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Mechanisms and kinetics of nitric and nitrous oxide production during nitrification in agricultural soil

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Abstract

Laboratory experiments were conducted with three California agricultural soils to examine substrate and process controls over temporal variability of NO and N_2O production during nitrification, and to quantify the kinetics of HNO_2-mediated chemical reactions. Gross NO production rates were highly correlated ($r^2 = 0.93$–0.97) with calculated concentrations of HNO_2, which were shown to originate from autotrophic microbial oxidation of NH_4^+ to NO_2^-. Production of NO was not correlated with NH_4^+ or NO_3^-, or with the overall nitrification rate. Distinct periods of high NO_2^- accumulation occurred below critical pH values in each soil, apparently due to inhibition of microbial NO_2^- oxidation. Data suggest that even during periods of relatively low NO_2^- accumulation and rapid overall nitrification, HNO_2-mediated reactions may have been the primary source of NO. Rate coefficients (k_{PNO}) relating NO production to HNO_2 concentrations were determined for sterile (λ-irradiated) soils, and were similar to k_{PNO} values in 2 of 3 nonsterile soils undergoing nitrification. Production of N_2O was correlated with HNO_2 ($r^2 = 0.88$–0.99) in sterile soils, and with NO_2^- and NO_3^- ($R^2 = 0.72$–0.91) in nonsterile soils. Experiments using ^{15}N confirmed that dissimilatory NO_3^- reduction contributed to N_2O production even under primarily aerobic conditions. Sterile k_{PNO} and k_{PN2O} values were correlated ($r^2 = 0.90$ and 0.82) with soil organic matter content. Overall, the results demonstrate that both steps of the nitrification sequence, together with abiotic reactions involving NO_2^-/HNO_2 need to be considered in developing improved models of NO and N_2O emissions from soils.

Keywords: acidity, chemodenitrification, denitrification, inhibition, nitrite, nitrous acid

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Introduction

Nitric oxide (NO) and nitrous oxide (N_2O) are important atmospheric trace gases (Crutzen 1979, 1981; Rodhe 1990) which can be produced and transformed by microbial and chemical processes in many ecosystems (Firestone & Davidson 1989). While agricultural soils have been recognized as a significant source of NO and N_2O, present estimates of the relative importance of these emissions on a regional and global scale are highly uncertain (Potter *et al.* 1996; Davidson & Kingerlee 1997). An improved understanding of the mechanisms controlling the soil–atmosphere exchange of NO and N_2O has been identified as a critical research need (Mosier *et al.* 1996; Matson 1997). Elucidation of mechanisms is required in order to establish parameters for process-based models, and will also assist development of management strategies for mitigating impacts of N losses from intensively fertilized systems (Matson *et al.* 1998).

NO and N_2O are produced during the transformation of soil N by the microbial processes of nitrification and denitrification and from abiotic reactions (Firestone & Davidson 1989). In agricultural soils, NO emissions have been found to correlate positively with fertilizer N inputs (Veldkamp & Keller 1997) and soil temperature (Williams *et al.* 1992). Most studies suggest that nitrification is the primary source of NO emissions, based on positive correlations with soil ammonium (NH_4^+) concentrations and increases following application of NH_4^+-based fertilizers (Slemr & Seiler 1991; Hutchinson & Brams 1992). Several other studies have found positive correlation of NO flux with soil NO_3^- concentrations (Williams & Fehsenfeld 1991; Thornton & Valente 1996). In general, however, researchers have been unable to develop useful models based on soil NO_3^- and/or NH_4^+ levels (Shepherd *et al.* 1991), and it is uncertain which

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index of N availability best captures the dependency of NO emissions (Hutchinson *et al.* 1997). Many studies relating variations in NO emissions to soil variables have used measurements of *net* production or emission rates, even though it is known that the high reactivity of NO in soil can potentially confound any relationships between *gross* NO production and controlling variables.

Process-based models have been proposed which describe NO and/or N_2O emission rates as a function of N substrate levels, gross N mineralization, denitrification and/or nitrification rates (e.g. Li *et al.* 1992; Potter *et al.* 1996; Riley & Matson 1998). Many of these models are specific formulations of the conceptual 'hole-in-the-pipe' model (Firestone & Davidson 1989), which proposed that a proportion of the N which flows through the nitrification and/or denitrification process 'leaks' out in the form of gaseous N oxides. Most if not all of the biotic mechanisms which have been implicated in contributing to NO and N_2O production in soils are known to involve nitrite (NO_2^-) as a central substrate or reactant (Conrad 1995). In aqueous systems at $pH < 5$, the protonated form of NO_2^-, nitrous acid (HNO_2, $pKa = 3.3$), becomes more predominant, and decomposes spontaneously to yield NO (Pauling 1970). In soils, HNO_2 can also react with organic constituents to form both NO and N_2O (Stevenson 1994). The importance of HNO_2-mediated reactions in controlling NO emissions in acid soils has been suggested by field studies (Serca *et al.* 1994). It has also been proposed that the existence of highly acidic clay surfaces and microsites, even in nonacid soils, can promote more significant rates of HNO_2 formation and decomposition than suggested by bulk soil pH measurements (Nelson 1982). In theory, reactions involving NO_2^-/HNO_2 represent a potentially important route by which N flowing through the two-step nitrification sequence, i.e. $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$, can partition to gaseous forms, consistent with the hole-in-the-pipe model. However, the temporal dynamics of NO_2^- concentrations together with pH have rarely been monitored in studies examining controls over NO and N_2O production, so these hypotheses have not been thoroughly tested.

The objectives of this study were therefore (i) to examine the temporal dynamics of and relationships between N substrate levels, nitrification rates and gross NO and N_2O production rates in agricultural soils under conditions favouring nitrification (ii) to examine the role of NO_2^-/HNO_2 and the importance of abiotic processes occurring simultaneously with nitrification, and (iii) to characterize some aspects of the NO_2^-/HNO_2-mediated kinetics of NO and N_2O production.

Materials and methods

Soils

Three agricultural soils from the Sacramento Valley of California were selected to represent a range of clay and organic matter contents (Table 1). Soils were sampled from the top 10 cm, and were air dried, ground mechanically, passed through a 2-mm sieve, and stored at room temperature.

Nitrification experiments

Prior to each nitrification experiment, ~1.5 kg of soil was flushed with an aqueous solution designed to approximate soil solution concentrations for a Sacramento Valley agricultural soil (Wolt 1994), with the addition of a NH_4^+ fertilizer source (2.5 mM $CaCl_2$, 2.5 mM $CaSO_4$, 1.25 mM $MgSO_4$, 0.625 mM K_2SO_4, and 7.5 mM $(NH_4)_2SO_4$). Soils were flushed slowly with solution until effluent NH_4^+ concentrations reached influent levels, and were then drained under suction. Tensions of −100, −200 and −500 mbar were applied to the Lang, Reiff and Yolo soils to achieve gravimetric water contents

Table 1 Some properties of soils used in laboratory experiments

Soil series texture	Lang loamy sand	Reiff sandy loam	Yolo silt loam
USDA classification	Psammaquent	Xerofluvent	Xerorthent
Sand (%)	74	62	36
Silt (%)	22	28	46
Clay (%)	4	10	18
pH (*1 : 1 KCl*)	5.6	6.5	6.3
Organic C^1 (%)	0.32	0.88	1.40
Total N^2 (%)	0.03	0.05	0.10
CEC (*μeq/g*)	70	170	230
recent use	alfalfa/tomatoes	corn/tomatoes	annual row crops

^{1}Dichromate oxidation; ^{2}Total Kjeldahl N.

of 0.14, 0.16 and 0.22 (± 0.01) g H_2O g^{-1} soil, respectively (% saturation ranged from 40 to 42%). Drained soils were passed through a 3-mm sieve and put into acrylic cylinders which were flushed continuously with humidified ambient air streams in a temperature-controlled room (25 °C) for 40–60 days. The systems were subsampled at 2–3 day intervals for determination of inorganic N concentrations, pH, water content, gross NO and N_2O production rates and first-order NO consumption rate coefficient. Two replications of the nitrification experiments were done for each soil (termed 'expt 1' and 'expt 2'). All statistical analyses were performed using Statgraphics (Manugistics, Inc.) statistical software.

