

Nitrite intensity explains N management effects on N₂O emissions in maize



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ABSTRACT

It is typically assumed that the dependence of nitrous oxide (N₂O) emissions on soil nitrogen (N) availability is best quantified in terms of ammonium (NH₄⁺) and/or nitrate (NO₃⁻) concentrations. In contrast, nitrite (NO₂⁻) is seldom measured separately from NO₃⁻ despite its role as a central substrate in N₂O production. We examined the effects of three N fertilizer sources and two placement methods on N₂O and N dynamics in maize over two growing seasons. Cumulative N₂O emissions were well-correlated with NO₂⁻ intensity (NO2I) but not with NO₃⁻ (NO3I) or NH₄⁺ (NH4I) intensity. By itself, NO2I explained more than 44% of the overall variance in N₂O. Treatment effects on N₂O and NO2I were similar. When conventional urea (U) was applied using mid-row banding (MRB), both N₂O and NO2I increased by a factor of about 2 compared to broadcast/incorporated (BI). When polymer-coated urea (PCU) was the N source, MRB placement increased both N₂O and NO2I compared to BI only in the wetter of the two years. When urea with microbial inhibitors (IU) was the N source, N₂O and NO2I were lowest across both years and were less affected by placement than U or PCU. A 50/50 mix of IU and U reduced N₂O and NO2I compared to U alone, suggesting that a mixed N source may provide an economical N₂O mitigation strategy. Our results show that practices which reduce NO₂⁻ accumulation have the potential to also reduce N₂O emissions, and that separate consideration of NO₃⁻ and NO₂⁻ dynamics can provide more insight than their combined dynamics as typically quantified.

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1. Introduction

Increasing use of N fertilizers and manures in agriculture has been contributing to rising atmospheric levels of the potent ozone depleting and greenhouse gas nitrous oxide (N₂O) (Cavigelli et al., 2012). Modification of N fertilizer formulation (i.e. source) and/or method of application (i.e. placement) have the potential to affect N₂O emissions (e.g. Halvorson and Del Grosso, 2013). Practices that increase the proportion of applied N that is utilized by the crop while minimizing the availability of soil inorganic N species (ammonium

[NH₄⁺], nitrite [NO₂⁻], and/or nitrate [NO₃⁻]) would be expected to reduce N₂O emissions (Firestone and Davidson, 1989). Better understanding of how specific management practices affect soil N availability is therefore critical in developing effective N₂O mitigation strategies. Studies relating soil N availability to N₂O emissions commonly focus on NO₃⁻ (e.g. Zebarth et al., 2012) and in some cases NH₄⁺ (e.g. Venterea et al., 2010). Substantially less effort has been devoted to measuring soil NO₂⁻ despite the fact that NO₂⁻ is a substrate for N₂O produced via nitrifier-denitrification (Wrage et al., 2001), chemodenitrification (Stevenson and Swaby, 1964) and heterotrophic denitrification (Zumft, 1997). In addition to its potential role in regulating N₂O emissions, soil NO₂⁻ in its protonated form (nitrous acid, HNO₂) can be released as a gas to the atmosphere where it regulates hydroxyl radical formation (Su et al., 2011) and/or react within the soil to form nitric oxide (NO) gas which can regulate tropospheric ozone and/or contribute to atmospheric N deposition (Venterea and Rolston, 2000a).

Several studies have reported soil NO₃⁻ “intensity” (NO3I) calculated as the time-weighted sum of soil NO₃⁻ concentrations which is interpreted to represent the cumulative exposure of soil microbial populations to NO₃⁻ and which has in some cases been

Abbreviations: ca-N₂O, cumulative area-based growing season N₂O emissions; cy-N₂O, cumulative yield-based growing season N₂O emissions; PCU, polymer-coated urea; IU, urea containing urease and nitrification inhibitors; U, urea; IU/U, a 50/50 mix of IU and U; NO2I, soil nitrite intensity; NO3I, soil nitrate intensity; NO23I, soil nitrite plus nitrate intensity; NH4I, soil ammonium intensity; FIEF, fertilizer-induced emission factor; NFRE, nitrogen fertilizer recovery efficiency; BI, broadcast/incorporation; MRB, mid-row banding; ND, nitrifier denitrification; CD, chemodenitrification; HD, heterotrophic denitrification.

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positively correlated with N₂O emissions (Zebarth et al., 2008a, 2008b, 2012; Burton et al., 2008a, 2008b; Engel et al., 2010). However, studies reporting soil NO₃⁻ concentration or intensity data almost always utilize analytical methods that quantify the sum of NO₂⁻ plus NO₃⁻ (Mulvaney, 1996). While NO₂⁻ and NO₃⁻ can be analyzed separately, this requires additional steps and precautions that are usually omitted (Stevens and Laughlin, 1995).

