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

RODNEY T. VENTEREA† and DENNIS E. ROLSTON
Department of Land, Air and Water Resources, University of California, Davis, One Shields Avenue, Davis, CA 95616

Abstract

Laboratory experiments were conducted with three California agricultural soils to examine substrate and process controls over temporal variability of NO and N₂O production during nitrification, and to quantify the kinetics of HNO₂-mediated chemical reactions. Gross NO production rates were highly correlated (r² = 0.93–0.97) with calculated concentrations of HNO₂, which were shown to originate from autotrophic microbial oxidation of NH₄⁺ to NO₂⁻. Production of NO was not correlated with NH₃⁺ or NO₃⁻, or with the overall nitrification rate. Distinct periods of high NO₂⁻ accumulation occurred below critical pH values in each soil, apparently due to inhibition of microbial NO₂⁻ oxidation. Data suggest that even during periods of relatively low NO₂⁻ accumulation and rapid overall nitrification, HNO₂-mediated reactions may have been the primary source of NO. Rate coefficients (kₚNO) relating NO production to HNO₂ concentrations were determined for sterile (λ-irradiated) soils, and were similar to kₚNO values in 2 of 3 nonsterile soils undergoing nitrification. Production of N₂O was correlated with HNO₂ (r² = 0.88–0.99) in sterile soils, and with NO₂⁻ and NO₃⁻ (R² = 0.72–0.91) in nonsterile soils. Experiments using ¹⁵N confirmed that dissimilatory NO₃⁻ reduction contributed to N₂O production even under primarily aerobic conditions. Sterile kₚNO and kₚN₂O values were correlated (r² = 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 NO₂⁻/HNO₂ need to be considered in developing improved models of NO and N₂O emissions from soils.

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

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Introduction

Nitric oxide (NO) and nitrous oxide (N₂O) 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 N₂O, 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 N₂O 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 N₂O 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 (NH₄⁺) concentrations and increases following application of NH₄⁺-based fertilizers (Slemr & Seiler 1991; Hutchinson & Brans 1992). Several other studies have found positive correlation of NO flux with soil NO₃⁻ concentrations (Williams & Felshenfeld 1991; Thornton & Valente 1996). In general, however, researchers have been unable to develop useful models based on soil NO₃⁻ and/or NH₄⁺ levels (Shepherd et al. 1991), and it is uncertain which
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 N₂O 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 N₂O production in soils are known to involve nitrite (NO₂⁻) as a central substrate or reactant (Conrad 1995). In aqueous systems at pH < 5, the protonated form of NO₂⁻, nitrous acid (HONO₂ pKa = 3.3), becomes more predominant, and decomposes spontaneously to yield NO (Pauling 1970). In soils, HONO₂ can also react with organic constituents to form both NO and N₂O (Stevenson 1994). The importance of HONO₂-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 nonacidic soils, can promote more significant rates of HONO₂ formation and decomposition than suggested by bulk soil pH measurements (Nelson 1982). In theory, reactions involving NO₂⁻/HONO₂ represent a potentially important route by which N flowing through the two-step nitrification sequence, i.e. NH₄⁺ → NO₂⁻ → NO₃⁻, can partition to gaseous forms, consistent with the hole-in-the-pipe model. However, the temporal dynamics of NO₂⁻ concentrations together with pH have rarely been monitored in studies examining controls over NO and N₂O 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 N₂O production rates in agricultural soils under conditions favouring nitrification (ii) to examine the role of NO₂⁻/HONO₂ and the importance of abiotic processes occurring simultaneously with nitrification, and (iii) to characterize some aspects of the NO₂⁻/HONO₂-mediated kinetics of NO and N₂O 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 NH₄⁺ fertilizer source (2.5 mM CaCl₂, 2.5 mM CaSO₄, 1.25 mM MgSO₄, 0.625 mM K₂SO₄ and 7.5 mM (NH₄)₂SO₄). Soils were flushed slowly with solution until effluent NH₄⁺ 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>
<thead>
<tr>
<th>Soil series texture</th>
<th>Lang loamy sand</th>
<th>Reiff sandy loam</th>
<th>Yolo silt loam</th>
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<tbody>
<tr>
<td>USDA classification</td>
<td>Psammaquent</td>
<td>Xerofluvent</td>
<td>Xerorthent</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>74</td>
<td>62</td>
<td>36</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>22</td>
<td>28</td>
<td>46</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>4</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>pH (1:1 KCl)</td>
<td>5.6</td>
<td>6.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Organic C (mg/kg)</td>
<td>0.32</td>
<td>0.88</td>
<td>1.40</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.03</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>CEC (mg/g)</td>
<td>70</td>
<td>170</td>
<td>230</td>
</tr>
<tr>
<td>recent use</td>
<td>alfalfa/tomatoes</td>
<td>corn/tomatoes</td>
<td>annual row crops</td>
</tr>
</tbody>
</table>