Soil chemical analysis

At each sampling time, two replicate subsamples were extracted with 0.5 M K_2SO_4 solution at a liquid:soil mass ratio of ~10 : 1. For low-level NO_2^- analysis (1 μg N g^{-1} soil), lower liquid:soil ratios were used so that levels as low as 0.01 μg N g^{-1} soil could be detected. Soils were extracted on a reciprocating shaker followed by centrifugation. Within 2 h of sampling, supernatant was removed for NO_2^- analysis by the modified Griess–Ilosvay method (Keeney & Nelson 1982). Remaining solution was stored at 4 °C and analysed within 10 d for total (NO_3^-+ NO_2^-)-N using cadmium (Cd) reduction followed by the modified Griess-Ilosvay method, and for NH^+_4-N using the indophenol blue method (Keeney & Nelson 1982). Reagent additions and colourimetric analyses for NO_2^-, NO_3^- and NH_4^+ were done manually (Hitachi 100–30 spectrophotometer). The assumption that the NO_2^- assay responded to total (NO_2^-+ HNO_2)-N concentration was tested by analysis of aqueous KNO_2 solutions containing 2.3 μg cm^{-3} of total (NO_2^-+ HNO_2)-N, adjusted to either pH 4 (at which ~20% of the total N is in the form of undissociated HNO_2) or pH 7 (< 0.05% is in form of HNO_2). No significant differences in analysed concentrations (< 1.5%) were found, indicating that the method did not discriminate between NO_2^- and HNO_2.

Preliminary experiments were carried out to evaluate methods for determining soil pH. Results using 1 M KCl as an extraction solvent were 0.1–0.5 units lower than with 0.01 M $CaCl_2$, but were more reproducible (standard deviation = ± 0.03 units and ± 0.07 units, respectively). Therefore, 1 M KCl was used for pH analysis in subsequent experiments. A separate subsample (4–12 g) was mixed with an equal mass of 1 M KCl solution, stirred manually and allowed to settle for 1 h before removal of supernatant for pH measurement. Gravimetric water content was determined by weighing a separate subsample before and after drying at 105 °C for 8–24 h.

The pH-dependent equilibrium between NO_2^- and HNO_2 was assumed:

$$H^+ + NO_2^- \rightleftharpoons HNO_2. \qquad (1)$$

Concentrations of HNO_2-N were calculated using the measured total (NO_2^-+ HNO_2)-N molar concentrations, soil pH (KCl) and the acid dissociation constant (pKa = 3.3, Van Cleemput & Samater 1996) for (1) in the equilibrium expression:

$$[\mathrm{HNO_2\text{-}N}] = \frac{[\mathrm{NO_2^-\text{-}N + HNO_2\text{-}N}]_{\mathrm{total}} \cdot 10^{-\mathrm{pH}}}{(10^{-\mathrm{pH}} + 10^{-\mathrm{pKa}})}. \qquad (2)$$

Soil pH values were used to calculate hydrogen ion (H^+) concentrations in (2). Because soil pH measurements vary with the type and concentration of extracting solution used, the soil:liquid ratio, and other factors (Bohn *et al.* 1985; Sumner 1994), the calculated HNO_2 concentrations are specific to the pH method employed.

Gross NO and N_2O production rates

A dynamic chamber system based on that of Remde *et al.* (1989) was used for determination of gross NO production rate (Fig. 1). The reaction chamber consisted of an acrylic cylinder (76 mm ID × 100 mm high) with O-ring-sealed end caps. A stainless steel screen installed horizontally across the mid-section supported a stainless steel screen sample holder. A gas stream entered the chamber through ports on the top and on one side, and exited through a port on the opposite side (FEP tubing and stainless steel fittings were used). Concentrations of NO in the influent stream were controlled by regulating the ratio of humidified air and standardized NO gas entering a static mixing tube, using variable area flow regulators. Total flow rate was determined for each measurement using a soap-film flowmeter and digital stopwatch (± 0.01 s). Preliminary experiments showed that the reaction chamber system (with no soil) behaved as a well-mixed reactor, and that no measurable losses of NO occurred across the empty chamber when influent NO gas concentrations were < 12 ng NO-N cm^{-3} (20 μL L^{-1}). For each sample, a thin layer (2–8 mm depth) of soil was placed inside the chamber. Typically, 3–4 levels of influent NO concentration were used for each test within the range of 0–5 ng N cm^{-3}. At each influent NO level, the net NO production rate (P_{net}, ng N g $soil^{-1}$ h^{-1}), was determined from:

$$P_{\mathrm{net}} = \frac{q}{m_{\mathrm{s}}}(C_{\mathrm{e}} - C_{\mathrm{i}}), \qquad (3)$$

where C_e=chamber effluent NO concentration (ng N cm^{-3}), C_i=chamber influent NO concentration (ng N

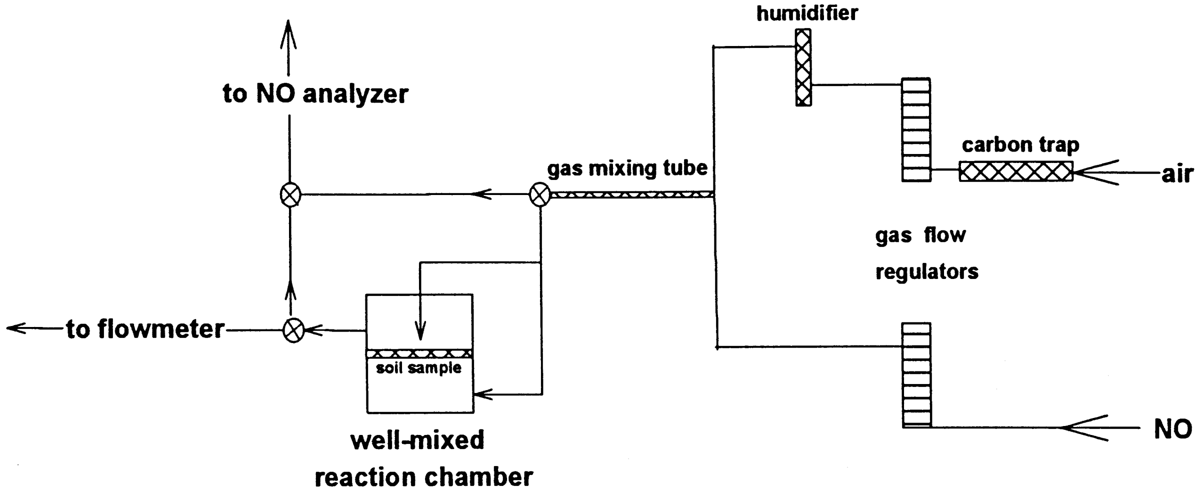

Fig. 1 Test system for measurement of gross NO production rate.

cm^{-3}), q = air flow rate (cm^3 h^{-1}), m_s=oven dry soil mass (g). Following Remde *et al.* (1989), the assumption of simultaneous zero-order gross NO production and first-order NO consumption were made, so that for each level:

$$P_{\text{net}} = P_{\text{NO}} - k_c C_e. \qquad (4)$$

Regression of P_{net} vs. C_e was used to determine the gross NO production rate (P_{NO}, ng N g soil^{-1} h^{-1}) and apparent first-order consumption rate coefficient (k_c, cm^{-3} g soil^{-1} h^{-1}) for each sample. Concentrations of NO were determined with a chemiluminescent analyser (Sievers Instruments 270B, Boulder, CO), utilizing O_3 oxidation of NO. The NO analyser was calibrated weekly using gas standards of NO in N_2 (Scott-Marin, Riverside CA and Puritan-Bennet, Lenexa, KS) which were diluted with air using the gas mixing system.

Gross N_2O production rates (P_{N2O}) were measured by incubation of subsamples (3–12 g) in 230-cm^3 glass canning jars equipped with Mininert gas sampling ports (Dynatech Precision Sampling Corporation, Baton Rouge LA). Headspace N_2O concentrations were measured typically after 0, 1 and 3 h of incubation, by injection into a gas chromatograph (Hewlett-Packard 6890/^{63}Ni electron capture detector) which was calibrated weekly using 0.31 and 1.5 µL L^{-1} standards. Headspace O_2 partial pressures were always $\geqslant$ 19.5 kPa. Production rates were determined from the increase in concentration, headspace volume and mass of soil, and were corrected for N_2O dissolved in the soil water assuming gas–liquid equilibrium (Moraghan & Buresh 1977). The P_{N2O} measurements were made in expt 2 with Lang, Reiff and Yolo soils, and also in expt 1 with Lang soil.

Nitrification inhibition experiments

Periodically during the nitrification experiments, subsamples (25–75 g soil) in 230-cm^3 glass canning jars were exposed to an atmosphere containing 10 Pa of acetylene (C_2H_2), which inhibits the NH_4^+-oxidizing activity of autotrophic nitrifiers without affecting the mineralization of organic N (Ryden 1982). After 8–12 h of C_2H_2 treatment, the soils were flushed with humidified air exiting from the corresponding incubation cylinder for the following 24–48 h. Subsamples of C_2H_2-treated soil were then removed for analysis of inorganic N concentrations and gas production rates.

