Global Change Biology (2007) **13,** 1798–1809, doi: 10.1111/j.1365-2486.2007.01389.x

# Nitrite-driven nitrous oxide production under aerobic soil conditions: kinetics and biochemical controls

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**Abstract**

**Nitrite ($NO_2^-$) can accumulate during nitrification in soil following fertilizer application. While the role of $NO_2^-$ as a substrate regulating nitrous oxide ($N_2O$) production is recognized, kinetic data are not available that allow for estimating $N_2O$ production or soil-to-atmosphere fluxes as a function of $NO_2^-$ levels under aerobic conditions. The current study investigated these kinetics as influenced by soil physical and biochemical factors in soils from cultivated and uncultivated fields in Minnesota, USA. A linear response of $N_2O$ production rate ($P_{N_2O}$) to $NO_2^-$ was observed at concentrations below 60 μg N $g^{-1}$ soil in both nonsterile and sterilized soils. Rate coefficients ($K_p$) relating $P_{N_2O}$ to $NO_2^-$ varied over two orders of magnitude and were correlated with pH, total nitrogen, and soluble and total carbon (C). Total C explained 84% of the variance in $K_p$ across all samples. Abiotic processes accounted for 31–75% of total $N_2O$ production. Biological reduction of $NO_2^-$ was enhanced as oxygen ($O_2$) levels were decreased from above ambient to 5%, consistent with nitrifier denitrification. In contrast, nitrate ($NO_3^-$)-reduction, and the reduction of $N_2O$ itself, were only stimulated at $O_2$ levels below 5%. Greater temperature sensitivity was observed for biological compared with chemical $N_2O$ production. Steady-state model simulations predict that $NO_2^-$ levels often found after fertilizer applications have the potential to generate substantial $N_2O$ fluxes even at ambient $O_2$. This potential derives in part from the production of $N_2O$ under conditions not favorable for $N_2O$ reduction, in contrast to $N_2O$ generated from $NO_3^-$ reduction. These results have implications with regard to improved management to minimize agricultural $N_2O$ emissions and improved emissions assessments.**

*Keywords:* anhydrous ammonia, fertilizer, greenhouse gas, nitric oxide, nitrification, nitrifier denitrification, pH, $Q_{10}$, soil carbon, urea

*Received 12 January 2007 and accepted 19 March 2007*

## Introduction

Improved understanding of controls over soil nitrous oxide ($N_2O$) production may help to develop agricultural practices that minimize $N_2O$ emissions and also improve emissions estimates at ecosystem and larger scales. Studies have examined substrate-specific kinetics associated with $N_2O$ derived from denitrification under anaerobic conditions (Dendooven *et al*., 1994; Holtan-Hartwig *et al*., 2000). Nitrification and related biochemical processes have also been identified as $N_2O$ sources under aerobic conditions (Firestone & Davidson, 1989). Nitrification-derived $N_2O$, and all known mechanisms of soil $N_2O$ production, involve the biochemical or chemical reduction of nitrite ($NO_2^-$) (Stevens & Laughlin, 1998; Wrage *et al*., 2001). Despite the central role of $NO_2^-$, there is little kinetic information relating $NO_2^-$ availability to $N_2O$ production rates under aerobic conditions, or how these kinetics are affected by biochemical or physical factors.

Most soils produce $NO_2^-$ following fertilizer application, at least to some degree. Morrill & Dawson (1967) found that 72 of 92 soils exhibiting nitrification accumulated $NO_2^-$ temporarily when perfused with ammonium ($NH_4^+$) salt solutions. Anhydrous ammonia ($NH_3$) and urea, which together account for 80% of worldwide nitrogen (N) fertilizer use (IFA, 2006), generate $NO_2^-$ levels exceeding 50 μg N $g^{-1}$ soil (Chapman & Liebig, 1952; Chalk *et al*., 1975; Venterea & Rolston, 2000a). Concentrations exceeding 50 μg N $g^{-1}$ soil have also been found in soils amended with cattle urine

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(Monaghan & Barraclough, 1992). Lower levels ($3\,ng\,N\,g^{-1}$ to $3\,\mu g\,N\,g^{-1}$) have been measured in N-amended grassland and forest soils (Burns *et al*., 1995; Venterea *et al*., 2003). It is believed that $NO_2^-$ does not accumulate substantially in unfertilized soil, although its measurement in unfertilized soil is hampered by the need for very low levels of detection. Kinetic nitrification models predict some degree of $NO_2^-$ accumulation in response to external $NH_4^+$ inputs, although it is not known if mineralization of soil N alone could have this effect (Paul & Domsch, 1972; Venterea & Rolston, 2000b).

Knowledge regarding $NO_2^-$-mediated $N_2O$ production in soil is based in large part on studies in pure microbiological and chemical systems. Aerobic nitrifying bacteria including *Nitrosomonas europaea* and *Nitrosovibrio* sp. that oxidize $NH_4^+$ to $NO_2^-$ can also utilize $NO_2^-$ as an electron acceptor and in the process generate $N_2O$ (Ritchie & Nicholas, 1972; Poth & Focht, 1985; Remde & Conrad, 1990). Most data indicate that 'nitrifier denitrification' proceeds readily at ambient oxygen ($O_2$) concentration, but is enhanced as $O_2$ levels decrease. In addition to strictly biological production, Stevenson & Swaby (1964) showed that $N_2O$ is chemically produced following $NO_2^-$ addition to acidic soil organic matter fractions. Stevenson *et al*. (1970) later demonstrated the feasibility of these reactions under neutral to slightly acidic conditions more representative of soil. Reaction pathways proposed in earlier studies have been partly confirmed using $^{15}N$ nuclear magnetic resonance (Thorn & Mikita, 2000). The importance of abiotic reactions in regulating field $N_2O$ emissions in soil fertilized with anhydrous $NH_3$ has been suggested by Venterea & Rolston (2000a).

The aim of the current study was to quantify $N_2O$ production kinetics and examine biochemical controls under laboratory conditions simulating $NO_2^-$ accumulation in cultivated and uncultivated soils. The kinetic parameters obtained were analyzed in relation to soil biochemical properties and used in a simplified model to estimate potential field $N_2O$ emissions originating from $NO_2^-$-mediated reactions.

## Materials and methods

### *Sites and soils*

Soil samples were collected at the University of Minnesota's Research and Outreach Station in Rosemount, MN (44°45′N, 93°04′W). Annual 30-year mean precipitation and temperature are 879 mm and 6.4 °C, respectively. Soils were classified as Waukegan silt loam (fine-silty over sandy or sandy-skeletal mixed, superactive mesic Typic Hapludoll) containing 22% sand, 55% silt, and 23% clay in the upper 5 cm. Samples from cultivated fields were collected from plots within a long-term tillage and crop rotation management study (Venterea *et al*., 2005a). Samples were also collected from a woodland located within 1 km of the research plots that had not been cultivated in at least the past 50 years. Sampling locations were within 100 m of agricultural fields and in an area mapped with the same soil unit as the research field (USDA, 1983). Nine sampling locations (six cultivated and three uncultivated) were selected across a range of tillage treatments, landscape positions, and depths. Samples were collected from four depth intervals at each location (0–5, 5–10, 10–20, and 20–30 cm), generating 24 cultivated and 12 uncultivated samples. Two locations within the cultivated fields (denoted as C1 and C2) and one location within the uncultivated fields (U1) were examined more intensively. In order to minimize sample storage time, these sites were sampled on multiple occasions (0–5 cm depth only). Most experiments were done within 15 days of sample collection. Soils were sieved (2 mm), manually homogenized, and refrigerated (4 °C) until used. An effort was made to collect soils at a time when they were relatively dry, so that following addition of solutions, soil moisture content would be 50–70% of water-holding capacity (WHC). In some cases, partial air drying at 25 °C was done with monitoring of soil mass to limit drying to the required extent.