It has been known for decades that soil NO₂⁻ tends to increase and then subside following application of ammonia (NH₃)-, NH₄⁺-, or urea-based fertilizers leading to a temporary decoupling of the two steps of nitrification resulting from a lag in the growth and/or activity of NO₂⁻ oxidizing microbes (NOs) relative to NH₄⁺ oxidizers (AOs) (Morrill and Dawson, 1967). Once present, NO₂⁻ can react quickly to form N₂O under a range of oxygen (O₂) conditions (Venterea, 2007). Even though NO₂⁻ is commonly present at much lower levels than NO₃⁻, their relative concentrations do not necessarily represent their relative importance in producing N₂O. In laboratory N addition experiments using several soils over a range of O₂ levels, Venterea (2007) found that substantially more N₂O was produced per unit of applied NO₂⁻ compared to NO₃⁻, ranging from 3 times greater at <0.1% O₂ to more than 100 times greater at O₂ ≥ 5%. Soil NO₂⁻ dynamics have been correlated with N₂O production in soils amended with bovine urine (Khan et al., 2011) and in manure composting operations (Fukumoto and Inubushi, 2010). Studies by Engel et al. (2010) and Venterea et al. (2010) have pointed to the potential importance of NO₂⁻ dynamics in mediating fertilizer management effects on N₂O emissions. However, concurrent *in situ* measurements of soil NO₂⁻ and N₂O dynamics are limited and relationships between them remain largely unexplored in fertilized cropping systems.

So-called “enhanced efficiency fertilizers” (EEFs) designed to release N at a rate that is more synchronized with crop demand than conventional N sources have in some cases reduced N₂O emissions (Akiyama et al., 2010). The physical placement of N fertilizer can also affect N₂O emissions (Engel et al., 2010). However, both N source and placement have been inconsistent in their effects on N₂O emissions with studies reporting increased N₂O emissions with EEFs in some growing seasons (Sistani et al., 2011) or no effects of fertilizer placement (Burton et al., 2008a). The objectives of this study were to quantify the effects of three N sources and two placement methods on N₂O emissions and crop response in a maize cropping system over two consecutive seasons, and to examine relationships between N₂O emissions and soil N intensities calculated separately for NH₄⁺, NO₂⁻, NO₃⁻, and the sum of NO₂⁻ + NO₃⁻ as commonly reported.

2. Materials and methods

2.1. Experimental design

The experiment was conducted at the University of Minnesota Research Station in St. Paul (44.99° N, 93.17° W) where the soil is a Waukegon silt loam with 3.9% organic C, 23% sand and 22% clay in the upper 0.15 m. The 30-year average precipitation and temperature during Apr–Oct are 621 mm and 16.4 °C, respectively (MCWG, 2013). The experimental design was randomized complete block, two-way factorial with three levels of N source and two levels of placement applied in three blocks, each containing six 5.3 m by 5 m plots. In both years, a control treatment receiving no N fertilizer was also included in each block to allow for calculation of fertilizer-induced N₂O emissions factor (FIEF) and N fertilizer recovery efficiency (NFRE). An eighth plot was included in each block in 2012 to provide for an additional treatment (as described below) that was analyzed separately from the two-year experiment. Maize (*Zea mays* L.) was planted at 95,660 seeds ha⁻¹ on 25 Apr 2011 and 30

Apr 2012. The N sources were conventional urea (U), polymer-coated urea (PCU), and urea containing microbial inhibitors (IU) all in granular form. The PCU product (ESN[®]) was manufactured and provided by Agrium Advanced Technologies (Loveland, CO), and the IU product (Super U[®]) was manufactured and provided by Koch Agronomic Services (Wichita, KS). The IU product contained the urease inhibitor N-(n-Butyl)-thiophosphoric triamide (NBPT) and the nitrification inhibitor dicyandiamide (DCD). The placement treatments were broadcasting with incorporation (BI) and mid-row banding (MRB) each of which was implemented using all three N sources. In 2012, an additional treatment (referred to as IU/U) used a 50:50 w/w mix of IU and U applied using MRB only. All treatments (except control) received 180 kg N ha⁻¹ according to recommended rates for the region (Randall et al., 2008). Fertilizer was applied when maize was at the V4–V6 stage (on 1 Jun 2011 and 5 Jun 2012). For the BI treatments, fertilizer granules were hand-applied uniformly across the plot and incorporated immediately after application using a cultivator which tilled all plots (including those to receive the MRB treatments) to a depth of 50–80 mm while avoiding the seed row. For the MRB treatments, furrows centered in and running parallel to the crop rows (50 mm deep and 50 mm wide) were prepared immediately following tillage using a hoe; fertilizer granules were hand-applied and furrows were then back-filled.

2.2. Nitrous oxide emissions

Soil-to-atmosphere N₂O fluxes were measured using non-steady state chambers designed and used in accordance with Rochette and Bertrand (2008) and Parkin and Venterea (2010). One chamber anchor (0.69 m × 0.34 m) encompassing >90% of the inter-row width was installed in each plot to a depth of 0.10 m centered between rows with the short side parallel to the row. Sampling was conducted once weekly in May and Sep and twice weekly in Jun through Aug of both years, for a total of 33 and 30 sampling dates in 2011 and 2012, respectively, and a total of 1413 individual N₂O chamber measurements. Approximately 45% of sampling events occurred within 24 h of rain events. On each sampling day, insulated and vented chamber tops (0.13 m high) were secured to anchors using 60-mm binder clips. Gas samples were collected 0, 0.5, 1.0 and 1.5 h after chamber placement using a polypropylene syringe. Samples were transferred to glass vials sealed with butyl rubber septa (Alltech, Deerfield, IL) and analyzed within 1 wk using a headspace autosampler (Teledyne Tekmar, Mason, OH) connected to a gas chromatograph (GC) (model 5890 Agilent/Hewlett–Packard, Santa Clara, CA) equipped with an electron capture detector. The GC was calibrated with analytical grade standards (Scott Specialty Gases, MI) each day when samples were analyzed. Gas concentrations in mixing ratios determined by the GC were converted to mass per volume concentrations using the ideal gas law and air temperature measured at the sampling time. Fluxes of N₂O were calculated from the rate of change in chamber N₂O concentration using methods designed to account for suppression of the surface-atmosphere concentration gradient (Venterea, 2010).