N mineralization experiments

Experiments were conducted to estimate the influence of processes other than autotrophic nitrification on NH_4^+ concentrations in the nitrification experiments, including the release of N from soil organic matter, immobilization by microbial incorporation, NH_3 volatilization and clay fixation. The net rate of increase in NH_4^+ concentration due to these processes collectively is defined here as the net mineralization rate (MR). Changes in NH_4^+ concentrations occurring in the C_2H_2 experiments (above) were within the range of the coefficient of variation (CV) for the NH_4^+ analysis (1–5% of initial concentrations) and therefore could not be used to calculate MRs. Accordingly, soil was flushed with the simulated soil solution containing no $(NH_4)_2SO_4$ and then incubated as in the nitrification experiments. Changes in NH_4^+ concentrations (15–70% of initial concentrations) occurring during 24–48 h after C_2H_2 treatment of these soils were used to calculate MRs.

Estimation of gross NH_4^+ oxidation rates

Estimates of gross autotrophic NH_4^+ oxidation rates (AOR) were made so that these rates could be compared to corresponding gas production rate measurements. For specific periods of each nitrification experiment, a net NH_4^+ depletion rate (ADR) was determined by regression of NH_4^+ concentrations vs. time, and the gross AOR was calculated from:

$$\mathrm{AOR} = \mathrm{ADR} + \mathrm{MR}. \tag{5}$$

Sterile soil experiments

Portions of each of soil were amended with solutions of sulphuric acid (H_2SO_4, 0.025–0.075 M). Two pH levels in addition to baseline levels were generated for each soil, corresponding approximately to: (i) the lowest pH, and (ii) an intermediate value between the baseline and lowest pH values observed during the nitrification experiments. Samples of unamended and H_2SO_4-amended soils were air dried and then exposed to radiation at a dose of 3 Mrads (at Phoenix Memorial Laboratories, University of Michigan, Ann Arbor). Irradiated soils were held for 4–12 months in glass jars or double-sealed plastic bags prior to use. The soils were then amended with the simulated soil solution used in the nitrification experiments to which varying concentrations of KNO_2 were added. Concentrations of KNO_2 were selected to achieve the same water content and range of soil NO_2^- and HNO_2 concentrations measured during the nitrification experiments. Solutions were added to the soil gravimetrically, mixed uniformly, and analysed within 10–20 min following addition of solution for NO_2^-, pH and gross NO and N_2O production rates. Solutions and materials were sterilized by autoclaving or washing with 95% ethanol. Similar experiments were done using soils treated with H_2SO_4 but not irradiated (nonsterile controls). The λ-irradiated and nonsterile control soils were kept in an air-dry state until addition of KNO_2 solutions immediately before making measurements. Sterile and nonsterile control soil experiments were carried out over a period of 3–4 weeks for each soil.

$^{15}NO_3^-$ reduction

Preliminary data analysis indicated that dissimilatory NO_3^--reduction may have been responsible for some of the N_2O produced during the nitrification experiments. This was further assessed using stable isotope (^{15}N) techniques. Portions of each soil were flushed with the simulated soil solution, allowed to air dry, and then amended with an aqueous solution of ^{15}N-labelled potassium nitrate (KNO_3) (51 atom% ^{15}N), to achieve NO_3^- concentrations of 70–100 μg N g^{-1} soil and at the same water contents used in the nitrification experiments. Soils were incubated as in the nitrification experiments for 2–3 days, and 20-g subsamples were then treated with 10 Pa of C_2H_2, followed by determination of gross N_2O production rate. Headspace gas samples were analysed for atom percentage ^{15}N in the N_2O and N_2 pools using continuous-flow isotope ratio mass spectrometery (IRMS) (Europa Hydra 20–20, PDZ Europa Ltd, UK). Soils were extracted in 0.5 M K_2SO_4 and atom percentage ^{15}N in the NO_3^- pools were determined according to methods described by Stevens & Laughlin (1994).

Results

Nitrification experiments

Inorganic N dynamics. Flushing with the salt/NH_4^+ solution resulted in uniform soil NH_4^+ concentrations for each soil (CV = 4–6%, *n* = 5). Initial concentrations of approximately 225, 580 and 800 μg NH_4^+-N g^{-1} soil were retained by Lang, Reiff and Yolo soils, respectively, varying in proportion to cation exchange capacity. These NH_4^+ levels are within ranges measured in field samples following application of banded fertilizer N (McIntosh & Frederick 1958; Chalk *et al.* 1975). Patterns of inorganic N dynamics (Fig. 2) were consistent in all experiments: (i) a short (3–4 d) period of increasing nitrification rate, (ii) a period of rapid and fairly constant nitrification, lasting for approximately 28, 10 and 22 d in Lang, Reiff and Yolo soils, respectively, followed by (iii) a period of reduced nitrification and NO_2^- accumulation.

(i) *Increasing nitrification rate period*. In all experiments, NO_3^- accumulation was evident within 2 d, and the rate of NO_3^- accumulation increased over the first 4 d. A peak in NO_2^- concentrations occurred in the Lang and Reiff experiments on days 4 and 2, reaching maximum concentrations of 0.06 and 1.1 μg N g^{-1} soil. In Yolo soil, NO_2^- levels were fairly constant (~ 0.15 μg N g^{-1}). Net N mineralization rates after 2 d of incubation in the C_2H_2-treated soils (not amended with N) were 63, 45 and 21 ng N g^{-1} soil h^{-1}, respectively, for Lang, Reiff and Yolo soils, and subsequently decreased (Table 2).

(ii) *Rapid nitrification period (low NO_2^- accumulation)*. After the first 3–4 d, concentrations of NH_4^+ and total $NO_2^- + NO_3^-$ changed linearly with respect to time ($r^2 > 0.95$) (Table 2). The NO_2^- concentrations were below detectable levels (0.01 μg N g^-) in Lang experiments, and were also low in Reiff and Yolo soils (0.1–0.4 μg N g^{-1}). Gross NH_4^+ oxidation rates (AORs) were 5–8 times greater in Reiff and Yolo as compared to Lang soil. Net $NO_2^- + NO_3^-$ accumulation rates were within 79–110% of calculated gross autotrophic NH_4^+ oxidation rates (AOR) (Table 2), with better agreement for Lang (96–110%) and Reiff (97–103%) than for Yolo (79–81%). The 20% imbalance in Yolo soil may have been due to NH_4^+ losses not accounted for in the mineralization experiments and/or losses of NO_3^- by denitrification.