Selected soil properties are shown in Table 1. Soil pH was determined in 1 M KCl extracts (1 : 1 by mass). This method generally yields pH values that are 0.1–1 units lower than other methods (Sumner, 1994). Soluble organic carbon (SOC) was determined by extracting 8 g soil with 32 mL of 10 mM $CaCl_2$, filtration of the extract through 0.4 μm polycarbonate filters followed by analysis using UV-persulfate oxidation (Phoenix 8000[1]; Tekmar-Dohrmann, Cincinnati, OH, USA). Total C and N content were determined following ball milling using an elemental analyzer (Model NA 1500 NC; Carlo Erba/Fisons, Milan, Italy). Soil $NH_4^+$ and $NO_3^-$ were determined by extracting 10 g soil with 40 mL of 2 M KCl followed by flow-injection analysis (QuickChem 8500; Lachat, Loveland, CO, USA). Soil $NO_2^-$ was determined by extraction of 10 g with 40 mL of 2 M KCl adjusted to pH 8, followed by shaking for 10 min and centrifugation at 1240 *g* for 10 min (Stevens & Laughlin, 1995). Supernatants were analyzed within 6 h of collection using the modified Griess–Ilosvay method with flow-injection analysis. Initial soil $NO_2^-$ concentrations were $<0.5\,\mu g\,N\,g^{-1}$ soil.

[1]Mention of product names is for the convenience of the reader and implies no endorsement on the part of the author or the USDA.

*Kinetic experiments*

Experiments were done in microcosms consisting of 10–20 g of soil in 160 mL glass serum bottles fitted with butyl rubber septum caps. Solutions containing varying concentrations of sodium or potassium nitrite ($NaNO_2$ or $KNO_2$) were mixed with soil to achieve the desired range of $NO_2^-$ concentrations and soil moisture. Solutions were added using a fine-tipped needle that delivered the liquid in a fine spray. Bottles contents were mixed manually immediately following addition of solutions and at 30 min intervals. Microcosms were injected with 27 mL of air or other gas initially to maintain positive pressure. Gas samples (9 mL) were withdrawn by syringe at three time points during the incubation (typically 30, 60, and 90 min) and transferred to 9 mL glass vials sealed with butyl rubber septa. Vial contents were analyzed for $N_2O$ by gas chromatography (GC) with electron capture detection (ECD) (Venterea *et al.*, 2005a) within 48 h of collection. The ECD was calibrated at least daily using certified $N_2O$ gas standards (American Gas Group, Toledo, OH, USA; Scott Specialty Gases, Plumsteadville, PA, USA). These incubation conditions yielded highly linear relationships between headspace $N_2O$ concentration [$N_2O$] and time. Approximately 98% of incubations with $NO_2^-$-amended soil yielded $r^2$ values $>0.99$. The $N_2O$ production rate ($P_{N_2O}$, $\mu g\,N\,dry\,g^{-1}\,h^{-1}$) was calculated from the slope of [$N_2O$] vs. time, headspace volume, and soil mass, accounting for equilibrium gas–liquid partitioning (Tiedje, 1994). All treatments were applied to two or three replicate microcosms. The efficiency of $NO_2^-$ recovery from spiked soil was determined in separate tests to be generally within $\pm 5\%$ of added amounts. The above protocols were repeated across a range of conditions, as described below.

*Response to $NO_2^-$ addition*

Experiments were done using soils C1, C2, and U1 following the addition of $NO_2^-$ over both a high-range (0–260 $\mu g\,N\,g^{-1}$ soil) and a low-range (0–60 $\mu g\,N\,g^{-1}$ soil) of soil $NO_2^-$ concentrations followed by incubation at 25 °C under ambient headspace $O_2$. Low-range experiments were done using all 36 nonsterile samples, and using subsamples of C1, C2, and U1 which had been sterilized either by $\gamma$-radiation (5 Mrad) or steam autoclaving (Table 1). Effectiveness of the sterilization techniques was evaluated by measuring denitrification enzyme activity (DEA) before and following treatments (Tiedje, 1994). Both techniques resulted in 99% inhibition of DEA.

Spiking solutions in the above experiments were generally added at a ratio of 0.5 mL : 10 g fresh soil, except for uncultivated surface soils that had higher organic C contents and WHCs, which generally received 2.0 mL : 10 g fresh soil. Lower solution/soil ratios were found in preliminary experiments to result in inadequate mixing of solution and soil. Solution addition resulted in soil moisture contents equivalent to 50–60% of WHC, except in uncultivated surface soils where moisture contents were $\sim$70% of WHC. Additional experiments examining $N_2O$ production at higher soil moisture levels and lower $O_2$ levels are described below.

*Response to soil moisture and $^{15}NO_2^-$ additions*

The WHC in samples C1, C2, and U1 were determined gravimetrically to be 0.358, 0.365, and 0.620 g $H_2O\,g^{-1}$ soil, respectively. Experiments were conducted using these samples at soil moisture contents ranging from 50% to 100% of WHC under ambient $O_2$ at 25 °C. Soils were amended with varying volumes of solutions containing varying concentrations of $NO_2^-$, designed to result in a uniform soil $NO_2^-$ concentration ($\sim$60 $\mu g\,N\,g^{-1}$) across soil moisture levels. Parallel microcosms were amended with the same level of $NO_3^-$ (instead of $NO_2^-$) across the same range of moisture conditions to examine potential $N_2O$ production via $NO_3^-$ reduction. Both sets of microcosms were preincubated for 24 h under a headspace containing 10 Pa of acetylene ($C_2H_2$) to inhibit nitrification-derived $N_2O$ production in the $NO_3^-$-amended soils. Preliminary experiments found that preincubation under $C_2H_2$ had no effect on $N_2O$ production in the $NO_2^-$-amended soils. Potential DEA was determined in separate subsamples by amending soils with 180 $\mu g\,C\,g^{-1}$ as glucose and 50 $\mu g\,NO_3^-$-$N\,g^{-1}$ followed by anaerobic incubation for 1.5 h.

Soils receiving the intermediate moisture level treatments were amended with $NO_2^-$ that was enriched with $^{15}N$ (minimum 98 atom%, Sigma Aldrich, St Louis, MO, USA). At the end of the incubation, separate 12 mL gas samples were taken from each microcosm and transferred to pre-evacuated septum-capped glass tubes (Labco Ltd, Buckinghamshire, UK) for analysis of $^{15}N$ and $^{14}N$ content of sample $N_2O$ by continuous flow isotope ratio mass spectrometry.