¹Dichromate oxidation; ²Total Kjeldahl N.
of 0.14, 0.16 and 0.22 (± 0.01) g H₂O 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 N₂O 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 time point, two replicate subsamples were extracted with 0.5 M K₂SO₄ solution at a liquid:soil mass ratio of ~10:1. For low-level NO₂⁻ 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 NO₂⁻ analysis by the modified Griess–Ilosvay method (Keeney & Nelson 1982). Remaining solution was stored at 4 °C and analysed within 10 d for total (NO₃⁻+NO₂⁻)-N using cadmium (Cd) reduction followed by the modified Griess-Ilosvay method, and for NH₄⁺-N using the indophenol blue method (Keeney & Nelson 1982). Reagent additions and colourimetric analyses for NO₂⁻, NO₃⁻ and NH₄⁺ were done manually (Hitachi 100–30 spectrophotometer). The assumption that the NO₂⁻ assay responded to total (NO₂⁻+HNO₂)-N concentration was tested by analysis of aqueous KNO₂ solutions containing 2.3 μg cm⁻³ of total (NO₂⁻+HNO₂)-N, adjusted to either pH 4 or pH 7 (< 0.05% is in form of HNO₂). No significant differences in analysed concentrations (<1.5%) were found, indicating that the method did not discriminate between NO₂⁻ and HNO₂.

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 CaCl₂ 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 NO₂⁻ and HNO₂ was assumed:

\[ \text{H}^+ + \text{NO}_2^- \rightleftharpoons \text{HNO}_2. \] (1)

Concentrations of HNO₂-N were calculated using the measured total (NO₂⁻+HNO₂)-N molar concentrations, soil pH (KCl) and the acid dissociation constant (pKₐ = 3.3, Van Cleemput & Samater 1996) for (1) in the equilibrium expression:

\[ [\text{HNO}_2^-\text{N}] = \frac{[\text{NO}_2^-\text{N} + \text{HNO}_2^-\text{N}]_{\text{total}} \cdot 10^{-p\text{H}}}{(10^{-p\text{H}} + 10^{-p\text{K}_a})}. \] (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 HNO₂ concentrations are specific to the pH method employed.

Gross NO and N₂O 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 (Pnet, ng N g⁻¹ h⁻¹), was determined from:

\[ P_{\text{net}} = \frac{q}{m_s}(C_e - C_i), \] (3)

where \( C_e = \) chamber effluent NO concentration (ng N cm⁻³), \( C_i = \) chamber influent NO concentration (ng N cm⁻³).
cm$^{-3}$, $q$ = air flow rate (cm$^3$ h$^{-1}$), $m_o$ = 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:

$$P_{net} = P_{NO} - k_c C_c.$$  (4)

Regression of $P_{net}$ vs. $C_c$ 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$_2$O 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$_2$O concentrations were measured typically after 0, 1 and 3 h of incubation, by injection into a gas chromatograph (Hewlett-Packard 6890/597Ni electron capture detector) which was calibrated weekly using 0.31 and 1.5 mL L$^{-1}$ standards. Headspace O$_2$ partial pressures were always $\geq$ 19.5 kPa. Production rates were determined from the increase in concentration, headspace volume and mass of soil, and were corrected for N$_2$O dissolved in the soil water assuming gas-liquid equilibrium (Moraghan & Buresh 1977). The $P_{N2O}$ measurements were made in exp 2 with Lang, Reiff and Yolo soils, and also in exp 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$_2$H$_2$), 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$_2$H$_2$ treatment, the soils were flushed with humidified air exiting from the corresponding incubation cylinder for the following 24-48 h. Subsamples of C$_2$H$_2$-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$_2$H$_2$ 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$_4$)$_2$SO$_4$ 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$_2$H$_2$ 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\textsubscript{4}\textsuperscript{+} depletion rate (ADR) was determined by regression of NH\textsubscript{4}\textsuperscript{+} concentrations vs. time, and the gross AOR was calculated from:

