. Nitric oxide emission from nitrapyrin-treated soil under the low water treatment [Fig. 4(a)] was also inhibited, but remained significantly higher than that from the two wetter treatments during both emission bursts, probably because the limited availability of water retarded the inhibitor's dissolution and diffusion. There were no significant changes in the NH_4^+ or NO_3^- concentrations of nitrapyrin-treated soil during 10 days of aerobic incubation [Fig. 4(d)–(f)], and CO_2 evolution was not significantly affected by the inhibitor, thus confirming that the chemical behaved as both a potent and specific inhibitor of chemoautotrophic NH_4^+ oxidation.

DISCUSSION

Biochemical source of gaseous N oxides

Chemoautotrophic NH_4^+-oxidizing bacteria were the predominant source of gaseous N oxides over the entire range of soil water potentials included in the experiment, thus confirming and extending recent findings (e.g. Lipschultz *et al.*, 1981; Johansson and Galbally, 1984; Anderson and Levine, 1986; Remde *et al.*, 1989; Tortoso and Hutchinson, 1990). The principal basis for this conclusion is that production of NO was reduced by nitrapyrin to low (but nonzero) mean rates of 0.14, 0.09 and 0.06 $\mu g\ N\ kg^{-1}\ h^{-1}$ for the low, medium and high water treatments, respectively (Fig. 4). These means exclude data for the first 16 h of incubation required for the inhibitor to dissolve and diffuse to the target organisms, as well as the last 60 h prior to rewetting the soil, when both NO and CO_2 evolution from the low water treatment were reduced to zero by the limited availability of water. Increasing the inhibitor concentration did not further reduce the rate of NO emission from soil with water content near field capacity (data not shown), suggesting that the small amounts of NO evolved from nitrapyrin-treated soil were not produced by autotrophic NH_4^+ oxidizers.

Because there was no apparent response of N oxide emissions to NH_4NO_3 in the presence of nitrapyrin, we concluded that denitrification made no significant contribution to NO or N_2O emissions from this soil, even when it was kept at *ca* -10 kPa water potential. Although soil gas diffusion rates were undoubtedly reduced at the higher water contents, the supply of O_2 was apparently not sufficiently limited to favor use of NO_3^-, rather than O_2 (which is preferred), as a terminal electron acceptor by the facultative anaerobes primarily responsible for denitrification. It should be emphasized, however, that this observation may not be repeated during incubation of finer-textured, more compact or deeper samples of soil at this relatively high water potential.

Because autotrophic nitrification and heterotrophic denitrification were apparently both inactive in nitrapyrin-treated soil, there was little opportunity for accumulation of the NO_2^- required by chemodenitrification, so we concluded that this process also made no significant contribution to the low rates of NO emission from these treatments. Relatively high soil pH (Table 1) further discounts the possibility of significant chemodenitrification. Of the known soil sources of gaseous N oxides, only heterotrophic nitrification was not excluded, and this process may have been responsible for the observed small emission rates (Schimel *et al.*, 1984; Robertson and Tiedje, 1987). The observation that NO and CO_2 evolution from the low water treatment simultaneously approached zero during the fifth day is consistent with this possibility.

Except during the initial bursts after both additions of water, NO emission rates generally followed changes in soil NH_4^+ concentration, thus providing additional evidence for the importance of nitrification as the source of gaseous N oxides. For example, the rate of post-burst NO emission from control soil under the medium and high water treatments was strongly correlated with soil NH_4^+ concentration ($R^2 = 0.71$ for measurements made after 2, 4, 7 and 10 days). When NH_4NO_3 was added, NO emissions still varied in concert with soil NH_4^+ concentration ($R^2 = 0.70$ for the same times and water treatments), but the regression coefficient (0.100 ± 0.017 SE μg NO-N $kg^{-1}\ h^{-1}$ per mg $NH_4^+ - N\ kg^{-1}$) was only about half as large as for control soil (0.207 ± 0.035 SE). This difference suggests the interesting possibility that the $NO:NO_3^-$ product ratio of nitrification may be higher at the low NH_4^+ concentrations commonly found in most soils. However, a more probable explanation is that gross nitrification rates are not linearly related to NH_4^+ concentration, particularly in

unfertilized soil where turnover of the NH_4^+ pool may occur as frequently as daily (Davidson *et al.*, 1990).

In the regressions described above we purposely excluded all data from the low water treatment and other data accumulated during water-induced emission bursts. Because some factor other than NH_4^+ availability controlled NO emission rates in these cases, including these data gave a much poorer fit. Williams *et al.* (1988) also observed that interacting control factors rendered soil NH_4^+ concentration a poor predictor of NO emissions at several diverse sites in Pennsylvania. In contrast to their results, however, we found this relation to be a better predictor than that of NO emissions to soil NO_3^- concentration. Hutchinson and Brams (1992), who measured NO and N_2O evolution from the field plots sampled for this experiment, also found that the emission rates were more strongly correlated with soil NH_4^+ concentration ($R^2 = 0.69$) than NO_3^- concentration ($R^2 = 0.34$). One possible reason for these opposing observations is that denitrification was the source of at least part of the NO measured by Williams *et al.* (1988), while O_2 diffusion rates in the well-drained sandy loam we studied were never restricted enough to support denitrification activity. An alternative explanation that does not require assumption of a denitrification source is that in comparisons across widely-divergent ecosystem types, N oxide emissions may be related to NO_3^- concentration simply because this ion generally accumulates where N availability exceeds C availability to soil microorganisms, a condition that also favors a leaky N cycle (Hutchinson and Davidson, 1992).