2.3. Grain yield and above-ground N uptake

After crops reached physiological maturity (3 Oct 2011 and 9 Sep 2012), ears and stover were manually harvested from all plants over a distance of 1.5 m in the middle two rows of each plot. Ears were air dried and shelled, and then grain, cob and stover were further dried for 3 d at 65 °C and weighed to obtain dry matter yields of each component. Sub-samples were ground with a ball mill and analyzed for N content with an elemental N analyzer (VarioMax;

Elementar, Hanau, Germany). Total N content in above-ground biomass was calculated from the sum of N masses harvested in grain, cob, and stover from each plot.

2.4. Climate and soil data

Air temperature and precipitation data were collected from a weather station located 1 km from the research field operated by the Minnesota Climatology Working Group (MCWG, 2013). Soil temperature was measured during each N₂O flux measurement event using temperature probes (Fisher, Hampton, NH) inserted to 50 mm within 1 m of chambers. Soil water content and bulk density were determined on samples collected from one fertilized plot within each block using 50-mm diameter × 50-mm deep metal tubes within 1 h of each flux measurement. Bulk density values were used together with gravimetric water content to estimate percent water-filled pore space (WFPS). Additional soil samples were collected for inorganic N analysis at approximately weekly frequency starting one week prior to fertilizer application until early Aug and thereafter at approximately two-week intervals on a total of 11 dates in 2011 and 13 dates in 2012. Soil N concentrations and N₂O fluxes were measured on the same day 7 times each year. On each sampling date, one sample from each plot was collected from the “center” position (0.33–0.43 m from the row) corresponding to the location of fertilizer placement in the MRB treatments, and two separate samples were collected from random locations distributed across the “off-center” position (0.05–0.25 m from the row). Samples from the two off-center positions were composited prior to analysis. Samples were taken at two depth intervals (0–0.1 m and 0.1–0.2 m) from each position for a total of 2172 individual samples over two years using a 19-mm diameter coring tool (Oakfield Apparatus, Inc., Oakfield, WI). Samples were processed and analyzed for NO₂⁻ and NO₃⁻ on the same day of sample collection. Samples were manually homogenized and 10-g subsamples were extracted in polypropylene tubes using 40 mL of 2 M KCl with pH adjusted to 8.0–8.5 placed on a reciprocating shaker at 120 rpm for 10 min (Stevens and Laughlin, 1995). Extracts were immediately filtered and analyzed separately for total NO₂⁻ + NO₃⁻ and NO₂⁻ using the Greiss–Ilosvay method with and without cadmium reduction of NO₃⁻ to NO₂⁻, respectively (Mulvaney, 1996) modified for use with flow-through injection analysis (FIA) (Lachat, Loveland, CO). Soil NO₃⁻ concentrations were determined by difference. The remaining sample was refrigerated for 24–48 h after which sub-samples were extracted in polypropylene tubes using 40 mL of 2 M KCl (pH 5.6) placed on a reciprocating shaker at 120 rpm for 1 h followed by filtration and analysis for NH₄⁺ using the sodium salicylate–nitroprusside method (Mulvaney, 1996) modified for use with FIA. An additional subsample (~5 g) was dried at 105 °C and all results reported on a dry soil basis. Extracts were diluted in 2 M KCl if necessary to reduce concentrations to within the range of analytical standards (<20 µg N mL⁻¹). Method detection limits for all inorganic N species were approximately 0.1 µg N g⁻¹ soil.

2.5. Data analysis

Nitrous oxide fluxes measured on each sampling date were used to determine cumulative area-based growing season N₂O emissions (ca-N₂O) by trapezoidal integration versus time. Values of FIEF and NFRE were calculated by subtracting ca-N₂O or above-ground N uptake, respectively, in each control plot from that measured in each fertilized plot within the same block and then expressing the difference as a percentage of applied fertilizer (180 kg N ha⁻¹). Cumulative yield-based N₂O emissions (cy-N₂O) were calculated by dividing ca-N₂O by grain yield for each plot. Soil N intensities were calculated separately for NH₄⁺ (NH4I), NO₂⁻ (NO2I), NO₃⁻ (NO3I), and

the sum of NO₂⁻ + NO₃⁻ (NO23I) using trapezoidal integration of concentration versus time, the latter index being what is commonly reported. Each intensity index was initially calculated separately for center and off-center sampling positions in all treatments. As expected and consistent with the fertilizer application methods, paired *t*-tests showed that all intensity indices for uniformly applied BI treatments did not differ by sampling position while for the MRB treatments intensity indices were greater in the center compared with off-center position (*P* < 0.001). Thus, for the MRB treatments, reported intensity values were determined using the spatially weighted mean of soil N concentrations measured at the center and off-center positions such that concentrations measured in the two positions represented 13% (0.10 m/0.76 m) and 87% (0.66 m/0.76 m) of the entire inter-row region, respectively. Each intensity index was calculated separately for the 0–0.1 m and 0–0.2 m depths, the latter value using the mass-weighted mean of soil concentrations at both depths. All dependent variables were evaluated for normality and were log-transformed if necessary prior to evaluation of treatment effects using Proc Mixed in SAS (Littell et al., 2006). Effects of year (Y), N source (S), and placement (P) for 2011–2012 were determined using block (B) and B-by-Y as random effects and S, P, and Y as fixed effects. Effects of S within the MRB placement treatments in 2012 were also evaluated separately using B as a random effect and S as a fixed effect. Means comparisons were conducted using contrast statements in SAS. Regression analyses were conducted using Statistix (version 9, Analytical Software, Tallahassee FL). Results of single-factor linear regression analysis are expressed as the square of the Pearson product-moment correlation coefficient (*r*²). Results of multiple-factor linear regression analysis are expressed as the square of the coefficient of multiple correlation (*R*²). Log or other transformation of variables prior to regression did not generally improve linearity or distribution of residuals, so regression results using untransformed variables are reported. Regression relating N₂O to soil N yielded nearly identical *P* and *r*² values using either soil N concentrations for the 0–0.1 m depth or the 0–0.2 m depth, so the latter are reported. Observations from the control treatments were included in all regression analyses.