$$\text{AOR} = \text{ADR} + \text{MR}. \quad (5)$$

**Sterile soil experiments**

Portions of each of soil were amended with solutions of sulphuric acid (H\textsubscript{2}SO\textsubscript{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\textsubscript{2}SO\textsubscript{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 solution used in the nitrification experiments to which varying concentrations of KNO\textsubscript{2} were added. Concentrations of KNO\textsubscript{2} were selected to achieve the same water content and range of soil NO\textsubscript{2}\textsuperscript{-} and HNO\textsubscript{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\textsubscript{3}\textsuperscript{-}, pH and gross NO and N\textsubscript{2}O production rates. Solutions and materials were sterilized by autoclaving or washing with 95% ethanol. Similar experiments were done using soils treated with H\textsubscript{2}SO\textsubscript{4} but not irradiated (nonsterile controls). The λ-irradiated and nonsterile control soils were kept in an air-dry state until addition of KNO\textsubscript{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.

\(^{15}\text{NO}_3\textsuperscript{-} \text{reduction}\)

Preliminary data analysis indicated that dissimilatory NO\textsubscript{3}\textsuperscript{-}reduction may have been responsible for some of the N\textsubscript{2}O produced during the nitrification experiments. This was further assessed using stable isotope \(^{15}\text{N}\) techniques. Portions of each soil were flushed with the simulated soil solution, allowed to air dry, and then amended with an aqueous solution of \(^{15}\text{N}\)-labelled potassium nitrate (KNO\textsubscript{2}) (51 atom% \(^{15}\text{N}\)), to achieve NO\textsubscript{3}\textsuperscript{-} concentrations of 70–100 μg N g\textsuperscript{-}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\textsubscript{2}H\textsubscript{2}, followed by determination of gross N\textsubscript{2}O production rate. Headspace gas samples were analysed for atom percentage \(^{15}\text{N}\) in the N\textsubscript{2}O and N\textsubscript{2} pools using continuous-flow isotope ratio mass spectrometry (IRMS) (Europa Hydra 20–20, PDZ Europa Ltd, UK). Soils were extracted in 0.5 m K\textsubscript{2}SO\textsubscript{4} and atom percentage \(^{15}\text{N}\) in the NO\textsubscript{3}\textsuperscript{-} pools were determined according to methods described by Stevens & Laughlin (1994).

**Results**

**Nitrification experiments**

**Inorganic N dynamics.** Flushing with the salt/NH\textsubscript{4}\textsuperscript{+} solution resulted in uniform soil NH\textsubscript{4}\textsuperscript{+} concentrations for each soil (CV = 4–6%, n = 5). Initial concentrations of approximately 225, 580 and 800 μg NH\textsubscript{4}\textsuperscript{+}-N g\textsuperscript{-}1 soil were retained by Lang, Reiff and Yolo soils, respectively, varying in proportion to cation exchange capacity. These NH\textsubscript{4}\textsuperscript{+} 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 20, 10 and 22 d in Lang, Reiff and Yolo soils, respectively, followed by (iii) a period of reduced nitrification and NO\textsubscript{3}\textsuperscript{-} accumulation.

(i) **Increasing nitrification rate period.** In all experiments, NO\textsubscript{3}\textsuperscript{-} accumulation was evident within 2 d, and the rate of NO\textsubscript{3}\textsuperscript{-} accumulation increased over the first 4 d. A peak in NO\textsubscript{3}\textsuperscript{-} 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\textsuperscript{-}1 soil. In Yolo soil, NO\textsubscript{3}\textsuperscript{-} levels were fairly constant (∼0.15 μg N g\textsuperscript{-}1). Net N mineralization rates after 2 d of incubation in the C\textsubscript{2}H\textsubscript{2}-treated soils (not amended with N) were 63, 45 and 21 ng N g\textsuperscript{-}1 soil h\textsuperscript{-1}, respectively, for Lang, Reiff and Yolo soils, and subsequently decreased (Table 2).