NO emissions response to wetting dry soil

In contrast to the results described above, water-induced bursts of NO evolution bore no relation to soil NH_4^+ availability. For example, there were no measurable differences in NH_4^+ concentration among unfertilized soils under the three water treatments that might explain differences in their responses to either addition of water (Fig. 3). Nevertheless, the bursts were virtually eliminated by nitrapyrin, indicating involvement by autotrophic NH_4^+ oxidizers. Davidson (1992) proposed that the burst of NO emissions induced by wetting dry soil may result from chemodenitrification of NO_2^- in transit from NH_4^+ oxidizers to NO_2^- consumers (primarily *Nitrobacter*), but his data did not exhibit the NO_2^- concentration dependence expected for such a mechanism. For example, he reported that a preliminary treatment of the incubated soil with acetylene caused only a 23% reduction in post-incubation soil NO_2^- concentration, but a several-fold reduction in NO emission throughout his 24 h experiment. In addition, the response of NO emissions to added NO_2^- (2 mg $N\,kg^{-1}$) was disproportionately large compared to the emission rate he measured from soil containing 1.3 mg $NO_2^- - N\,kg^{-1}$ apparently produced by NH_4^+-oxidizing bacteria. In our study, soil NO_2^- concentrations never exceeded 90 μg $N\,kg^{-1}$, and although they were higher after 1, 2, 4, 7 and 10 days incubation than in samples extracted immediately after initial wetting, there were no other significant differences among sampling times or N and water treatments. As a result, our data add no support for Davidson's (1992) hypothesis, but neither do they eliminate this possibility because (1) soil sampling times may not have been appropriate to reveal the dynamics of rapidly-changing soil NO_2^- pool sizes and transformation rates, (2) bulk soil analyses may have provided a poor measure of microsite NO_2^- concentrations near NH_4^+-oxidizer colonies, and (3) although soil pH decreased in proportion to the amount of nitrification that occurred, even the minimum observed bulk soil pH of 6.9 was high compared to that where chemodenitrification has been shown to be an important process.

Rewetting the soil after 7 days incubation caused a second large burst of NO evolution from only the lowest water treatment, indicating that the occurrence of such a response depended on the degree of dryness attained, rather than the amount of time elapsed, since the previous burst. To elicit a wetting response, it appeared that the soil had to become sufficiently dry to influence the survival or metabolic activity of NO-producing organisms, such as occurred in the low water treatment during the last 60 h prior to rewetting. In the medium water treatment where mean soil water content decreased to 3.1% after 7 days of incubation, there was no similar approach to zero by CO_2 or NO emissions, yet there was a tenuous response of NO emissions to rewetting [Fig. 4(b)]. Apparently, desiccation effects on the NO-producing organisms were just beginning to materialize. Soil water content in this case was similar to its 2.8% water content during storage at which there was a large response of NO emissions to the initial addition of water, thereby suggesting a potential interaction between exposure time and exposure concentration in establishing the conditions prerequisite for supporting a burst of gaseous N oxide emissions following the next addition of water. Alternatively, other environmental limitations on microbial growth (e.g. long-term exposure to low temperature) may have substituted for limited water availability in predisposing the organisms to a burst of gaseous N oxide evolution when favorable growth conditions returned at the start of the experiment. Our study was not designed to discriminate between these alternative hypotheses.

$NO : NO_3^-$ ratio of nitrification products

The apparent antagonistic relationship between the water content and NO emission rate of control soil during the first 2 days of incubation (Fig. 3) was hypothesized earlier to be due to soil water's effect on gas phase transport rates. This effect is shown more dramatically in Table 2, where the total amount of N evolved as NO from control or NH_4NO_3-treated soil

Table 2. Total amount of N evolved as NO expressed as a fraction of the total amount of N oxidized to NO_3^- during 10 days incubation of control and NH_4NO_3-amended soil under three water treatments

Initial soil water content (%)	N evolved as NO / N oxidized to NO_3^-	
	Control	NH_4NO_3
4	0.035	0.053
7	0.017	0.029
10	0.012	0.019

under each water treatment is expressed as a fraction of the total amount of N oxidized to NO_3^- during 10 days of incubation. For both N treatments there was nearly a 3-fold difference in the $NO:NO_3^-$ product ratio of nitrification between the low and high water treatments. Because we found no evidence that O_2 was limiting under any of our experimental treatments, we assumed that gas diffusion rates were not severely restricted even under the high water treatment, so the data in Table 2 stress just how critical the transport dependence is of the NO yield of nitrification in soil. Corroborative evidence was provided by Hutchinson and Andre (1989) and Remde *et al.* (1989). The transport dependence of NO yield probably results from its propensity for rapid oxidation to NO_2^- in aerobic environments, reduction to N_2O in anaerobic environments, assimilation in N-limited environments etc.

Data in Table 2 suggest that any factor that influences soil gas diffusion rates will also affect both the magnitude and composition of its gaseous N oxide emissions. Therefore, extreme caution must be exercised in extrapolating the results of laboratory soil incubation studies to the natural environment. Such laboratory studies typically employ shallow layers of sieved soil maintained at moderate soil water content, which may support vastly different gas transport rates than the same soil in the field. The data also emphasize the advantages of open (flow-through) incubation systems for studying aerobic processes involved in the production and emission of gaseous N oxides from soil; in closed incubation systems headspace gases remain in contact with the soil where they are subject to further reaction.

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Trade names and company names are included for the reader's convenience, and such inclusion does not constitute any preferential endorsement by the USDA of products named over similar products available on the market.

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