3. Results

3.1. Climate and crop responses

The 2012 growing season was drier than normal, in particular during 20 Jun through 1 Oct when only 174 mm of rain was recorded compared to 471 mm in 2011 (Fig. 1a). Soil WFPS at the time of N₂O flux sampling was ≥40% on only 6 dates in 2012 compared to 20 dates in 2011, while air and soil temperatures were similar in 2011 and 2012. Soil temperature and WFPS measured at the time of N₂O flux sampling were only weakly correlated with N₂O flux (*r*² ≤ 0.1). Consistent with the drier conditions in 2012, grain yields were on average ~30% less than in 2011 and there was a non-significant trend for lower NFRE in 2012 (Table 1). There were no significant effects of placement or N source on crop response (Table 1).

3.2. Nitrous oxide emissions

Daily N₂O fluxes increased within 2 wks after fertilizer application and remained above baseline levels until early Aug each year (Fig. 1b–c). There were significant N source (S)-by-placement (P), S-by-year (Y), and Y-by-P interaction effects on ca-N₂O and cy-N₂O (Table 1, Fig. 2a). In 2011, MRB placement with each N source had greater ca-N₂O than the corresponding BI placement, and IU-MRB had lower ca-N₂O than PCU-MRB and U-MRB (Fig. 2a). In 2012, placement had a significant effect on ca-N₂O only when U was the

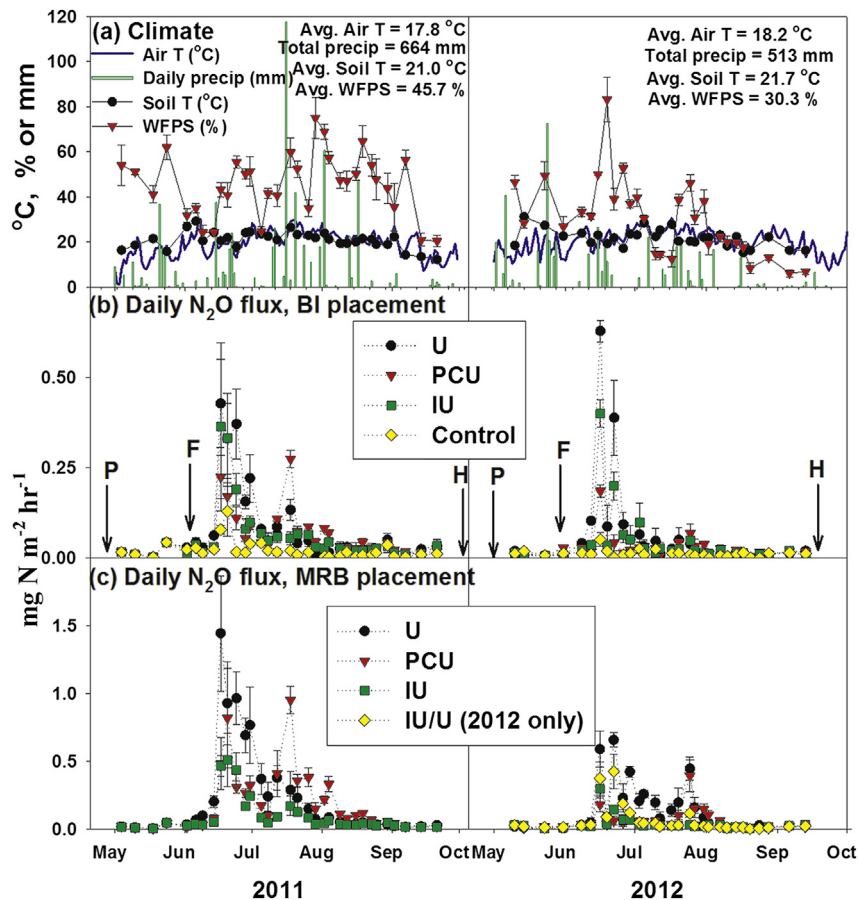


Fig. 1. (a) Daily precipitation and air temperature, and mean (with std error) soil temperature and water-filled pore space (WFPS) at time of N₂O sampling; and mean (with std error) daily N₂O fluxes for (b) broadcast incorporation (BI) placement using urea (U), polymer-coated urea (PCU), urea with microbial inhibitors (IU), and in the zero-N control treatment, and (c) mid-row banding (MRB) placement using U, PCU, IU, and 50/50 mix of IU and U (IU/U) (2012 only); arrows indicate dates of planting (P), fertilizer application (F) and harvest (H).