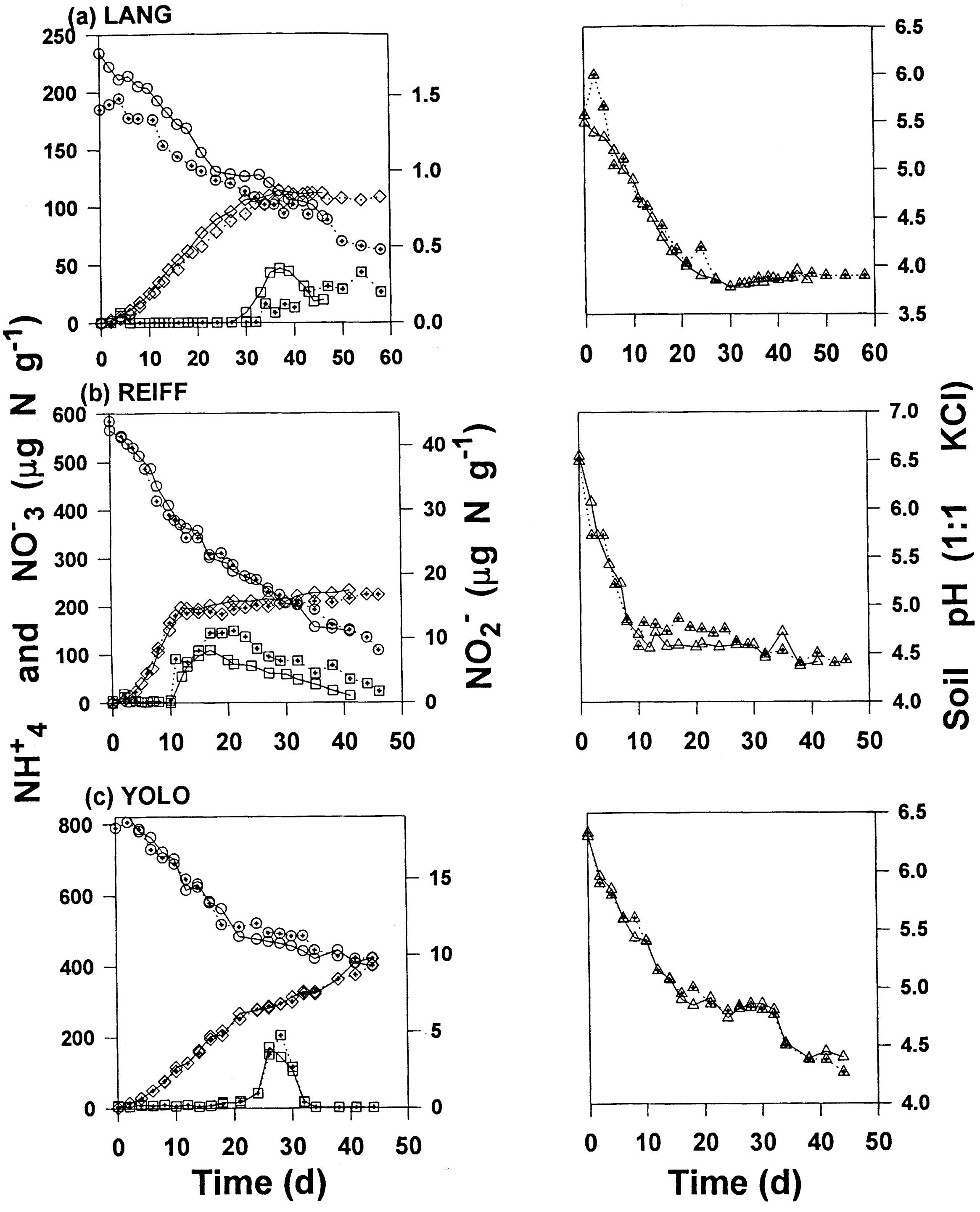

Fig. 2 Inorganic N concentrations and soil pH in (a) Lang (b) Reiff and (c) Yolo soil nitrification experiments. Each point is mean of 2 replicates. ○ = NH_4^+-N, ◇ = NO_3^- N, □ = NO_2^-N, △ = soil pH. Open symbols, solid lines=expt 1; dotted symbols, dashed lines=expt 2.

(iii) *NO_2^- accumulation/reduced nitrification rate period.* In all experiments, rates of both NH_4^+ and NO_2^- oxidation subsequently decreased coincident with an abrupt increase in NO_2^- concentrations (Table 2, Fig. 2). Rates of accumulation of NO_2^- + NO_3^- were markedly decreased in relation to NH_4^+ oxidation rates (Table 2)

Table 2 N transformation rates in nitrification experiments (in ng N g^{-1} h^{-1})

	Net mineralization[1]	Net accumulation[2]		Net depletion[2]	Gross oxidation[3]	
	NH_4^+	NO_3^-	$NO_2^- + NO_3^-$	NH_4^+	NH_4^+	*n*
High nitrification rate phase						
Lang 1	4 (± 0.4)	176	176	172	176	11
Lang 2		155	155	130	134	11
Reiff 1	17 (± 3.5)	807	807	767	784	5
Reiff 2		995	996	1008	1025	4
Yolo 1	6 (± 1.7)	582	581	730	736	9
Yolo 2		566	566	689	695	9
NO_2^- accumulation/reduced nitrification phase						
Lang 1	5 (± 0.4)	13.6 (0.41)	13.6 (0.43)	94 (0.94)	99	8
Lang 2		6.5 (0.18)	6.2 (0.17)	77 (0.92)	82	10
Reiff 1	−2 (± 0.5)	44.9 (0.91)	51.9 (0.93)	338	336	13
Reiff 2		41.8 (0.93)	50.0	301	299	16
Yolo 1	−12 (± 2.8)	221 (0.91)	231 (0.91)	251 (0.91)	239	6
Yolo 2		210	210	243 (0.80)	231	6

[1] Net mineralization rates (MR) in C_2H_2-treated, unfertilized soil (± 1 SD, $n = 2$).

[2] Accumulation and depletion rates from concentration vs. time regression; $r^2 \geqslant 0.95$ (unless noted in parentheses).

[3] Gross NH_4^+ oxidation rate (AOR) = net NH_4^+ depletion rate + net NH_4^+ mineralization rate.

in Lang and Reiff (8–17% of AORs), while in Yolo soil approximately 90% of the oxidized NH_4^+ was recovered as $NO_2^- + NO_3^-$ (Table 2).

Net mineralization. The AORs (Table 2) were calculated from (5) using the net N mineralization rates (MRs) measured in the C_2H_2-treated unfertilized soils. A major assumption of this estimate is that MRs were not influenced by N concentrations and chemical changes occurring in the N-amended soils. It is not clear how the combination of lower pH and higher NH_4^+ levels in the N-amended systems may have affected rates of NH_3 volatilization compared to the unamended systems. In any case, the impacts of such effects on gross AOR estimates are likely to have been < 10%, since MRs were only 0.5–6% of net NH_4^+ depletion rates.

Soil pH. Prior to the NO_2^- accumulation periods, pH decreased linearly with time ($r^2 = 0.91$–0.98). Oxidation of approximately 60, 120 and 250 µg NH_4^+-N g^{-1} soil were required per unit decrease in pH for Lang, Reiff and Yolo, respectively. In all experiments, the onset of NO_2^- accumulation occurred when the soil pH reached a critical value which was fairly consistent across replications, but which varied between soil types (Fig. 2). The critical pH values were 3.8, 4.6 and 4.9 (1 M KCl) for Lang, Reiff and Yolo soils, respectively. Corresponding pH values (0.01 M $CaCl_2$) were 4.2, 4.7 and 5.3.

Gross NO production rates. Mean NO production rates (P_{NO}) were in the range of 2.6–5.2 ng N g^{-1} soil h^{-1} during high nitrification rate phases (Fig. 3), representing 0.3–2.3% of gross AORs. These rates are similar to values previously observed in wetted agricultural soils incubated aerobically (Remde *et al.* 1989) and in a grassland soil following amendment with NH_4NO_3 (Hutchinson *et al.* 1993). For all soils, P_{NO} increased significantly ($P < 0.01$) during the NO_{2^-} accumulation/reduced nitrification phases (Fig. 3). In all experiments, the timing of the abrupt increase in NO production coincided with the critical soil pH and paralleled the accumulation of NO_2^-. The P_{NO} data pooled from all phases of expts 1 and 2 were well-correlated with HNO_2 for Lang, Reiff and Yolo (Fig. 4, nonsterile soil data). For the Lang data (which had below-detectable levels of NO_2^- in some cases) regression analyses performed using either 0 or the detection limit (0.01 µg N g^{-1}) for these values gave practically identical results. Use of NH_4^+ or NO_3^- as independent variables alone or in combination in regression models yielded relatively poor fits to the NO production rate data ($r^2 < 0.5$).

Gross N_2O production rates. During the high nitrification rate periods, mean P_{N2O} values (Fig. 4) were 0.5–1.6% of the gross AORs, similar to results of a previous study of N_2O production under conditions favouring nitrification (Goodroad & Keeney 1983). The P_{N2O} increased during the NO_2^- accumulation/reduced nitrification periods in all experiments, accounting for 5–9% of the AORs (Fig. 3). Correlation of P_{N2O} with HNO_2 was fairly strong for Yolo ($r^2 = 0.866$), but less strong for Lang and Reiff (Fig. 4, nonsterile soil