(ii) **Rapid nitrification period (low NO\textsubscript{3}\textsuperscript{-} accumulation).** After the first 3–4 d, concentrations of NH\textsubscript{4}\textsuperscript{+} and total NO\textsubscript{2}+NO\textsubscript{3}\textsuperscript{-} changed linearly with respect to time \((r^2 > 0.95)\) (Table 2). The NO\textsubscript{2}\textsuperscript{-} concentrations were below detectable levels (0.01 μg N g\textsuperscript{-}1) in Lang experiments, and were also low in Reiff and Yolo soils (0.1–0.4 μg N g\textsuperscript{-}1). Gross NH\textsubscript{4}\textsuperscript{+} oxidation rates (AORs) were 5–8 times greater in Reiff and Yolo as compared to Lang soil. Net NO\textsubscript{2}+NO\textsubscript{3}\textsuperscript{-} accumulation rates were within 79–110% of calculated gross autotrophic NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} losses not accounted for in the mineralization experiments and/or losses of NO\textsubscript{3}\textsuperscript{-} by denitrification.
(iii) $\text{NO}_2^-$ accumulation/reduced nitrification rate period. In all experiments, rates of both $\text{NH}_4^+$ and $\text{NO}_2^-$ oxidation subsequently decreased coincident with an abrupt increase in $\text{NO}_2^-$ concentrations (Table 2, Fig. 2). Rates of accumulation of $\text{NO}_2^- + \text{NO}_3^-$ were markedly decreased in relation to $\text{NH}_4^+$ oxidation rates (Table 2).
Table 2 N transformation rates in nitrification experiments (in ng N g$^{-1}$ h$^{-1}$)

<table>
<thead>
<tr>
<th></th>
<th>Net mineralization$^1$</th>
<th>Net accumulation$^2$</th>
<th>Net depletion$^2$</th>
<th>Gross oxidation$^3$</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>NO$_3^-$</td>
<td>NO$_3^-$ + NO$_2^-$</td>
<td>NH$_4^+$</td>
<td></td>
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<tr>
<td>High nitrification rate phase</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lang 1</td>
<td>4 (± 0.4)</td>
<td>176</td>
<td>176</td>
<td>172</td>
<td>176</td>
</tr>
<tr>
<td>Lang 2</td>
<td>155</td>
<td>155</td>
<td>130</td>
<td>134</td>
<td>11</td>
</tr>
<tr>
<td>Reiff 1</td>
<td>17 (± 3.5)</td>
<td>807</td>
<td>807</td>
<td>767</td>
<td>784</td>
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<tr>
<td>Reiff 2</td>
<td>995</td>
<td>996</td>
<td>1008</td>
<td>1025</td>
<td>4</td>
</tr>
<tr>
<td>Yolo 1</td>
<td>6 (± 1.7)</td>
<td>582</td>
<td>581</td>
<td>730</td>
<td>736</td>
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<tr>
<td>Yolo 2</td>
<td>566</td>
<td>566</td>
<td>689</td>
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<td>NO$_2^-$ accumulation/reduced nitrification phase</td>
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<td></td>
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<tr>
<td>Lang 1</td>
<td>5 (± 0.4)</td>
<td>13.6 (0.41)</td>
<td>13.6 (0.43)</td>
<td>94 (0.94)</td>
<td>99</td>
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<tr>
<td>Lang 2</td>
<td>6.5 (0.18)</td>
<td>6.2 (0.17)</td>
<td>77 (0.92)</td>
<td>82</td>
<td>10</td>
</tr>
<tr>
<td>Reiff 1</td>
<td>2 (± 0.5)</td>
<td>44.9 (0.91)</td>
<td>51.9 (0.93)</td>
<td>338</td>
<td>336</td>
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<tr>
<td>Reiff 2</td>
<td>41.8 (0.93)</td>
<td>50.0</td>
<td>301</td>
<td>299</td>
<td>16</td>
</tr>
<tr>
<td>Yolo 1</td>
<td>12 (± 2.8)</td>
<td>221 (0.91)</td>
<td>231 (0.91)</td>
<td>251 (0.91)</td>
<td>239</td>
</tr>
<tr>
<td>Yolo 2</td>
<td>210</td>
<td>210</td>
<td>243 (0.80)</td>
<td>231</td>
<td>6</td>
</tr>
</tbody>
</table>

$^1$Net mineralization rates (MR) in C$_2$H$_5$-treated, unfertilized soil (± 1 SD, n = 2).
$^2$Accumulation and depletion rates from concentration vs. time regression; $r^2 ≥ 0.95$ (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$_3^- +$ NO$_2^-$. (Table 2).

Net mineralization. The AORs (Table 2) were calculated from (5) using the net N mineralization rates (MRs) measured in the C$_2$H$_5$-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$_4$NO$_3$ (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$_2$O 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$_2$O 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$_2$O 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.

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;
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 ($r^2<0.18$).
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$_2$O production generally decreased by only 50–60% after C$_2$H$_2$ treatment (Table 3).