N source (Fig. 2a). Averaged across N sources and placements, ca-N₂O and FIEF were significantly greater in 2011 (the wetter year) compared to 2012; however, due to higher yields in 2011, cy-N₂O did not differ by year (Table 1). Averaged over years and placements, ca-N₂O and FIEF increased in the order IU < PCU < U, and U had greater cy-N₂O than PCU and IU (Table 1). In a separate analysis of the 2012 MRB treatments, ca-N₂O, cy-N₂O and FIEF were significantly greater with U compared to PCU, IU, and the 50/50 mix of IU/U (Table 1).

3.3. Soil N dynamics and intensity

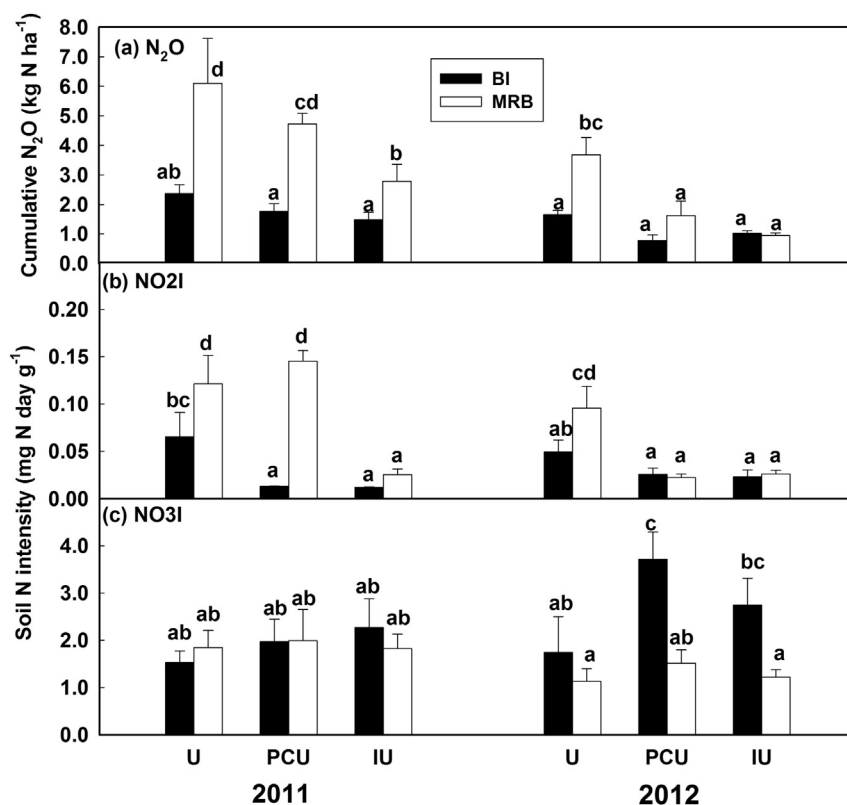
Consistent with the differences in fertilizer placement methods, soil concentrations of NH₄⁺, NO₂⁻, and NO₃⁻ in the uniformly-applied BI treatments (averaged across the center and off-center sampling positions) were lower than those observed in the center position in the MRB treatments (Figs. 3–4). Inorganic N levels in the off-center sampling position in the MRB treatments (not shown) were generally similar to levels observed in the control treatment. All N species showed a response to fertilizer addition resulting in greater soil concentrations compared to the control treatment. Soil NO₃⁻ levels increased in the control treatment after tillage in June likely due to mineralization of soil organic N. Because tillage coincided with N fertilization in both the control and fertilized treatments, it cannot be excluded that some of the increase in NO₃⁻ in the fertilized treatments was attributable to mineralization of soil organic N. Mean soil NH₄⁺ levels tended to be higher with U compared to PCU

and IU, except for sampling events after mid-July of both years where NH₄⁺ levels with PCU (and in some cases with IU and IU/U) exceeded those with U. Soil NO₂⁻ levels also tended to be higher with U except in the MRB treatment in 2011 where they were similar in U and PCU. Soil NO₃⁻ levels displayed a less consistent pattern among N sources, although in most cases NO₃⁻ levels with PCU tended to be greater than with other N sources after mid-July. Soil N concentrations in samples from the 0.1–0.2 m depth (data not shown) displayed similar dynamics to samples from the 0–0.1 m depth (shown in Figs. 3–4). On the 7 dates each year where soil N and N₂O flux were measured on the same day, soil NO₂⁻ concentrations were highly correlated ($P < 0.0001$) with N₂O flux ($r^2 = 0.419$ and 0.219 in 2011 and 2012, respectively). There were also significant ($P < 0.05$) correlations between N₂O flux and NO₃⁻ and the sum of NO₂⁻ + NO₃⁻ in 2011 and NH₄⁺ in 2012, but in all cases r^2 values were <0.05.