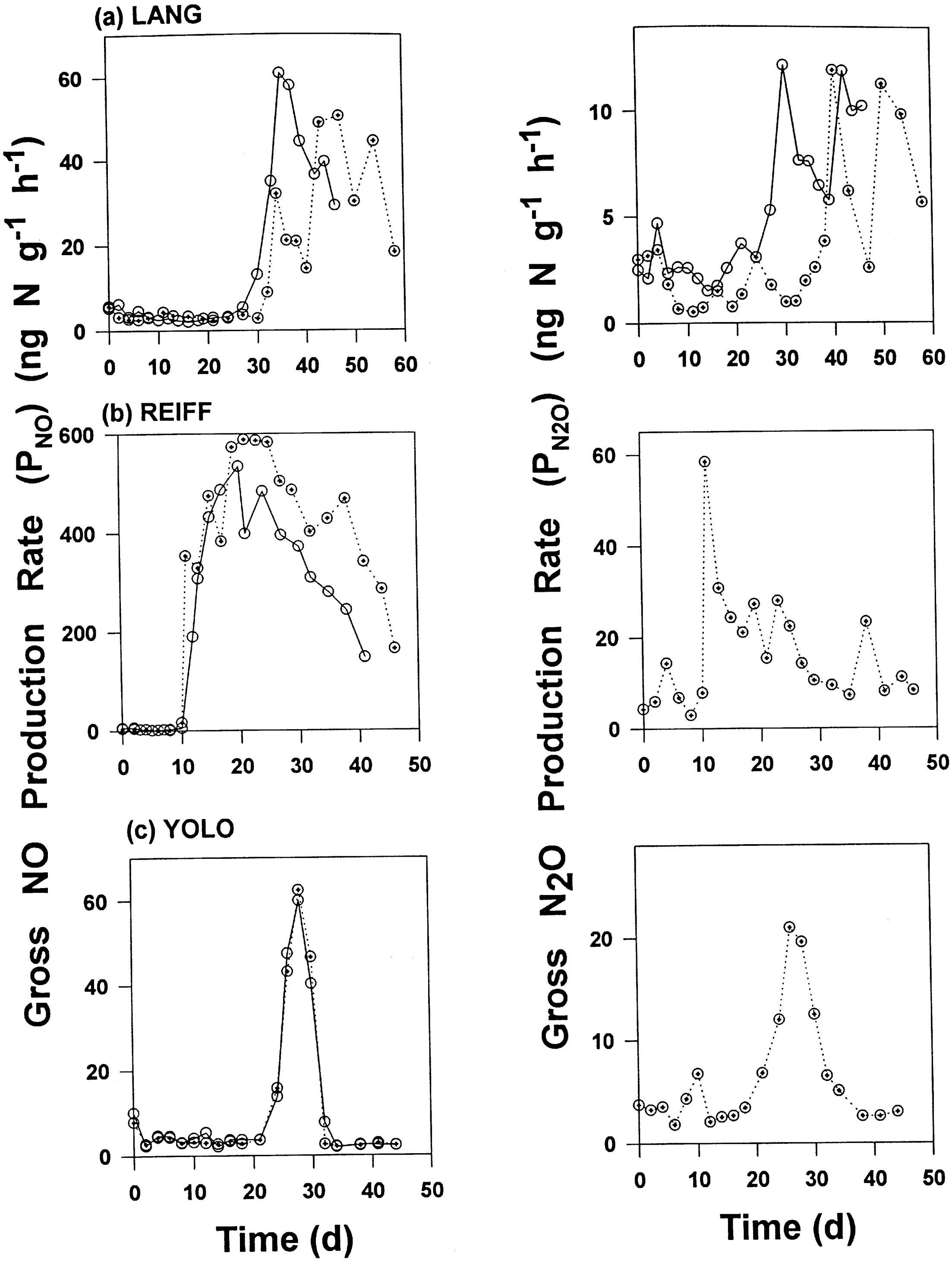

Fig. 3 Gross NO and N_2O production rates in (a) Lang (b) Reiff and (c) Yolo soil nitrification experiments. Open symbols, solid lines = expt 1; dotted symbols, dashed lines = expt 2.

data). No significant correlations ($r^2 < 0.37$) were found with NH_4^+ or NO_3^-. Multiple regression models containing NO_2^- and NO_3^- provided better fits to the P_{N2O} data (Fig. 5).

NO consumption. First-order NO consumption rate coefficients (k_c) were within the range of previous measurements made on a variety of soils under aerobic conditions (Baumgartner & Conrad 1992;

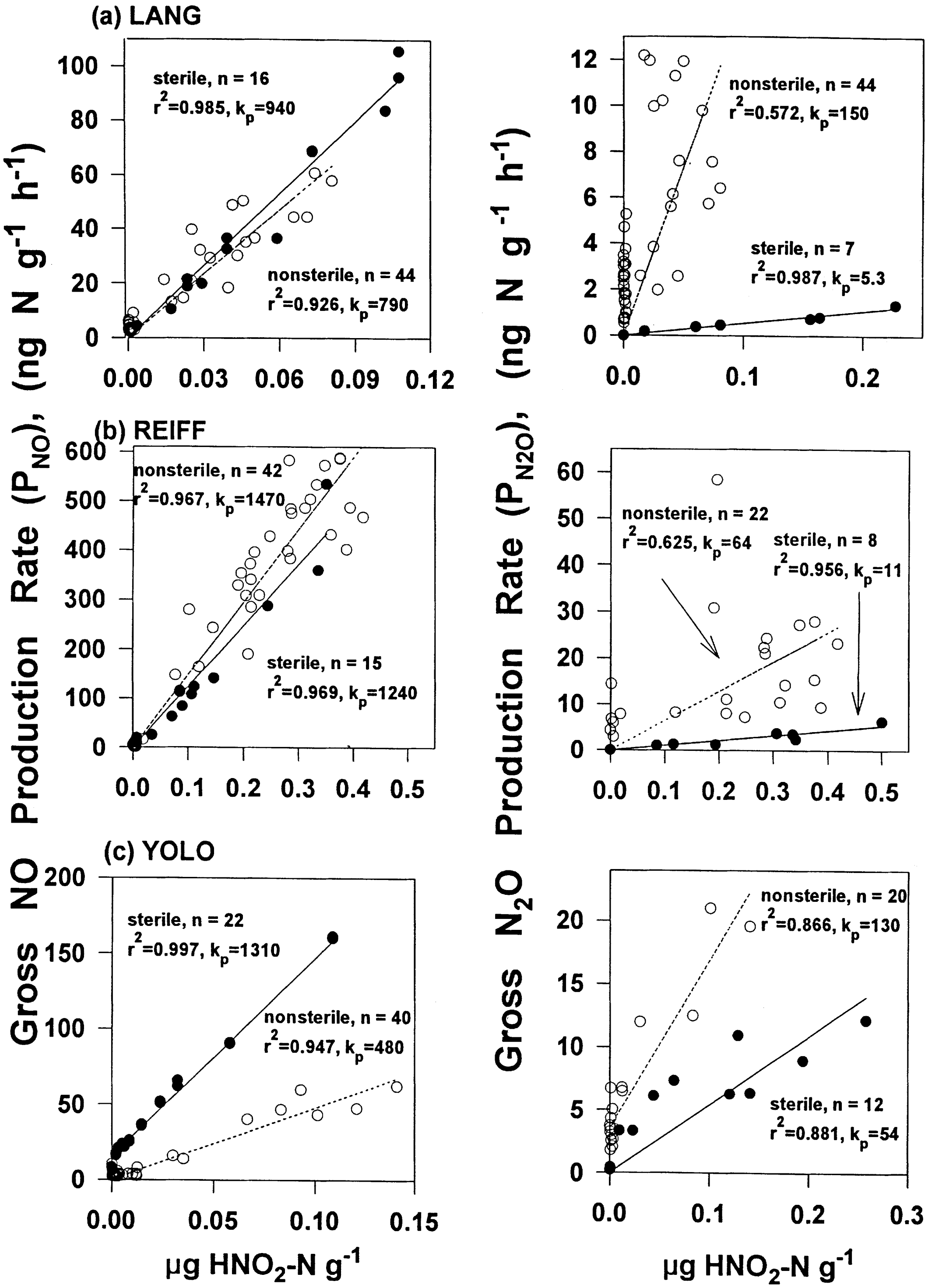

Fig. 4 Gross NO and N_2O production as function of HNO_2 in (a) Lang (b) Reiff and (c) Yolo soil. ○, dashed regression lines = nitrifying nonsterile soil; ●, solid regression lines = sterile (λ-irradiated) soil; k_p [=] ng N µg HNO_2-N^{-1} h^{-1}.

Koschorreck & Conrad 1997). Mean k_c values were significantly higher ($P < 0.01$) in Reiff ($\bar{x} = 39.6 \pm 27.6$, $n = 42$) compared to Lang ($\bar{x} = 18.0 \pm 9.0$, $n = 44$) and Yolo ($\bar{x} = 16.2 \pm 11.4$, $n = 40$) (cm^3 g^{-1} soil h^{-1}). There were no strong correlations between k_c and any measured variables ($r^2 < 0.18$).