$^{15}$NO$_3^-$ reduction

Significant enrichments in atom % of $^{15}$N were evident in N$_2$O evolved from soils amended with K$^{15}$NO$_3$, indicating that NO$_3^-$ reduction was the primary source of N$_2$O in soils treated with 10 Pa C$_2$H$_2$. 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$_2$O pools were 96, 80 and 71%, respectively, of the theoretical values assuming that increases in headspace N$_2$O 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$_2$H$_2$ penetration to microsites.

Sterile soil experiments

Production of NO was detected within seconds after the addition of KNO$_2$ solutions to both $\lambda$-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:

$$P_{NO} = \frac{d[NO]}{dt} = k_{PNO}[HNO_2]$$

Values of $k_{PNO}$ were on the order of 1 µg NO-N per µg HNO$_2$-N per h in $\lambda$-irradiated soil (Fig. 4). The nonsterile control data (not shown) were similar to the $\lambda$-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 C₂H₂-inhibition experiments

<table>
<thead>
<tr>
<th>Day</th>
<th>NO₂⁻ (µg N g⁻¹)</th>
<th>²Δ(NO₂⁻ + NO₃⁻)</th>
<th>PNO (ng N g⁻¹ h⁻¹)</th>
<th>PN₂O (ng N g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₂H₂</td>
<td>Air</td>
<td>C₂H₂</td>
<td>Air</td>
</tr>
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<td>&lt;0.01</td>
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</tr>
<tr>
<td></td>
<td>19</td>
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<td>0.03</td>
<td>+1.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>-1.0</td>
</tr>
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<td>-0.9</td>
</tr>
<tr>
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<td>13</td>
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<td>7.9</td>
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</tr>
<tr>
<td></td>
<td>29</td>
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<td>6.2</td>
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</tr>
<tr>
<td>Yolo</td>
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<td>0.17</td>
<td>-1.3</td>
</tr>
<tr>
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<td>8</td>
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<tr>
<td></td>
<td>24</td>
<td>&lt;0.01</td>
<td>3.5</td>
<td>-1.5</td>
</tr>
</tbody>
</table>

¹NO₂⁻ concentration remaining in soil after C₂H₂ treatment.
²Change in NO₂⁻ + NO₃⁻ concentration during incubation period.

![Fig. 6 Effect of 10 Pa C₂H₂ on NO₂⁻ and gross NO production rate (P₂O₂) in Reiff soil. Each point is mean of 2 replicates. Open symbols = C₂H₂-treated soil; closed symbols = untreated soil.](image)

Fig. 6 Effect of 10 Pa C₂H₂ on NO₂⁻ and gross NO production rate (P₂O₂) in Reiff soil. Each point is mean of 2 replicates. Open symbols = C₂H₂-treated soil; closed symbols = untreated soil.

Linear increases in headspace N₂O concentration were observed in jars containing NO₂⁻ amended, λ-irradiated soils over periods of 1–3 h. Rates of abiotic N₂O production were highly correlated with HNO₂ (Fig. 4). Apparent N₂O production rate coefficients [k₂O₂, corresponding to k₂NO in eqn (6)] were a small fraction (0.6–4%) of the sterile k₂NO values. Rates of gross N₂O production per unit of HNO₂ were higher in the nitrifying nonsterile compared to sterile soils in all cases, with significant N₂O production observed at NO₂⁻ and HNO₂ concentrations near zero (Fig. 4). The k₂NO values for nonsterile control soils (data not shown) were 5–30% higher than λ-irradiated soils, with less linearity with respect to HNO₂ concentration (r² = 0.60–0.75), suggesting that the effects of λ radiation caused some attenuation of N₂O production, and/or that biological activity occurring in the nonsterile control soil in the 1–3 h following wetting produced some N₂O. Soils amended with NO₂⁻-free deionized H₂O demonstrated very low rates of NO and N₂O 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 NO₂⁻ were detected in some of the deionized H₂O-amended soils).

Discussion

NO₂⁻ accumulation

Concentrations of NO₂⁻ 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 NH₄⁺ oxidizers, as has been previously observed (Morrill & Dawson 1967). During the high NO₂⁻ 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 HNO₂ (Hunik et al. 1992, 1993). Reduced pH can also inhibit NH₄⁺ oxidation by influencing substrate availability (Prosper 1989), and NO₂⁻ oxidation by apparently enhancing end-product inhibition (Hunik et al. 1993). The onset of high NO₂⁻ accumulation occurred after oxidation of approximately 100, 200 and 300 µg NH₄⁺-N g⁻¹ soil in Lang, Reiff and Yolo soils.
Localized concentrations of >1000 μg NH₄⁺-N g⁻¹ (initially) and >200 μg NO₃⁻-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 NO₃⁻ 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 NO₃⁻ oxidation in acid soils (Prosser 1989). The differences observed in critical pH values between soils, and the subsequent temporal patterns of NO₃⁻ concentration, may have reflected differences in acid-tolerant or the dominant NO₃⁻ 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 HNO₂ concentrations, and reported values of k_pNO and k_pNO₂, are specific to the soil pH method used.