*Response to $O_2$ availability*

Soils C1, C2, and U1 were incubated under headspace $O_2$ concentrations of $<0.1\%$, 5%, 10%, 15%, 21%, and 100% at 25 °C. Initial headspace $O_2$ concentration was controlled using a 10-port vacuum/pressurization manifold equipped with a digital vacuum-pressure gauge (DPG-1000; Omega Engineering, Stamford, CT,

**Table 1** Properties of soil samples used in kinetic experiments

| | Soil pH (1 : 1 M KCl) | SOC ($\mu g\,C\,g^{-1}$) | Total C ($mg\,C\,g^{-1}$) | Total N ($mg\,N\,g^{-1}$) | $NH_4^+$ ($\mu g\,N\,g^{-1}$) | $NO_3^-$ ($\mu g\,N\,g^{-1}$) |
|---|---|---|---|---|---|---|
| Cultivated | 4.9–6.0 | 4.5–13 | 17–31 | 1.3–2.7 | 0.3–2.8 | 0.86–31 |
| Uncultivated | 4.8–5.7 | 7.2–130 | 10–77 | 0.79–5.6 | 1.1–6.3 | <0.1–1.5 |
| C1 (cultivated)† | | | | | | |
| Nonsterile | 5.1 (0.02) | 6.5 (0.24)a | 25 (0.65) | 2.1 (0.06) | 1.1 (0.02)a | 1.3 (0.53) |
| $\gamma$-irradiated | 5.1 (0.02) | 150 (0.45)b | 26 (0.11) | 2.2 (0.02) | 11 (0.01)b | – |
| Autoclaved | 5.2 (0.03) | 1100 (12)c | 25 (0.21) | 2.2 (0.02) | 32 (1.9)c | – |
| C2 (cultivated) | | | | | | |
| Nonsterile | 5.3 (0.04) | 8.2 (0.25)a | 31 (0.51) | 2.5 (0.02) | 0.46 (0.08)a | 1.5 (0.51) |
| $\gamma$-irradiated | 5.4 (0.02) | 280 (3.3)b | 30 (0.79) | 2.5 (0.03) | 19 (0.14)b | – |
| Autoclaved | 5.4 (0.01) | 1500 (9.5)c | 31 (0.32) | 2.6 (0.03) | 57 (2.5)c | – |
| U1 (uncultivated) | | | | | | |
| Nonsterile | 5.6 (0.03) | 12 (4.8)a | 67 (0.72)b | 4.7 (0.04) | 1.9 (0.03)a | 1.0 (0.02) |
| $\gamma$-irradiated | 5.5 (0.13) | 790 (100)b | 70 (1.7)b | 4.8 (0.10) | 81 (0.53)b | – |
| Autoclaved | 5.6 (0.03) | 4000 (6.9)c | 63 (2.3)a | 4.5 (0.15) | 140 (5.5)c | – |

Ranges are reported for nonsterile cultivated ($n = 24$) and uncultivated ($n = 12$) samples.
†For comparisons among nonsterile, $\gamma$-irradiated and autoclaved soils, values followed by same letter are not significantly different ($P < 0.05$).
Specific values (means and standard errors, $n = 3$) are reported for samples C1, C2, and U1.
–, not measured; SOC, soluble organic carbon; N, nitrogen.

**Table 2** Descriptive statistics for first-order $N_2O$ production rate coefficient ($K_p$) and single-factor linear correlation results relating $K_p$ to selected chemical properties across all soils and depths

| | $K_p$ | | | | Correlation results | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Min | Max | pH | $10^{-pH}$ | SOC | Total C | Total N |
| | $10^{-4}\,h^{-1}$ | | | | $r$† | | | | |
| Cultivated ($n = 24$) | 4.43 | 4.05 | 1.04 | 15.4 | −0.49*‡ | 0.45* | 0.60** | 0.51* | 0.61** |
| Uncultivated ($n = 12$) | 28.1 | 31.5 | 3.69 | 103 | −0.57* | 0.60* | ns | 0.89*** | 0.87*** |
| All ($n = 36$) | 12.3 | 21.2 | 1.04 | 103 | −0.48** | 0.61*** | ns | 0.89*** | 0.84*** |

†Pearson product-moment correlation coefficient.
‡$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
ns, not significant; SOC, soluble organic carbon; N, nitrogen.

USA). Microcosms (except the 21% treatment) were connected to the manifold by inserting needles through the septa, followed by evacuation to <0.1 bar. Bottles were then pressurized with pure $N_2$ or pure $O_2$ to 1 bar, vented to just above atmospheric pressure, and the cycle was repeated a total of three times. Using $N_2$, this procedure produced headspace $O_2$ levels <0.1%. The 5%, 10%, and 15% treatments were pressurized with $N_2$ and then manually injected with an aliquot of pure $O_2$ using a syringe to achieve the desired $O_2$ levels. Before headspace manipulation, soils were amended with $60\,\mu g\,NO_2^-\text{-}N\,g^{-1}$ soil. Soils C1 and U1 were incubated at 60% and 70% of WHC, respectively. Soil C2 was incubated at 60% and 70% of WHC. Parallel sets of microcosms were amended with $60\,\mu g\,NO_3^-\text{-}N\,g^{-1}$ soil under the same $O_2$ and WHC conditions to examine the potential for $N_2O$ production driven by $NO_3^-$ reduction. Headspace $O_2$ levels were determined in the same 9 mL samples collected for $N_2O$ analysis using an automated valve to split a subsample to a separate GC with thermal conductivity detection. The change in headspace $O_2$ during incubation was negligible (<1% $O_2$). Responses to subambient vs. ambient $O_2$ were examined in sterilized subsamples.

### *Response to NO addition*

Separate microcosms containing soils C1, C2, and U1 amended with $H_2O$ to achieve water contents of 60%, 60%, and 75% of WHC, respectively, were injected with nitric oxide (NO) gas and then incubated at headspace $O_2$ levels of 21% and 5% at 25 °C. NO gas was injected

 

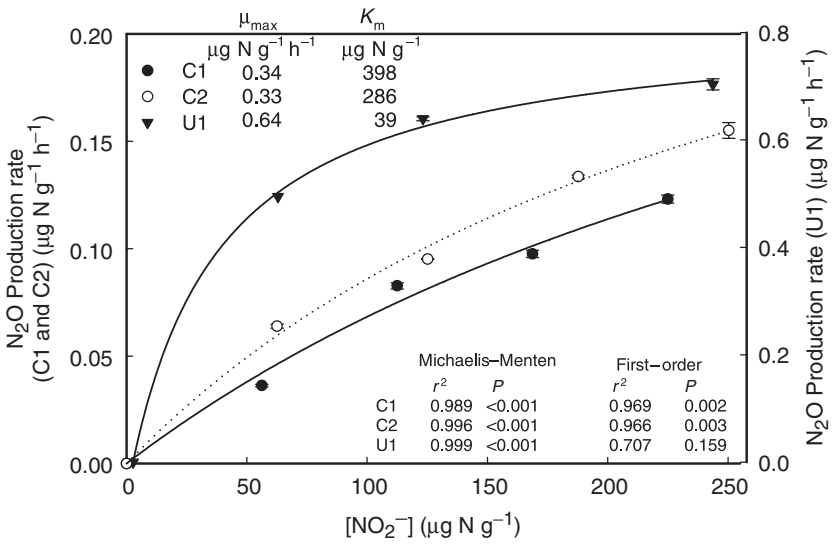


**Fig. 1** Nitrous oxide ($N_2O$) production rates vs. nitrite concentration [$NO_2^-$] in soils C1 and C2 (left-hand axis) and U1 (right-hand axis) in the high-range $NO_2^-$-addition experiments. Apparent half-saturation concentrations ($K_m$) and maximum production rate ($\mu_{max}$) values, and correlation coefficients ($r^2$) for Michaelis–Menten and first-order models are shown. Bars represent standard errors of three replicate subsamples.