Treatment effects on soil nitrite intensity (NO₂I) were similar to the pattern observed for ca-N₂O, while treatment effects on NH₄I and NO₃I displayed different trends than N₂O (Table 2, Fig. 2b–c). In 2011, MRB placement using U and PCU had greater NO₂I than the corresponding BI placement, and there was a trend for greater NO₂I using MRB-IU than BI-IU ($P = 0.15$); in 2012, placement had a significant effect on NO₂I only for U as was the case for N₂O. Averaged across years and placements, NO₂I increased in same order as ca-N₂O (IU < PCU < U), and in a separate analysis of the MRB treatments in 2012, NO₂I displayed the same pattern of differences as N₂O (Table 2). Observed values of ca-N₂O were significantly

Table 1Effects of year, N source, and placement on N₂O emissions, fertilizer-induced emissions factor (FIEF), grain yield and N fertilizer recovery efficiency (NFRE).^a

Source of variation	N ₂ O emissions			Crop response	
	Area-based	Yield-based	FIEF	Grain yield	NFRE
	kg N ha ⁻¹	kg N Mg ⁻¹	%	Mg ha ⁻¹	%
2011–2012					
Year (Y)					
2011	3.3 (0.4) b	0.24 (0.03)	1.4 (0.2) b	14.1 (0.3) b	65 (7.5)
2012	1.6 (0.2) a	0.17 (0.03)	0.7 (0.1) a	10.1 (0.4) a	39 (5.7)
Significance	**	NS	**	**	NS
N source (S) ^b					
Control	0.5 (0.1)	0.06 (0.01)	—	9.6 (0.8)	—
U	3.4 (0.2) c	0.30 (0.02) b	1.6 (0.1) c	11.6 (0.7)	42 (13)
PCU	2.3 (0.2) b	0.18 (0.01) a	1.0 (0.1) b	12.7 (0.8)	56 (7.8)
IU	1.6 (0.2) a	0.13 (0.01) a	0.6 (0.1) a	12.0 (0.7)	59 (12)
Significance	***	***	***	NS	NS
Placement (P) ^c					
BI	1.6 (0.1) a	0.13 (0.01) a	0.6 (0.1) a	12.1 (0.5)	51 (8.3)
MRB	3.3 (0.4) b	0.28 (0.04) b	1.6 (0.2) b	12.1 (0.5)	53 (5.8)
Significance	***	***	***	NS	NS
Interactions					
S × P	**	*	*	NS	NS
S × Y	*	*	*	NS	NS
Y × P	*	*	NS	NS	NS
S × Y × P	NS	NS	NS	NS	NS
2012 ^d					
N source (S)					
U	3.4 (0.5) b	0.40 (0.11) b	1.7 (0.3) b	9.7 (1.7)	48.9 (19.2)
PCU	1.6 (0.4) a	0.15 (0.04) a	0.7 (0.20) a	11.5 (0.8)	46.2 (13.8)
IU	1.0 (0.1) a	0.10 (0.01) a	0.3 (0.07) a	9.9 (0.9)	42.4 (18.7)
IU/U	1.6 (0.3) a	0.15 (0.04) a	0.7 (0.17) a	10.6 (0.7)	51.2 (12.7)
Significance	**	*	**	NS	NS

****P* < 0.001, ***P* < 0.01, **P* < 0.05, NS = not significant.^a Means (std errors) followed by same letter are not significantly different.^b S treatments were urea (U), polymer-coated urea (PCU), urea plus inhibitors (IU), and 50:50 mix of IU/U (in 2012). Control was not included in analysis to maintain balanced design.^c P treatments are broadcast with incorporation (BI) and mid-row banding (MRB).^d Separate analysis of N source effects on MRB treatments only during 2012.**Fig. 2.** Mean (with std error) (a) cumulative area-based growing season N₂O emissions (ca-N₂O), (b) soil nitrite intensity (NO₂I) and (c) soil nitrate intensity (NO₃I) with broadcast incorporation (BI) and mid-row banding (MRB) placements using urea (U), polymer-coated urea (PCU), and urea with microbial inhibitors (IU). Bars with the same letters are not significantly different (*P* < 0.05).

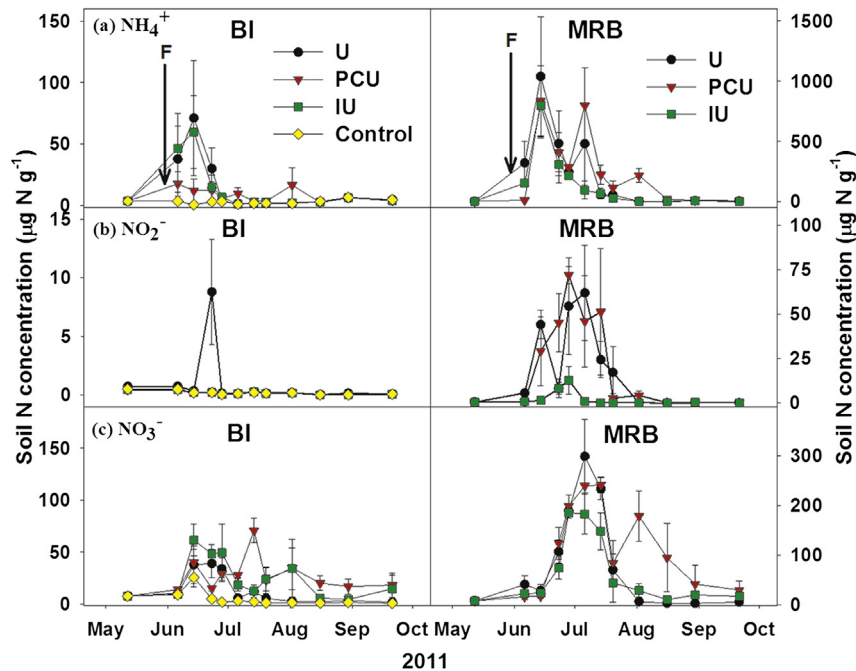


Fig. 3. Mean (with std error) soil concentrations of (a) NH_4^+ , (b) NO_2^- and (c) NO_3^- in samples from the 0–0.1 m depth with broadcast incorporation (BI) placements using urea (U), polymer-coated urea (PCU), urea with microbial inhibitors (IU), and in the zero-N control treatment and for mid-row banding (MRB) placements using U, PCU and IU. Arrows indicate date of fertilizer application (F). Data for BI are average values of samples collected from center and off-center locations; data for MRB are from center sampling positions.