**NO and N₂O production**

The data suggest that the primary mechanism of NO production involved NO₃⁻ derived from autotrophic NH₄⁺ oxidation which equilibrated with H⁺ in soil solution or near charged surfaces to form HNO₂. The aqueous decomposition of NO is thought to proceed according to:

\[ 3\text{HNO}_2 \rightarrow 2\text{NO} + \text{HNO}_3 + \text{H}_2\text{O}, \]

or similar reactions (Van Cleemput & Baert 1976). Reactions of HNO₂ 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 HNO₂ 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 HNO₂-mediated reactions during the high NO₂⁻ accumulation periods, it also suggests that these may have been the primary source even when NO₂⁻ concentrations were low. During high nitrification rate periods, residual NO₂⁻ 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 NO₂⁻ through some finite region is required, providing the opportunity for abiotic HNO₂ and NO production, even when bulk NO₂⁻ concentrations are low relative to NH₄⁺ or NO₃⁻. This may explain the difficulty encountered in studies attempting to correlate NO emissions with NH₄⁺ or NO₃⁻, without considering NO₂⁻. The k_pNO values obtained in sterile and nonsterile soils indicate that at low NO₂⁻ concentrations and only slightly acidic pH, significant NO production can be attributed to abiotic reactions. For example, at 0.5 μg NO₂⁻·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 attributed to nitrification in recent studies (Bollman & Conrad 1998). While initial NO₂⁻ levels are often reported in NO studies, it is not clear to what extent the dynamics (e.g., after wetting and incubation) of NO₂⁻ concentrations are responsible for observed NO production.

No trends in residuals were apparent in regression results of \( P_{NO} \) vs. HNO₂ for the nonsterile Reiff data \( (r^2 = 0.967, \ n = 42) \), suggesting that HNO₂ was the primary driving factor. For Lang and Yolo soils, when HNO₂ levels were <5 ng N g⁻¹ soil, simple regression models (with HNO₂) consistently under-predicted \( P_{NO} \) by 1–6 ng NO-N g⁻¹ h⁻¹. Incorporation of NH₄⁺ concentration into multiple regression models eliminated these trends \( (R^2 = 0.942 \ and \ 0.960 \ for \ Lang \ and \ Yolo) \). This suggests that NH₄⁺ 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 NH₄⁺ to NO₂⁻ has been suggested, although its role is this regard has not been confirmed (Hooper 1982). There is no evidence that rates of gross NH₄⁺ 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 (NO₂⁻) by competing biological and chemical reactions. Yolo nitrifying soil showed the closest agreement between NH₄⁺ oxidation and NO₃⁻ accumulation rates during the NO₂⁻ accumulation (high NO production) period (Table 2), indicating that significant microbial NO₂⁻ oxidation continued throughout this period. Microbial NO₂⁻ utilization occurring simultaneously with abiotic HNO₂ reduction could have competed for available NO₂⁻ 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$_3^-$ to N$_2$O could have had the same effect.

Multiple processes were important in controlling N$_2$O production. Abiotic N$_2$O 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 $k_{\text{N$_2$O}}$ values. The $^{15}$N data together with the two-factor (NO$_3^-$, NO$_2^-$) regression results (Fig. 5) and the C$_2$H$_2$ 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. $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O}$, was responsible for a significant fraction of the N$_2$O produced, presumably due to microbial activity in anoxic soil microenvironments. This has been suggested by prior studies (Goodroad & Keeney 1983) where N$_2$O production was correlated with NO$_3^-$ under relatively dry conditions. Increases in N$_2$O production rates after exposure to higher levels (10 kPa) of C$_2$H$_2$, which inhibits N$_2$O 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$_2$O $\rightarrow$ N$_2$. Overall, the data indicate that biological reductive processes were more important in controlling N$_2$O production as compared to NO production, since NO production in C$_2$H$_2$-treated soils was generally below detectable levels (Table 3).

Concluding remarks

The central role of NO$_3^-$ and HNO$_2$ in controlling NO and N$_2$O 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$_4^+$ or NH$_3^+$-based fertilizers can inhibit NO$_3^-$ 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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