to achieve initial concentrations of 0, 100, and 200 ppm using a 1000 ppm NO standard tank (balance He; Scott-Marrin, Riverside, CA, USA). The NO additions were equivalent to approximately 0%, 1.5%, and 3 μg NO-N $g^{-1}$. Levels of NO added were based on measurements of headspace NO concentrations in separate $NO_2^-$-addition experiments. Maximum NO concentrations of 40–120 ppm were measured 30, 60, and 90 min after additions of 60 μg $NO_2^-$-N $g^{-1}$ to soils C1, C2, and U1. Headspace NO concentrations were determined in 5 mL gas samples injected to a $NO_x$-free air stream (1 L $min^{-1}$) flowing to a chemiluminescent $NO_x$ analyzer (LMA-3D; Unisearch Associates, Ontario, Canada). Peak areas were integrated using data acquisition software, and concentrations determined by comparison with standards prepared using certified NO gas mixtures.

### *Response to temperature and $O_2$ availability*

Low-level $NO_2^-$ addition experiments were conducted using soils C1, C2, and U1 incubated at 5, 15, 25, and 35 °C and at headspace $O_2$ levels of 5% and 21% at each temperature. Incubations were conducted concurrently in separate temperature-controlled chambers. Soils C1 and U1 were incubated at 60% of WHC and soil U1 at 70% of WHC.

### *$N_2O$ reduction*

The potential for $N_2O$ reduction at varying headspace $O_2$ levels was examined in separate experiments. A preliminary incubation was conducted to remove ambient soil $NO_3^-$ so that $N_2O$ reduction could be directly measured in subsequent incubations (Holtan-Hartwig *et al.*, 2000). Subsamples (15 g) of three cultivated soils sampled from 0 to 5 cm depth were each placed into three replicate 160 mL serum bottles and amended with 1.0 mL of a 15 mM glutamic acid to achieve moisture contents equivalent to ~80% of WHC. The microcosms were evacuated followed by pressurization with pure $N_2$ (cycle repeated three times). Following 72 h of incubation, microcosms were again evacuated and filled with pure $N_2$ (three cycles) and sampled for $N_2O$ after 1 and 2 h to confirm that $N_2O$ accumulation was negligible (< 3 ng N $g^{-1}$ $h^{-1}$). Bottles were amended with 10 Pa of $C_2H_2$ and incubated anaerobically for another 24 h. A final set of evacuation/pressurization cycles was then applied, this time using a gas mixture containing 1000 ppm of $N_2O$ in $N_2$ (Scott Specialty Gases). After the final pressurization cycle, the bottles were vented to slightly above ambient pressure, and injected with pure $O_2$ to establish initial headspace $O_2$ levels of 5.0%, 2.5%, and < 0.1% (no $O_2$ addition) followed by incubation at 25 °C with sampling for headspace $N_2O$ following 0.5, 1.5, 4.5, and 7.5 h.

### *Model extrapolations*

The coefficients determined above were applied in a simplified $N_2O$ emissions model. Assuming steady-state and vertically uniform soil profile conditions with regard to soil gas diffusivity, temperature, bulk density, and water content, and the absence of any $N_2O$

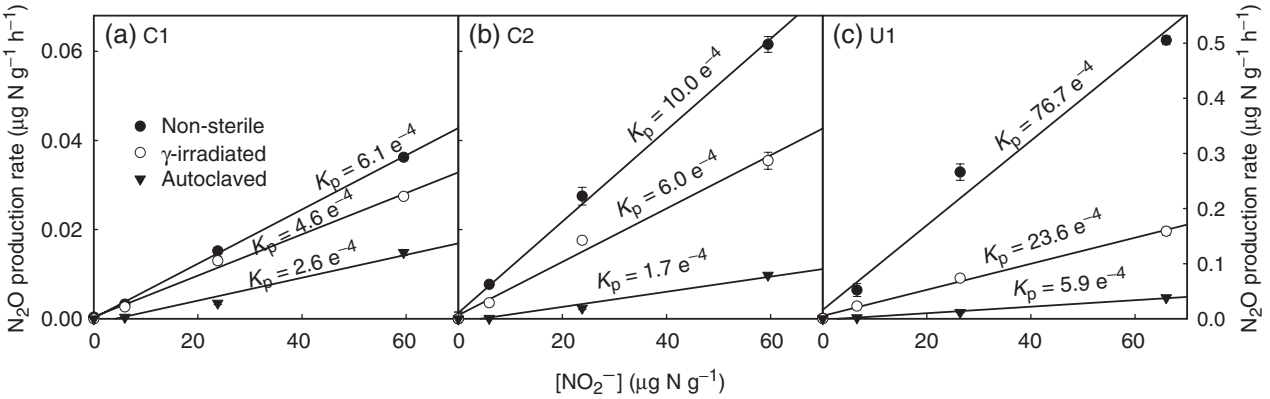


**Fig. 2** Nitrous oxide ($N_2O$) production rates vs. nitrite concentration [$NO_2^-$] in nonsterile, $\gamma$-irradiated, and autoclaved soils (a) C1 (left-hand axis), (b) C2 (left-hand axis), and (c) U1 (right-hand axis) in the low-range $NO_2^-$-addition experiments. Bars represent standard errors of three replicate subsamples. $K_p$ values were determined from slope per Eqn (4).

consumption, the equation governing vertical $N_2O$ diffusive transport is

$$-D_p \frac{d^2[N_2O]}{dz^2} = \rho P_{N_2O}, \quad (1)$$

where $D_p$ is the soil–gas diffusion coefficient ($cm^3$ gas $cm^{-1}$ soil $h^{-1}$), [$N_2O$] is the soil–gas $N_2O$ concentration (μg N $cm^{-3}$ gas), $\rho$ is soil bulk density (g $cm^{-3}$), and $z$ is depth (cm soil) (Hillel, 1982). Equation (1) can be integrated once to determine the vertical $N_2O$ concentration gradient at the soil surface ($z = 0$) and then combined with Fick's equation to yield an expression for the $N_2O$ flux ($F_{N_2O}$, μg N $cm^{-2}$ $h^{-1}$) that is independent of $D_p$:

$$F_{N_2O} = \rho \int_{z_a}^{z_b} P_{N_2O}\, dz, \quad (2)$$

Eqn (2) assumes that there is a gas-impermeable (no-flux) boundary at some depth in the soil and that $N_2O$ production occurs in a vertical band of thickness ($z_b$–$z_a$). Additional details of the model application are described in 'Results.'