correlated with NO_2I but not with NH_4I , NO_3I or $\text{NO}_2\text{3I}$ (Table 3). In both years, one of the U-MRB treatment replicates displayed greater N_2O emissions than all others and exclusion of these data points resulted in greater r^2 values for the MRB data and for all treatments taken together (Fig. 5a,b). For BI data from 2011 (the wetter year), multiple regression analysis using NO_3I and NO_2I as

separate independent variables explained slightly more of the variation ($R^2 = 0.463$) compared to using NO_2I as a single independent variable ($r^2 = 0.448$) (Fig. 6). Multiple regression using subsets of data other than the 2011 BI treatments did not explain any more of the variation in $\text{ca-N}_2\text{O}$ than NO_2I used as a single independent variable.

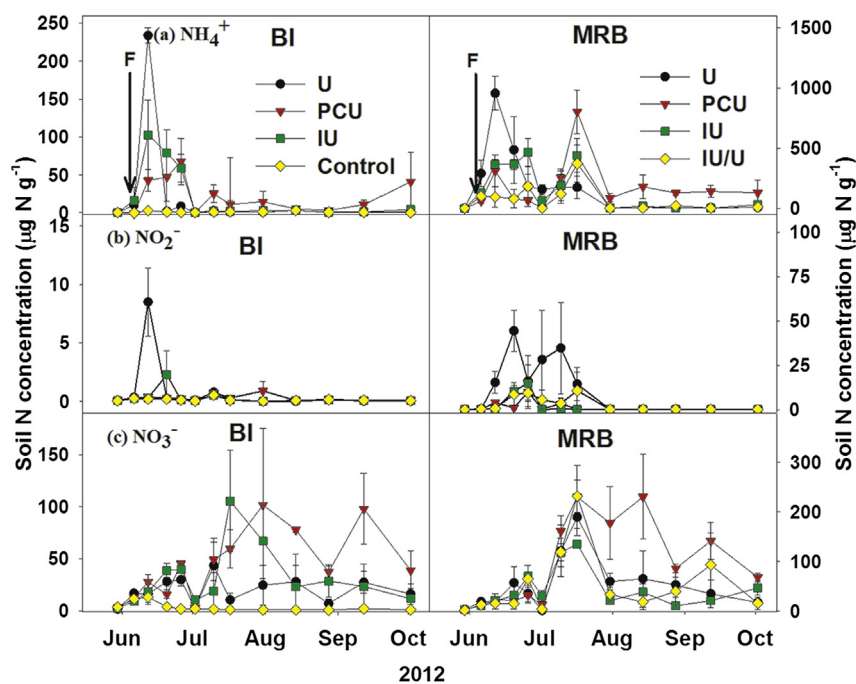


Fig. 4. Mean (with std error) soil concentrations of (a) ammonium (NH_4^+), (b) nitrite (NO_2^-) and (c) nitrate (NO_3^-) in samples from the 0–0.1 m depth for broadcast incorporation (BI) placements using urea (U), polymer-coated urea (PCU), urea with microbial inhibitors (IU) and in the zero-N control treatment and for mid-row banding (MRB) placements using U, PCU, IU and 50/50 mix of IU and U (IU/U). Arrows indicate date of fertilizer application (F). Data for BI are average values of samples collected from center and off-center locations; data for MRB are from center sampling positions.

Table 2
Effects of year, N source, and placement on ammonium (NH₄I), nitrite (NO₂I), and nitrate (NO₃I) intensities.^a

Source of variation	NH ₄ I		NO ₂ I		NO ₃ I	
	0–0.10 m	0–0.20 m	0–0.10 m	0–0.20 m	0–0.10 m	0–0.20 m
	mg N d g ⁻¹					
	2011–2012					
Year (Y)						
2011	2.01 (0.32)	1.26 (0.17)	0.11 (0.027)	0.064 (0.014)	2.41 (0.25)	1.91 (0.17)
2012	2.45 (0.20)	1.55 (0.11)	0.061 (0.014)	0.040 (0.008)	2.60 (0.45)	2.01 (0.28)
Significance	NS	NS	NS	NS	NS	NS
N source (S) ^b						
Control	0.24 (0.043)	0.30 (0.046)	0.014 (0.001)	0.014 (0.001)	0.44 (0.068)	0.52 (0.018)
U	2.41 (0.40)	1.46 (0.21)	0.14 (0.026) c	0.083 (0.013) c	1.81 (0.27) a	1.56 (0.21) a
PCU	2.28 (0.32)	1.42 (0.18)	0.083 (0.031) b	0.052 (0.017) b	3.18 (0.53) b	2.30 (0.34) b
IU	1.99 (0.26)	1.34 (0.16)	0.028 (0.005) a	0.022 (0.003) a	2.53 (0.42) ab	2.02 (0.26) ab
Significance	NS	NS	***	***	*	*
Placement (P) ^c						
BI	1.84 (0.25) a	1.19 (0.13) a	0.045 (0.011) a	0.031 (0.006) a	3.12 (0.41) b	2.33 (0.26) b
MRB	2.62 (0.26) b	1.62 (0.14) b	0.12 (0.026) b	0.073 (0.013) b	1.89 (0.23) a	1.59 (0.15) a
Significance	*	*	***	***	**	**
Interactions						
S × P	NS	NS	*	*	NS	NS
S × Y	NS	NS	*	*	NS	NS
Y × P	NS	NS	**	**	**	**
S × Y × P	NS	NS	*	*	NS	NS
	2012 ^d					
N source (S)						
U	2.44(0.45) bc	1.33 (0.21) b	0.16 (0.052) b	0.10 (0.023) b	1.16 (0.44)	1.13 (0.27)
PCU	3.06 (0.10) c	1.96 (0.19) c	0.028 (0.007) a	0.022 (0.004) a	2.01 (0.37)	1.52 (0.28)
IU	2.18 (0.22) b	1.59(0.25) bc	0.036 (0.007) a	0.026 (0.004) a	1.03 (0.10)	1.22 (0.16)
IU/U	1.30 (0.32) a	0.79 (0.16) a	0.058 (0.016) a	0.038 (0.009) a	1.44 (0.38)	1.26 (0.30)
Significance	**	**	*	**	NS	NS