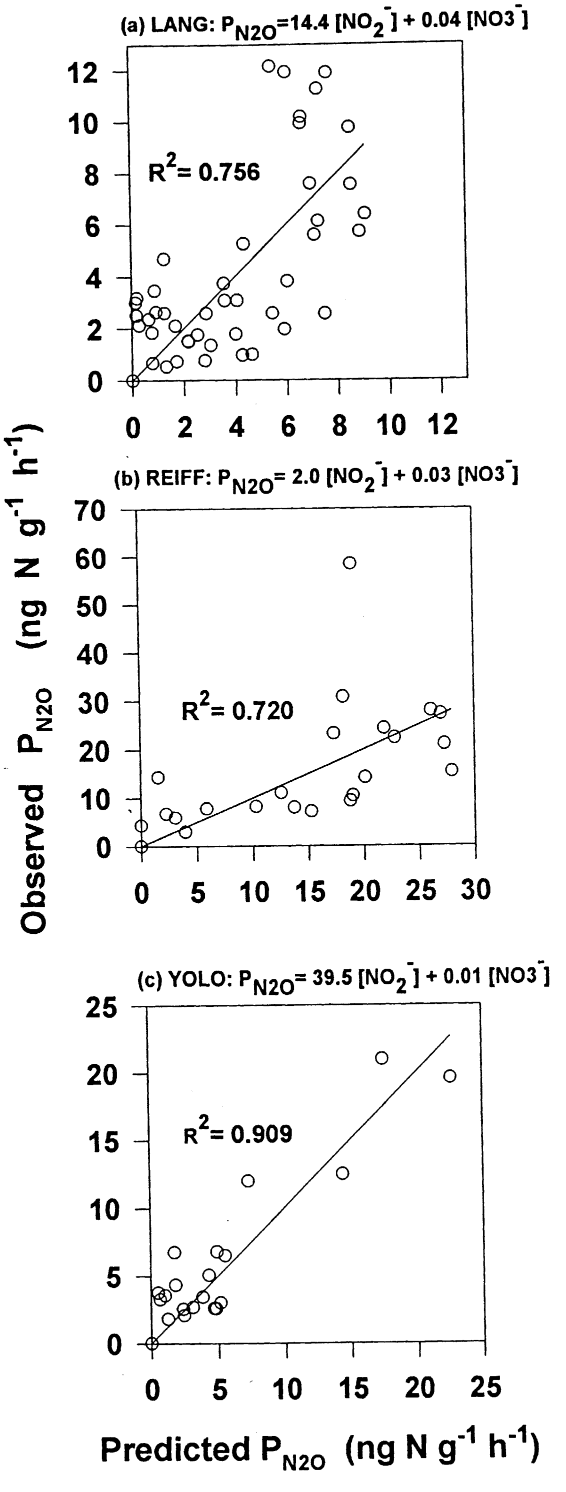

Fig. 5 Results of multiple regression models using NO_2^- and NO_3^- to predict gross N_2O production rates in (a) Lang (b) Reiff and (c) Yolo soil nitrification experiments. 1 : 1 (perfect fit) lines are shown.

C_2H_2 inhibition experiments

Treatment with 10 Pa of C_2H_2 resulted in the depletion of NO_2^- concentrations and significant reductions in $NO_2^- + NO_3^-$ accumulation (Table 3). Production of NO decreased in proportion to NO_2^- concentrations (Fig. 6), demonstrating that the accumulated NO_2^- was derived primarily from nitrification, and that this NO_2^- was the primary source of NO production. Trace levels of NO_2^- remaining in the Day 13 Reiff sample may have been due to incomplete inhibition of nitrification and/or insufficient incubation time to allow for the NO_2^- initially present to be consumed, and/or possibly to NO_2^- derived from NO_3^- reduction. N_2O production generally decreased by only 50–60% after C_2H_2 treatment (Table 3).

$^{15}NO_3^-$ reduction

Significant enrichments in atom % of ^{15}N were evident in N_2O evolved from soils amended with $K^{15}NO_3$, indicating that NO_3^- reduction was the primary source of N_2O in soils treated with 10 Pa C_2H_2. Atom percentage ^{15}N values of 22, 25 and 21% were measured in Lang, Reiff and Yolo soils, respectively (compared to natural abundance of 0.366%). The measured atom % ^{15}N values in the N_2O pools were 96, 80 and 71%, respectively, of the theoretical values assuming that increases in headspace N_2O concentration directly reflected measured atom percentage ^{15}N in the NO_3^- pools. The lower than expected values could have been due to incomplete inhibition of nitrification, which may have been more pronounced in Reiff and Yolo because of higher clay and organic matter content which could have restricted C_2H_2 penetration to microsites.

Sterile soil experiments

Production of NO was detected within seconds after the addition of KNO_2 solutions to both λ-irradiated and nonsterile control soils, and steady NO concentrations were measured within 10–15 min in the reaction chamber effluent gas stream. The P_{NO} values were highly correlated with HNO_2 concentrations in all soils (Fig. 4, sterile soil data), but not with NO_2^- ($r^2 = 0.008$, 0.415 and < 0.001). The observed proportionality between P_{NO} and HNO_2 can be defined as an overall apparent NO production rate coefficient:

$$P_{NO} = \frac{d[NO]}{dt} = k_{PNO}[HNO_2] \qquad (6)$$

Values of k_{PNO} were on the order of 1 μg NO-N per μg HNO_2-N per h in λ-irradiated soil (Fig. 4). The nonsterile control data (not shown) were similar to the λ-irradiated soil data, with k_{PNO} values of 1.0, 1.3 and 1.4 μg NO-N per μg HNO_2-N per h obtained for Lang, Reiff and Yolo soils, respectively, with similarly high correlation between HNO_2 and P_{NO} ($r^2 > 0.94$). The k_{PNO} values were

Table 3 Results of C_2H_2-inhibition experiments

		(μg N g^{-1})				(ng N g^{-1} h^{-1})			
		NO_2^-		$^2\Delta(NO_2^- + NO_3^-)$		P_{NO}		P_{N2O}	
	Day	$^1C_2H_2$	Air	C_2H_2	Air	C_2H_2	Air	C_2H_2	Air
Lang	4	< 0.01	< 0.01	+ 0.35	+ 4.4	0.5	2.6	0.4	0.9
	19	< 0.01	0.03	+ 1.0	+ 8.3	< 0.3	2.4	1.8	1.4
	30	< 0.01	< 0.01	− 1.0	+ 9.1	< 0.3	12	4.7	12
Reiff	2	< 0.01	0.20	− 0.9	+ 8.3	< 0.3	3.8	2.6	5.9
	13	0.20	7.9	− 2.5	− 0.3	2.2	329	12	31
	29	< 0.01	6.2	− 1.0	− 1.1	< 0.3	486	12	10
Yolo	2	< 0.01	0.17	− 1.3	+ 14	< 0.3	2.7	1.5	3.2
	8	< 0.01	0.11	− 1.4	+ 28	< 0.3	3.0	2.3	4.3
	24	< 0.01	3.5	− 1.5	+ 11	< 0.3	16	4.5	12

[1] NO_2^- concentration remaining in soil after C_2H_2 treatment.
[2] Change in $NO_2^- + NO_3^-$ concentration during incubation period.

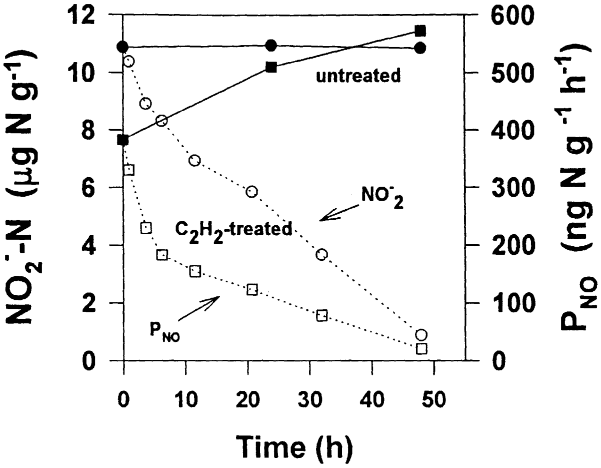

Fig. 6 Effect of 10 Pa C_2H_2 on NO_2^- and gross NO production rate (P_{NO}) in Reiff soil. Each point is mean of 2 replicates. ○ = NO_2^-; □ = P_{NO}; open symbols = C_2H_2-treated soil; closed symbols = untreated soil.

similar in the nitrifying nonsterile and sterile soils for Lang and Reiff, but significantly lower in the Yolo nitrifying nonsterile compared to sterile soil (Fig. 4).

Linear increases in headspace N_2O concentration were observed in jars containing NO_2^- amended, λ-irradiated soils over periods of 1–3 h. Rates of abiotic N_2O production were highly correlated with HNO_2 (Fig. 4). Apparent N_2O production rate coefficients [k_{PN2O}, corresponding to k_{PNO} in eqn (6)] were a small fraction (0.6–4%) of the sterile k_{PNO} values. Rates of gross N_2O production per unit of HNO_2 were higher in the nitrifying nonsterile compared to sterile soils in all cases, with significant N_2O production observed at NO_2^- and HNO_2 concentrations near zero (Fig. 4). The k_{PN2O} values for nonsterile control soils (data not shown) were 5–30% higher than λ-irradiated soils, with less linearity with respect to HNO_2 concentration ($r^2 = 0.60$–0.75), suggesting that the effects of λ radiation caused some attenuation of N_2O production, and/or that biological activity occurring in the nonsterile control soil in the 1–3 h following wetting produced some N_2O. Soils amended with NO_2^--free deionized H_2O demonstrated very low rates of NO and N_2O production (< 1 and < 0.5 ng N g^{-1} h^{-1}, respectively), indicating that the effect of wetting *per se* had negligible impact on gas production (trace levels of NO_2^- were detected in some of the deionized H_2O-amended soils).