## Results

### *Response to $NO_2^-$ addition*

Addition of $NO_2^-$ to soils C1, C2, and U1 over the high concentration range followed by aerobic incubation yielded $N_2O$ production rates that could be described using Michaelis–Menten kinetic models (Fig. 1) (Pauling, 1970), i.e.,

$$P_{N_2O} = \left(\frac{\mu_{max}[NO_2^-]}{K_m + [NO_2^-]}\right). \quad (3)$$

Apparent half-saturation concentrations ($K_m$) obtained by nonlinear regression were more than six times higher in soils C1 and C2 than U1. The maximum production rate ($\mu_{max}$) in U1 was more than double that in C1 and C2. Owing to high $K_m$ values in soils C1 and C2, linear models (i.e. first-order with respect to $NO_2^-$) described the data reasonably well ($r^2 > 0.96$, $P < 0.01$).

Over the low $NO_2^-$ concentration range, first-order models were sufficient to describe the data in all 24 cultivated soils ($r^2 > 0.99$), as shown for soils C1 and C2 in Figs. 2a and b. In the 16 uncultivated soils, first-order models were also reasonably effective ($r^2 > 0.93$) as shown in Fig. 2c for soil U1. To describe responses at $NO_2^- \leq 60$ μg N $g^{-1}$, a first-order rate constant $K_p$ ($h^{-1}$) was defined from the slope of $P_{N_2O}$ vs. $NO_2^-$ where

$$P_{N_2O} = K_p[NO_2^-]. \quad (4)$$

Values of $K_p$ in nonsterile C1, C2, and U1 soils were consistently greater than in sterilized soils, and $\gamma$-irradiated $K_p$ values were consistently greater than autoclaved values (Fig. 2). $K_p$ values in $\gamma$-irradiated soils were 25%, 40%, and 69% lower than in nonsterile soils for C1, C2, and U1, respectively.

Across all soils and depths, $K_p$ values ranged over two orders of magnitude, with a high degree of variance within the cultivated (CV = 98%) and uncultivated (CV = 112%) soils (Table 2). Significant linear correlations were evident between $K_p$ and pH, $10^{-pH}$, SOC, total C, and total N (Table 2). A linear multiple regression model explained 70% of the variance in $K_p$ within the cultivated soils (Fig. 3a). A nonlinear single-factor model explained 84% of the overall variance as a function of total C (Fig. 3b). The soil properties listed in Table 1 for soils C1, C2, and U1 correspond to the $K_p$ data included in Fig. 3 and Table 2. In data sets described below, some variation in $N_2O$ production rates in response to $NO_2^-$ additions were evident due

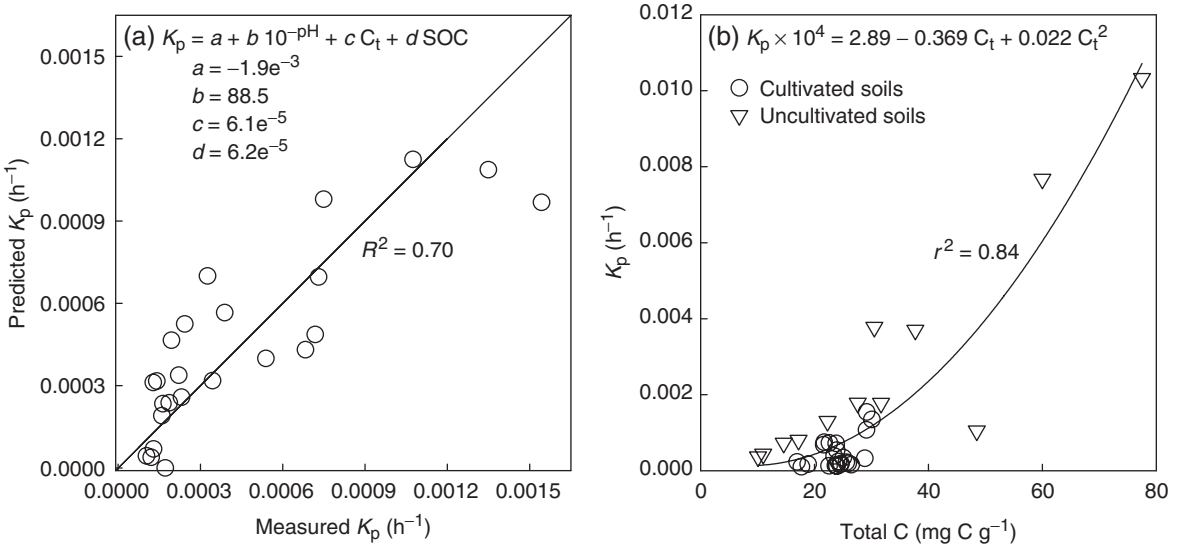


**Fig. 3** (a) Multiple regression model describing nitrous oxide ($N_2O$) production rate coefficient ($K_p$) as function of soil pH (1 : 1 M KCl), total carbon ($C_t$, mg C $g^{-1}$) and soluble organic carbon (SOC, μg C $g^{-1}$) in cultivated soils ($n = 24$), and (b) nonlinear model describing $K_p$ as function of total carbon in all cultivated and uncultivated soils ($n = 36$).

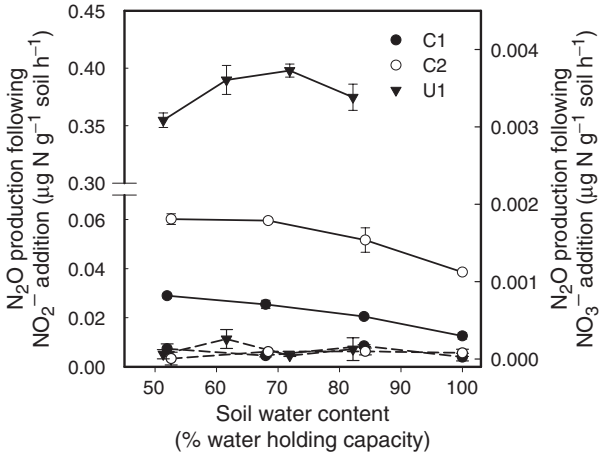


**Fig. 4** Nitrous oxide ($N_2O$) production rates following the addition of 60 μg N $g^{-1}$ as either $NO_2^-$ (left-hand axis, solid lines) or $NO_3^-$ (right-hand axis, dashed lines) in soil incubated at 21% $O_2$ at varying soil water content in soils C1, C2, and U1. Bars represent standard errors of three replicate subsamples.

to variation in properties among samples collected on different dates.

### *Response to moisture content and $^{15}NO_2^-$ additions*

In soils incubated at 21% $O_2$ following the addition of 60 μg $NO_2^-$-N $g^{-1}$ soil, the rate of $N_2O$ production in the two cultivated soils (C1 and C2) decreased as soil moisture increased above 50% of WHC (Fig. 4). In the uncultivated soil (U1), $P_{N_2O}$ was maximal in the range of 60–70% of WHC with decreased $N_2O$ production at lower and higher soil water contents. The decreased response at lower water content in U1 was likely due to inadequate mixing of solution with soil, as found in preliminary experiments at moistures <50% WHC (data not shown). The $^{15}N$ contents of the evolved $N_2O$ were used to calculate the $^{15}N$-enrichment of the $N_2O$ source pools. Source pool $^{15}N$ enrichments were consistent across soils, ranging from 95.6 to 95.9 atom%, indicating that >95% of the $N_2O$ originated from added $^{15}N$. Rates of $N_2O$ production following addition of 60 μg $NO_3^-$-N $g^{-1}$ were <1% of rates observed following the addition of the same amount of $NO_2^-$, and there was no apparent response to soil moisture in $NO_3^-$-amended soils under aerobic conditions (Fig. 4). $N_2O$ was readily produced in 1.5 h anaerobic incubations using separate subsamples amended with $NO_3^-$ and glucose. Potential DEA rates were 0.17, 0.43, and 0.94 μg N $g^{-1}$ $h^{-1}$, respectively, in soils C1, C2, and U1.

### *Response to $O_2$ availability*

All $NO_2^-$-amended soils displayed increases in $P_{N_2O}$ as headspace [$O_2$] decreased from 21 to <0.1% (Fig. 5). The rate of increase per unit decrease in [$O_2$] was fairly linear over the range 5–21% ($r^2 \geq 0.94$). The C2 soil incubated at 70% WHC displayed higher $N_2O$ production at [$O_2$] ≤10% compared with at 60% WHC (Fig. 5b). In contrast, $NO_3^-$-amended soils showed no response to varying $O_2$ except at <0.1%. Rates of $N_2O$ produced in $NO_2^-$-amended soils incubated at 100% $O_2$ were significantly lower ($P < 0.05$) than soils incubated at ambient $O_2$ (data not shown). Mean production rates

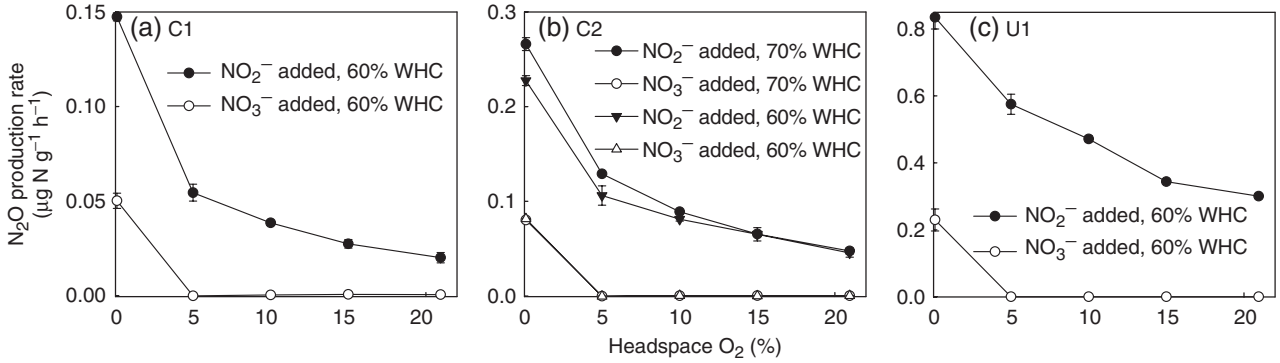


**Fig. 5** Nitrous oxide ($N_2O$) production rates after addition of 60 μg N g$^{-1}$ as either $NO_2^-$ (closed symbols) or $NO_3^-$ (open symbols) at varying levels of headspace oxygen ($O_2$) in soils (a) C1, (b) C2 and (c) U1. Bars represent standard errors of three replicate subsamples.

at 100% $O_2$ in soils C1, C2, and U1 were 13%, 42%, and 63% lower than at 21% $O_2$, and were similar to rates measured in $\gamma$-irradiated soils at 21% $O_2$. There was no response to $O_2$ in $NO_2^-$-amended sterilized soils (data not shown).

### *Response to NO addition*

In soils incubated at 5% $O_2$, $N_2O$ production increased from $<0.0005$ to 0.01–0.05 μg N g$^{-1}$ h$^{-1}$ as NO availability increased ($r^2 = 0.97$–$0.99$, Fig. 6). C2 and U1 exhibited similar responses, while a smaller response was evident in C1. At 21% $O_2$, $N_2O$ production rates at the highest level of NO addition were negligible ($<0.004$ μg N g$^{-1}$ h$^{-1}$) and not significantly different from rates in the absence of NO addition ($P > 0.3$).

### *Response to temperature and $O_2$ availability*

Temperature responses in C1, C2, and U1 at both 5% and 21% $O_2$ were well-described ($r^2 \geq 0.99$) by the Arrhenius equation

$$\ln K_p = A_o - \frac{E_a}{R}\frac{1}{T}, \quad (5)$$

where $R$ is the universal gas constant ($8.31 \times 10^{-3}$ kJ mol$^{-1}$ K$^{-1}$), $A_o$ is a coefficient representing various rate factors, and the activation energy ($E_a$, kJ mol$^{-1}$) can be estimated from a plot of ln $K_p$ vs. the reciprocal of the absolute temperature ($T$, K) (Pauling, 1970) (Fig. 7). $Q_{10}$ factors were also calculated from the data at 25 and 35 °C. A pattern of higher $E_a$ and $Q_{10}$ values (i.e. greater temperature sensitivity) at the lower $O_2$ level was consistent across soils (Fig. 7). When analyzed by two-way analyses of variance, this trend resulted in a significant temperature-by-$O_2$ interaction effect for all three soils ($P < 0.01$).

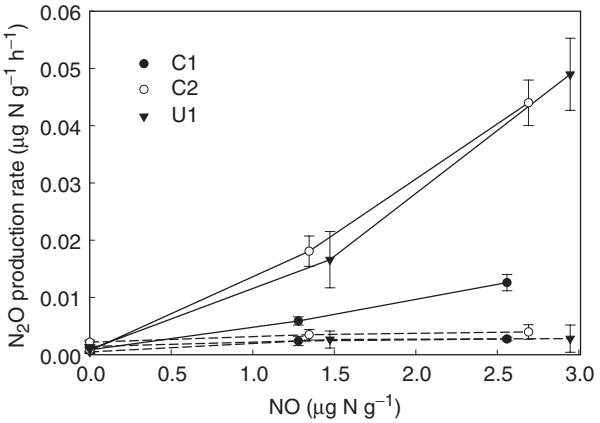


**Fig. 6** Nitrous oxide ($N_2O$) production rates following addition of nitric oxide (NO) gas in soils incubated at 21% $O_2$ (dashed lines) and 5% $O_2$ (solid lines) in soils C1, C2, and U1. Bars represent standard errors of three replicate subsamples.

### *$N_2O$ reduction*

In microcosms using three cultivated soils at 80% of WHC, $N_2O$ consumption increased as headspace $O_2$ levels decreased below 5% (Fig. 8). There was no evidence of $N_2O$ consumption at 5% $O_2$. These data therefore imply that no $N_2O$ reduction occurred in the other experiments with cultivated soils as presented in Figs 1–7, except for the anaerobic treatments ($O_2 < 0.1\%$, Fig. 7) and possibly in the aerobic incubations at WHC $>80\%$ (Fig. 4). Analogous interpretations cannot be made with regard to the uncultivated soils as $N_2O$ reduction was not measured.