Discussion

NO_2^- accumulation

Concentrations of NO_2^- were detectable during all periods of the nitrification experiments except for the high nitrification rate periods in Lang soil. During the early periods (0–4 d), *Nitrobacter* populations may have experienced slightly greater lag effects than NH_4^+ oxidizers, as has been previously observed (Morrill & Dawson 1967). During the high NO_2^- accumulation periods, the inhibitory factor was most likely the decreased soil pH (Fig. 2), which has been observed to inhibit both steps of nitrification by promoting the formation of HNO_2 (Hunik *et al.* 1992, 1993). Reduced pH can also inhibit NH_4^+ oxidation by influencing substrate availability (Prosser 1989), and NO_2^- oxidation by apparently enhancing end-product inhibition (Hunik *et al.* 1993). The onset of high NO^-_2 accumulation occurred after oxidation of approximately 100, 200 and 300 µg NH_4^+-N g^{-1} soil in Lang, Reiff and Yolo soils, respectively. Localized concentrations of > 1000 µg NH_4^+-N g^{-1} (initially) and > 200 µg NO_3^--N g^{-1} (after nitrification) are commonly found following the application of banded N fertilizer (McIntosh & Frederick 1958; Chalk *et al.* 1975), so it is likely that these processes could occur in localized regions.

The critical pH values at which significant inhibition of NO_2^- oxidation began were higher in the more highly buffered soils (i.e. Yolo>Reiff>Lang). Acid-tolerant or acidophilic strains of *Nitrobacter* have been implicated in controlling NO_2^- oxidation in acid soils (Prosser 1989). The differences observed in critical pH values between soils, and the subsequent temporal patterns of NO_2^- concentration, may have reflected differences in acid-tolerance of the dominant NO_2^- oxidizing bacterial populations in each soil, i.e. *Nitrobacter* populations in the less buffered soils may have been more tolerant of nitrification-induced pH decreases. It should be emphasized that the critical pH, calculated HNO_2 concentrations, and reported values of k_{PNO} and k_{PN2O}, are specific to the soil pH method used.

NO and N_2O production

The data suggest that the primary mechanism of NO production involved NO_2^- derived from autotrophic NH_4^+ oxidation which equilibrated with H^+ in soil solution or near charged surfaces to form HNO_2. The aqueous decomposition of NO is thought to proceed according to:

$$3HNO_2 \Rightarrow 2NO + HNO_3 + H_2O, \qquad (7)$$

or similar reactions (Van Cleemput & Baert 1976). Reactions of HNO_2 with phenolic and other functional constituents of soil organic matter leading to NO production have also been observed (Stevenson 1994). The positive correlation between k_{PNO} values (sterile soil) and soil organic matter ($r^2 = 0.90$) suggest that these reactions were important. Reactions of HNO_2 with reduced metal cations have also been implicated, but are considered to be insignificant under the oxidizing conditions of the present study (Nelson 1982).

While the data most clearly shows the importance of HNO_2-mediated reactions during the high NO_2^- accumulation periods, it also suggests that these may have been the primary source even when NO_2^- concentrations were low. During high nitrification rate periods, residual NO_2^- concentrations were always present in Reiff and Yolo soils. There is evidence that *Nitrosomonas* and *Nitrobacter* populations preferentially grow on oppositely charged surfaces, each tending to proliferate on surfaces to which its primary substrate is adsorbed (Underhill & Prosser 1987). Thus, prior to utilization by oxidative or reductive microbes, diffusion of nitrification-derived NO_2^- through some finite region is required, providing the opportunity for abiotic HNO_2 and NO production, even when bulk NO_2^- concentrations are low relative to NH_4^+ or NO_3^-. This may explain the difficulty encountered in studies attempting to correlate NO emissions with NH_4^+ or NO_3^-, without considering NO_2^-. The k_{PNO} values obtained in sterile and nonsterile soils indicate that at low NO_2^- concentrations and only slightly acidic pH, significant NO production can be attributed to abiotic reactions. For example, at 0.5 µg NO_2^-- N g^{-1} soil and pH (1 : 1 KCl) = 6.0, NO production in Reiff soil would be 1.2 ng N g^{-1} soil h^{-1}, which is within the range attributable to nitrification in recent studies (Bollman & Conrad 1998). While initial NO_2^- levels are often reported in NO studies, it is not clear to what extent the dynamics (e.g. after wetting and incubation) of NO_2^- concentrations are responsible for observed NO production.

No trends in residuals were apparent in regression results of P_{NO} vs. HNO_2 for the nonsterile Reiff data ($r^2 = 0.967$, $n = 42$), suggesting that HNO_2 was the primary driving factor. For Lang and Yolo soils, when HNO_2 levels were < 5 ng N g^{-1} soil, simple regression models (with HNO_2) consistently under-predicted P_{NO} by 1–6 ng NO-N g^{-1} h^{-1}. Incorporation of NH_4^+ concentration into multiple regression models eliminated these trends ($R^2 = 0.942$ and 0.960 for Lang and Yolo). This suggests that NH_4^+ concentrations exerted some influence over low-level NO production, although the mechanistic basis for this statistical result is not clear. The role of NO as an oxidative intermediate in autotrophic oxidation of NH_4^+ to NO_2^- has been suggested, although its role is this regard has not been confirmed (Hooper 1982). There is no evidence that rates of gross NH_4^+ oxidation (AORs) *per se* had a major influence on NO production, i.e. AORs in Reiff and Yolo soil were 5–8 times greater than in Lang soil during high nitrification periods (Table 2), yet P_{NO} values were not significantly different. Therefore, the data gives no support to models which calculate NO production rates as a fraction of gross or net nitrification rate.

A possible explanation for the discrepancy between k_{PNO} values in the Yolo nitrifying nonsterile vs. the sterile and nonsterile control soils (Fig. 4c) is the utilization of a central substrate (NO_2^-) by competing biological and chemical reactions. Yolo nitrifying soil showed the closest agreement between NH_4^+ oxidation and NO_3^- accumulation rates during the NO_2^- accumulation (high NO production) period (Table 2), indicating that significant microbial NO_2^- oxidation continued throughout this period. Microbial NO_2^- utilization occurring simultaneously with abiotic HNO_2 reduction could have competed for available NO_2^- and thereby attenuated abiotic NO production, as compared to the sterile and nonsterile control soils where nitrifying activity was likely to have been low to nonexistent. Reductive microbial transformations of NO_2^- to N_2O could have had the same effect.

Multiple processes were important in controlling N_2O production. Abiotic N_2O production is believed to involve reactions of HNO_2 with phenolic functional constituents of soil organic matter (Stevenson 1994). The role of organic matter is evident in the positive correlation ($r^2 = 0.82$) between organic matter content and observed k_{PN2O} values. The ^{15}N data together with the two-factor (NO_2^-, NO_3^-) regression results (Fig. 5) and the C_2H_2 results (Table 3) are conclusive evidence that, even under well-aerated conditions at 40–42% of water saturation, the dissimilatory NO_3^--reduction sequence, i.e. $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O$, was responsible for a significant fraction of the N_2O produced, presumably due to microbial activity in anoxic soil microenvironments. This has been suggested by prior studies (Goodroad & Keeney 1983) where N_2O production was correlated with NO_3^- under relatively dry conditions. Increases in N_2O production rates after exposure to higher levels (10 kPa) of C_2H_2, which inhibits N_2O reduction by denitrifying bacteria (Davidson *et al.* 1986) were observed in some instances during the nitrification experiments, but not consistently (data not shown). The ^{15}N experiments showed no enrichment of ^{15}N in N_2 pools, so it is not clear to what extent the reduction sequence was carried through to completion, i.e. $N_2O \rightarrow N_2$. Overall, the data indicate that biological reductive processes were more important in controlling N_2O production as compared to NO production, since NO production in C_2H_2-treated soils was generally below detectable levels (Table 3).

Concluding remarks

The central role of NO_2^- and HNO_2 in controlling NO and N_2O emissions during nitrification was demonstrated. The overall rate of nitrification *per se* did not control these emissions. Factors which affect the accumulation of NO_2^- and N oxide-forming reactions will significantly influence the proportion of nitrified N which leaks from the nitrification process. While microbial ecological factors may be important, soil pH, buffering capacity and organic matter content are more readily quantified variables which could be incorporated into predictive emissions models, along with the kinetic parameters defined in this study. Fertilizer management practices will directly influence these dynamics. Intensive applications of NH_3^- or NH_4^+-based fertilizers can inhibit NO_2^- oxidation and cause localized lowering of pH. Continued fertilizer use over several years, without liming, will further promote N oxide emissions by reducing background soil pH. The data also suggest that abiotic processes may be important in certain natural ecosystems (e.g. tropical soils) where levels of acidity and soil organic matter may be sufficient to promote HNO_2-mediated N trace gas production. The importance of these mechanisms across a range of ecosystems needs to be further investigated.