### *Model extrapolation*

Michaelis–Menten kinetic parameters obtained in the high-level $NO_2^-$ addition experiments (Fig. 1) were used to describe $P_{N_2O}$ in Eqn (2) assuming a 5 cm thick vertical band of $NO_2^-$ present in the soil profile. These

 

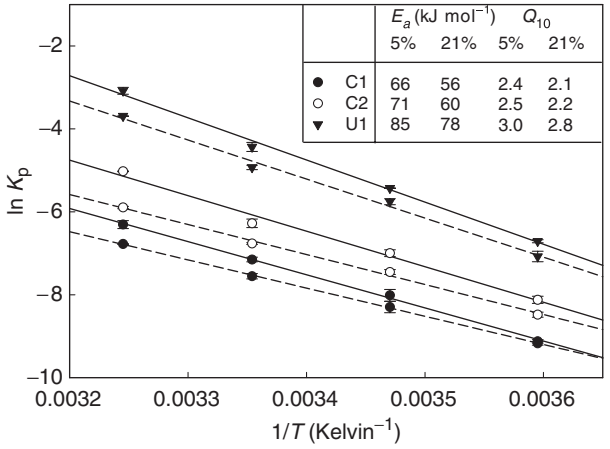


**Fig. 7** Nitrous oxide ($N_2O$) production rates following addition of 60 μg $NO_2^-$–N g$^{-1}$ in soils incubated at varying temperature at 21% $O_2$ (dashed lines) and 5% $O_2$ (solid lines) in soils C1, C2, and U1. Bars represent standard errors of two replicate subsamples.

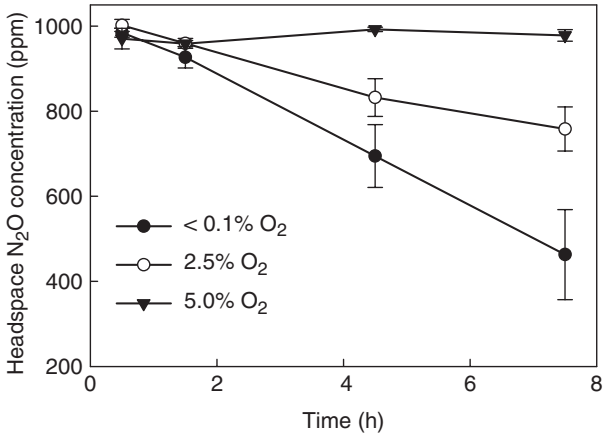


**Fig. 8** Depletion of nitrous oxide ($N_2O$) over time in headspace of microcosms incubated under varying levels of $O_2$. Bars represent standard errors of three cultivated soils.

measurements were made at 25 °C, which is representative of surface soil temperatures during May–July at this site (Venterea *et al*., 2005a). Assuming that $\mu_{max}$, $K_m$, and $[NO_2^-]$ are uniform in this band, Eqn (2) becomes

$$F_{N_2O} = L\rho\left(\frac{\mu_{max}[NO_2^-]}{K_m + [NO_2^-]}\right), \quad (6)$$

where $z_b$–$z_a = L = 5$ cm. Measured $\rho$ values for C1, C2, and U1 were 1.25, 1.35, and 0.9 g cm$^{-3}$, respectively. Eqn (6) predicts $N_2O$ fluxes of 1.0 and 1.4 kg N ha$^{-1}$ day$^{-1}$ in soils C1 and C2, respectively, at $[NO_2^-] = 100$ μg N g$^{-1}$, and a flux of 1.0 kg N ha$^{-1}$ day$^{-1}$ in soil U1 at $[NO_2^-] = 5$ μg N g$^{-1}$ (Fig. 9).

## Discussion

The kinetic data found here provide evidence that $NO_2^-$-driven reactions occurring under ambient to subambient $O_2$ have the potential to generate substantial fluxes, depending on soil $NO_2^-$ levels. Production rates following addition of 60 μg N g$^{-1}$ as $NO_2^-$ and incubated at 10–21% $O_2$ were comparable with $N_2O$ produced following $NO_3^-$ addition at <0.1% $O_2$ (Fig. 5). Soil $NO_2^-$ levels greater than 50 μg N g$^{-1}$ can persist for periods of weeks to months following anhydrous $NH_3$ or urea application (Chapman & Liebig, 1952; Chalk *et al*., 1975). A simplified model was used to put the kinetic measurements into context by estimating the order of magnitude of the resulting fluxes. The model estimates that $NO_2^-$ concentrations of 50–75 μg N g$^{-1}$ in a 5 cm thick band would generate steady-state fluxes of 0.6–1.1 kg N ha$^{-1}$ day$^{-1}$ using kinetic parameters obtained for cultivated soils at ambient $O_2$ and 25 °C. This range agrees very closely with peak $N_2O$ fluxes in anhydrous $NH_3$-fertilized fields (Bremner *et al*., 1981; Thornton & Valente, 1996; Venterea & Rolston, 2000a), and is comparable with fluxes attributed to anaerobic denitrification in other studies (Li *et al*., 1992; Riley & Matson, 2000).

An important aspect of $NO_2^-$-driven $N_2O$ production measured here under aerobic conditions is the low potential for $N_2O$ reduction, which only occurred at $O_2$ levels <5%. Thus, a large fraction of the $N_2O$ generated from these reactions would be subject to release to the atmosphere. In contrast, denitrification of $NO_3^-$ was only stimulated under low-$O_2$ conditions that also promoted $N_2O$ reduction. Similarly, Bollmann & Conrad (1998) found that denitrification-derived $N_2O$ exceeded $N_2O$ produced via nitrification only at $O_2$ <0.1%, although substrate-specific kinetics were not measured. While there is considerable diversity in the sensitivity of denitrification enzyme systems to $O_2$, pH, and other factors, $NO_3^-$ reduction is generally considered to be accompanied by at least the potential for $N_2O$ reduction (Tiedje, 1994; Stevens & Laughlin, 1998).

Biotic and abiotic processes acted simultaneously to generate $N_2O$. At ambient $O_2$, $N_2O$ production in γ-irradiated soils was 75%, 60%, and 31% of $N_2O$ production in nonsterile soils C1, C2, and U1, respectively (Fig. 2). The positive correlations between $K_p$ and total C and N, and negative correlations with pH, are also consistent with an abiotic component (Stevenson *et al*., 1970). It is possible that γ-radiation created artifacts by altering organic matter functional groups involved in nitrosation reactions initiating $N_2O$ production (Thorn & Mikita, 2000). This is suggested by the release of SOC following radiation and to a much greater extent following steam sterilization (Table 1). However, similar rates

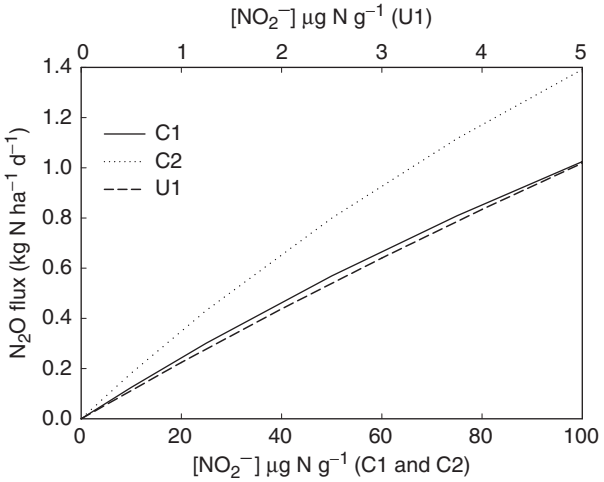


**Fig. 9** Steady-state fluxes predicted by nitrous oxide ($N_2O$) emissions model as a function of $NO_2^-$ concentration in soils C1 and C2 (bottom horizontal axis) and U1 (top horizontal axis).

of $N_2O$ production were observed in $\gamma$-irradiated soils and in nonsterile soils incubated at 100% $O_2$, which may have completely inhibited biological production. This suggests that rates of abiotic processes occurring in $\gamma$-irradiated soils were representative of abiotic process rates occurring in nonsterile soils. While the theoretically based Michaelis–Menten model was suitable for describing $N_2O$ production kinetics at 21% $O_2$, in this case the model should be interpreted as empirical since more than one fundamental process was at play.

Increased $N_2O$ production induced by lowering $O_2$ availability below ambient (Fig. 5) is consistent with nitrifier denitrification of $NO_2^-$ directly to $N_2O$, which tends to be enhanced at subambient $O_2$ (Poth & Focht, 1985; Remde & Conrad, 1990). Nitric oxide (NO) also can be produced via both abiotic and biotic means in the presence of $NO_2^-$ and $O_2$ (Remde & Conrad, 1990; Venterea *et al*., 2005b). Increasing reduction of NO to $N_2O$ was observed with decreasing $O_2$ availability (Fig. 6). Some fraction of the increased $N_2O$ production at subambient $O_2$ may therefore have been due to increased reduction of NO accumulating in the bottle headspace, mediated by either nitrifier denitrification or heterotrophic denitrification (Schafer & Conrad, 1993). Thus, the following processes were potentially active in these experiments, as shown in Fig. 10: direct biological reduction of $NO_2^-$ to $N_2O$ via nitrifier denitrification, direct abiotic $NO_2^-$ reduction to $N_2O$, biological $NO_2^-$ reduction to NO, and abiotic $NO_2^-$ reduction to NO, with NO subject to biological reduction to $N_2O$.

The lack of response to NO addition at 21% $O_2$ (Fig. 6) suggests that biological NO reduction to $N_2O$ only occurred at subambient $O_2$. These findings are consistent with Schafer & Conrad (1993) who demonstrated that NO

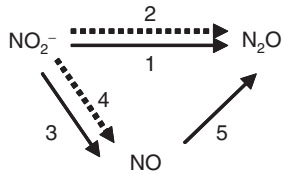


**Fig. 10** Pathways of nitrous oxide ($N_2O$) production occurring in the current experiments. Solid lines indicate biological reactions, dashed lines indicate chemical reactions: (1) direct biological reduction of $NO_2^-$ to $N_2O$ via nitrifier denitrification, (2) direct abiotic $NO_2^-$ reduction to $N_2O$, (3) biological $NO_2^-$ reduction to NO and (4) abiotic $NO_2^-$ reduction to NO, with (3) and (4) each followed by (5) biological NO reduction to $N_2O$.

was biologically reduced in autoclaved soil inoculated with the heterotrophic bacterium *Pseudomonas stutzeri* with increasing activity at subambient $O_2$. Schafer & Conrad (1993) also observed NO reduction even at 20% $O_2$ in soil initially maintained under anaerobic conditions and then supplied with glucose, but the activity gradually dissipated upon exposure to 20% $O_2$. No evidence of NO reduction to $N_2O$ at 21% $O_2$ was observed here in soils maintained under aerobic conditions. Thus, under fully aerobic incubation conditions, $N_2O$ production appeared to be limited to direct biotic and abiotic reduction from $NO_2^-$.

Based on the above discussion, a greater proportion of total $N_2O$ production at ambient $O_2$ was derived from chemical vs. biological reduction compared with at subambient $O_2$. The higher activation energies observed at 5% compared with 21% (Fig. 7) therefore imply a greater temperature sensitivity of the biological than the chemical reduction process. The greater temperature response observed in sample U1 compared with C1 and C2 is also consistent with this interpretation, as sample U1 had the greatest biological contribution to total $N_2O$ production at 21% $O_2$, i.e. 69% in U1 compared with 25% and 40% in C1 and C2, respectively.

The decreasing responses of $N_2O$ production to soil water content at a fixed soil $NO_2^-$ concentration (Fig. 4) are suggestive of diffusion limitations to surface-mediated reactions, similar to previous data for $NO_2^-$-mediated NO production (Venterea *et al*., 2005b). At increasing soil moisture, a greater proportion of $NO_2^-$ is in bulk solution and not in direct contact with reactive soil surfaces. There is no evidence that increased moisture resulted in decreased $O_2$ availability and increased $NO_2^-$ or NO reduction to $N_2O$. This might occur under field conditions with intact soil structure.

Neutralization of soil pH might be effective in reducing $N_2O$ emissions. Using the regression model in Fig. 3a, raising pH (1 : 1 M KCl) from 5.0 to 6.0 would decrease $K_p$ values by 85% for a soil having total C and SOC concentrations equal to the mean values

 

($24\,mg\,C\,g^{-1}$ and $7.3\,\mu g\,C\,g^{-1}$, respectively). This practice presumably would decrease the abiotic component of $N_2O$ production arising from reactions which are promoted under acidic conditions (Stevenson *et al*., 1970), assuming the same soil $NO_2^-$ levels. However, more alkaline soil conditions can promote increased levels of $NO_2^-$ accumulation due to increased toxicity of $NH_3$ to *Nitrobacter* (Van Cleemput & Samater, 1996) which could counteract this effect. There is also evidence based on thermodynamic considerations that nitrifier denitrification may be promoted at lower pH (Wrage *et al*., 2001), but more study is needed to understand the role of pH in regulating $N_2O$ produced under aerobic conditions.

## Concluding remarks

The potential importance of $NO_2^-$-driven reactions in generating $N_2O$ emissions on regional and global scales appears to be high given the widespread use of anhydrous $NH_3$ and urea, the two fertilizers believed to have the greatest potential for promoting soil $NO_2^-$ accumulation. Urea and anhydrous $NH_3$ accounted for 38% and 42%, respectively, of total fertilizer N applied annually worldwide in 2005 (IFA, 2006). Yet very few studies have attempted to measure soil $NO_2^-$ and $N_2O$ emissions concurrently. The highly dynamic nature of both $NO_2^-$ and pH, which together may exert strong control over $NO_2^-$-derived $N_2O$ production, require significant effort to capture the temporal variability of emissions following fertilizer applications (Venterea & Rolston, 2000a). Accurate determination of soil $NO_2^-$ is more challenging than other inorganic N forms due to its high reactivity (Stevens & Laughlin, 1995). The role of organic matter in promoting $NO_2^-$-driven reactions shown here suggests that agricultural management practices designed to increase soil C storage may have unintended consequences with regard to $N_2O$ production that could counteract greenhouse gas benefits. Further experiments are required to examine the significance of the reactions across a range of conditions. If ranges of $NO_2^-$ accumulation and resulting $N_2O$ emissions can be identified for specific agricultural management practices (e.g. fertilizer forms, tillage regimes) and/or soil properties (e.g. pH, total C), this information may be useful in improving emissions estimates and modeling efforts across scales.

## Acknowledgements

The author gratefully acknowledges S. Claussen, D. Dodor, M. Dolan, Z. Jorgensen, R. Schaubach, and A. Stanenas for assistance with experiments, D. Harris for conducting $^{15}N$ analyses, and R. Blackburn for performing $\gamma$-radiations. The manuscript was substantially improved following the suggestions of anonymous reviewers.

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