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References

Baumgartner M, Conrad R (1992) Effects of soil variables and season on the production and consumption of nitric oxide in oxic soils. *Biology and Fertility of Soils*, **14,** 166–174.

Bohn H, McNeal B, O'Connor G (1985). *Soil Chemistry*. John Wiley, New York.

Bollmann A, Conrad R (1998) Influence of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. *Global Change Biology*, **4,** 387–396.

Chalk PM, Keeney D, Walsh L (1975) Crop recovery and nitrification of fall and spring applied anhydrous ammonia. *Agronomy Journal*, **67,** 33–41.

Conrad R (1995) Metabolism of nitric oxide in soil and soil microorganisms and regulation of flux into the atmosphere. In: *Microbiology of Atmospheric Trace Gases/ Sources, Sinks and Global Change Processes* (eds Murrell JC, Kelly DP), pp. 167–201. Springer, New York.

Crutzen P (1979) The role of NO and NO_2 in the chemistry of the troposphere and stratosphere. *Annual Reviews of Earth Planetary Science*, **7,** 443–472.

Crutzen P (1981) Atmospheric chemical processes of the oxides of nitrogen, including nitrous oxide. In: *Denitrification, Nitrification and Atmospheric N_2O* (ed. Delwiche C), pp. 17–44. John Wiley, Chichester.

Davidson EA, Swank W, Perry T (1986) Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. *Applied and Environmental Microbiology*, **52,** 1280–1286.

Davidson EA, Kingerlee W (1997) A global inventory of nitric oxide emissions from soils. *Nutrient Cycling in Agroecosystems*, **48,** 37–50.

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. In: *Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere*, pp. 7–21 (eds Andreae M, Schimel D) John Wiley, Chichester.

Goodroad L, Keeney D (1983) Nitrous oxide production in aerobic soils under varying pH, temperature and water content. *Soil Biology and Biochemistry*, **16,** 39–43.

Hooper A (1982) Ammonia oxidation and energy transduction in the nitrifying bacteria. In: *Microbial Chemoautotrophy* (eds Strohl WR, Tuovinen OH), pp. 133–167. Ohio State University Press, Columbus, OH.

Hunik JH, Meijer HJG, Tramper J (1992) Kinetics of *Nitrosomonas* *europaea* at extreme substrate, product and salt concentrations. *Applied Microbiology and Biotechnology*, **37,** 802–807.

Hunik JH, Meijer HJG, Tramper J (1993) Kinetics of *Nitrobacter agilis* at extreme substrate, product and salt concentrations. *Applied Microbiology and Biotechnology*, **40,** 442–448.

Hutchinson G, Brams E (1992) NO versus N_2O emissions from an NH_4-amended Bermuda grass pasture. *Journal of Geophysical Research-Atmospheres*, **97,** 9889–9896.

Hutchinson G, Guenzi W, Livingston G (1993) Soil water controls on aerobic soil emission of gaseous nitrogen oxides. *Soil Biology and Biochemistry*, **25,** 1–9.

Hutchinson GL, Vigil MF, Doran JW, Kessavalou A (1997) Coarse-scale soil-atmosphere NO_x exchange modeling: status and limitations. *Nutrient Cycling in Agroecosystems*, **48,** 25–35.

Keeney D, Nelson D (1982) Nitrogen-Inorganic Forms. In: *Methods of Soil Analysis, Part 2 Chemical and Microbiological Properties* (eds Page A *et al.*), pp. 643–698. American Society of Agronomy, Madison, WI.

Koschorreck M, Conrad R (1997) Kinetics of nitric oxide consumption in tropical soils under oxic and anoxic conditions. *Biology and Fertility of Soils*, **25,** 82–88.

Li C, Frolking S, Frolking T (1992) A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity. *Journal of Geophysical Research – Atmospheres*, **97,** 9759–9776.

Matson P (1997) NOx emission from soils and its consequences for the atmosphere and biosphere: critical gaps and research directions for the future. *Nutrient Cycling in Agroecosystems*, **48,** 1–6.

Matson P, Naylor R, Ortiz-Monasterio I (1998) Integration of environmental, agronomic, and economic aspects of fertilizer management. *Science*, **280,** 112–115.

McIntosh T, Frederick L (1958) Distribution and nitrification of anhydrous ammonia in a Nicollet sandy clay loam. *Soil Science Society of America Proceedings*, **22,** 402–405.

Moraghan J, Buresh R (1977) Correction for dissolved nitrous oxide in nitrogen studies. *Soil Science Society of America Journal*, **41,** 1201–1202.

Morrill L, Dawson J (1967) Patterns observed for the oxidation of ammonium to nitrate by soil organisms. *Soil Science Society of America Journal*, **31,** 757–760.

Mosier A, Duxbury J, Freney J, Heinemeyer O, Minami K (1996) Nitrous oxide emissions from agricultural fields: assessment, measurement and mitigation. *Plant and Soil*, **181,** 95–108.

Nelson D (1982) Gaseous losses of nitrogen other than through denitrification. In: *Nitrogen in Agricultural Soils* (ed. Stevenson F), pp. 327–364. American Society of Agronomy, Madison, WI.

Pauling L (1970) *General Chemistry*. Dover, New York.

Potter CS, Matson PA, Vitousek PM, Davidson EA (1996) Process modeling of controls on nitrogen trace gas emissions from soils worldwide. *Journal of Geophysical Research-Atmospheres*, **101,** 1361–1377.

Prosser J (1989) Autotrophic nitrification in bacteria. *Advances in Microbial Physiology*, **30,** 125–181.

Remde A, Slemr F, Conrad R (1989) Microbial production and uptake of nitric oxide in soil. *FEMS Microbiology Ecology*, **62,** 221–230.

Riley W, Matson P (1998) A mechanistic model of biogenic N trace-gas effluxes from soil during nitrification and denitrification (Suppl.). *Eos, Transactions of the American Geophysical Union*, **79,** F163.

Rodhe H (1990) A comparison of the contribution of various gases to the greenhouse effect. *Science*, **248,** 1217–1219.

Ryden J (1982) Effects of acetylene on nitrification and denitrification in two soils during incubation with ammonium nitrate. *Journal of Soil Science*, **33,** 263–270.

Serca D, Delmas R, Jambert C, Labroque L (1994) Emissions of nitrogen oxides from an equatorial rain forest in central Africa: origin and regulation of NO emissions from soils. *Tellus*, **46B,** 243–254.

Shepherd M, Barzetti S, Hastie D (1991) The production of atmospheric NO_x and N_2O from a fertilized agricultural soil. *Atmospheric Environment*, **25A,** 1961–1969.

Slemr F, Seiler W (1991) Field study of environmental variables controlling the NO emissions from soil and the NO compensation point. *Journal of Geophysical Research-Atmospheres*, **96,** 13,017–13,031.

Stevens R, Laughlin R (1994) Determining nitrogen-15 in nitrite or nitrate by producing nitrous oxide. *Soil Science Society of America Journal*, **58,** 1108–1116.

Stevenson FJ (1994). *Humus Chemistry: Genesis, Composition, Reactions*. John Wiley, New York.

Sumner M (1994) Measurement of soil pH: problems and solutions. *Communications in Soil Science and Plant Analysis*, **25,** 859–879.

Thornton FC, Valente RJ (1996) Soil emissions of nitric oxide and nitrous oxide from no-till corn. *Soil Science Society of America Journal*, **60,** 1127–1133.

Underhill SE, Prosser JI (1987) Surface attachment of nitrifying bacteria and their inhibition by potassium ethyl xanthate. *Microbial Ecology*, **14,** 129–139.

Van Cleemput O, Samater A (1996) Nitrite in soils: accumulation and role in the formation of gaseous N compounds. *Fertilizer Research*, **45,** 81–89.

Van Cleemput O, Baert L (1976) Theoretical considerations on nitrite self-decomposition reactions in soils. *Soil Science Society of America Journal*, **40,** 322–324.

Veldkamp E, Keller M (1997) Fertilizer-induced nitric oxide emissions form agricultural soils. *Nutrient Cycling in Agroecosystems*, **48,** 69–77.

Williams E, Fehsenfeld F (1991) Measurement of soil nitrogen oxide emissions at three North American ecosystems. *Journal of Geophysical Research – Atmospheres*, **96,** 1033–1042.

Williams EA, Geunther A, Fehsenfeld F (1992) An inventory of nitric oxide emissions from soils in the United States. *Journal of Geophysical Research – Atmospheres*, **97,** 7511–7519.

Wolt J (1994). *Soil Solution Chemistry*. John Wiley, New York.