****P* < 0.001, ***P* < 0.01, **P* < 0.05, NS = not significant.

^a Means (std errors) followed by same letter are not significantly different.

^b S treatments were urea (U), polymer-coated urea (PCU), urea plus inhibitors (IU), and 50:50 mix of IU/U (2012). Control was not included in analysis to maintain balanced design.

^c P treatments are broadcast with incorporation (BI) and mid-row banding (MRB).

^d Separate analysis of N source effects on MRB treatments only during 2012.

4. Discussion

There are surprisingly few concurrent measurements of N₂O emissions and NO₂⁻ dynamics in fertilized agricultural soil. Venterea and Rolston (2000b) measured daily N₂O fluxes above 0.5 kg N ha⁻¹ d⁻¹ when soil NO₂⁻ exceeded 100 μg N g⁻¹ and found a significant correlation between N₂O and soil HNO₂, temperature, and WFPS in a multiple regression model. Engel et al. (2010) and Venterea et al. (2010) both found that fertilizer treatments with greater N₂O emissions also tended to have greater soil NO₂⁻ concentrations. This is the first study to quantify the relative strength in correlation between N₂O emissions and soil NH₄⁺, NO₂⁻, NO₃⁻, and NO₂⁻ + NO₃⁻ intensities under varying N fertilizer treatments.

Correlation between N₂O emissions and NO₂⁻ dynamics observed here is consistent with our understanding of underlying

mechanisms of N₂O production. Nitrite is a precursor substrate for N₂O produced via abiotic chemodenitrification (CD) (Stevenson and Swaby, 1964) and via nitrifier denitrification (ND) performed by AOs (Wrage et al., 2001). Studies have shown that ND can occur under fully to moderately oxic conditions (Ritchie and Nicholas, 1972; Goreau et al., 1980; Venterea, 2007) and under near-anoxic conditions that are also conducive to heterotrophic denitrification (HD) (Zhu et al., 2013). In addition, NO₂⁻ is more proximal to N₂O in the HD reduction sequence than NO₃⁻ (Zumft, 1997). In contrast to CD and ND, HD has a narrower window of O₂ conditions where it is feasible, generally requiring O₂ levels lower than about 0.5% (Tiedje, 1994; Zumft, 1997). In soil incubation studies, Bollmann and Conrad (1998) found that HD-derived N₂O exceeded nitrification-derived N₂O only at O₂ < 0.1%. Although WFPS may not be a completely reliable predictor of HD potential (Balaine et al., 2013), HD-derived N₂O production in soil is often expressed in terms of WFPS, with some studies suggesting that N₂O from HD occurs mainly when WFPS is above 70% (e.g. Dobbie et al., 1999). Based on WFPS, it is likely that ND and CD were more important in producing N₂O than HD in the current study. In 2011, 94% of observations of daily N₂O fluxes exceeding 0.1 mg N m⁻² h⁻¹ occurred when WFPS was below 60%, and in 2012, 100% of observations exceeding 0.1 mg N m⁻² h⁻¹ occurred when WFPS was below 53%. However, the role of HD-derived N₂O at least under some conditions is implied by the significance of NO₃⁻ in the multiple regression results for BI treatments in the wetter year (Fig. 6). The relative importance of CD versus ND is difficult to assess because both processes can occur over a wide range of O₂ status and elucidation

Table 3
Results of linear regression analysis (*r*² values) for cumulative N₂O emissions versus soil intensities of ammonium (NH₄I), nitrite (NO₂I), nitrate (NO₃I) and the sum of nitrite and nitrate (NO₂3I) for all treatments and analyzed separately for mid-row banding (MRB) and broadcast with incorporation (BI) treatments.

	All data		MRB		BI	
	2011	2012	2011	2012	2011	2012
NH ₄ I	0.177	0.025	0.160	0.063	0.141	0.323
NO ₂ I	0.508***	0.442***	0.446*	0.468**	0.448*	0.396*
NO ₃ I	0.022	0.083	0.137	0.002	0.228	0.001
NO ₂ 3I	0.038	0.074	0.161	0.009	0.248	0.001

****P* < 0.001, ***P* < 0.01, **P* < 0.05.

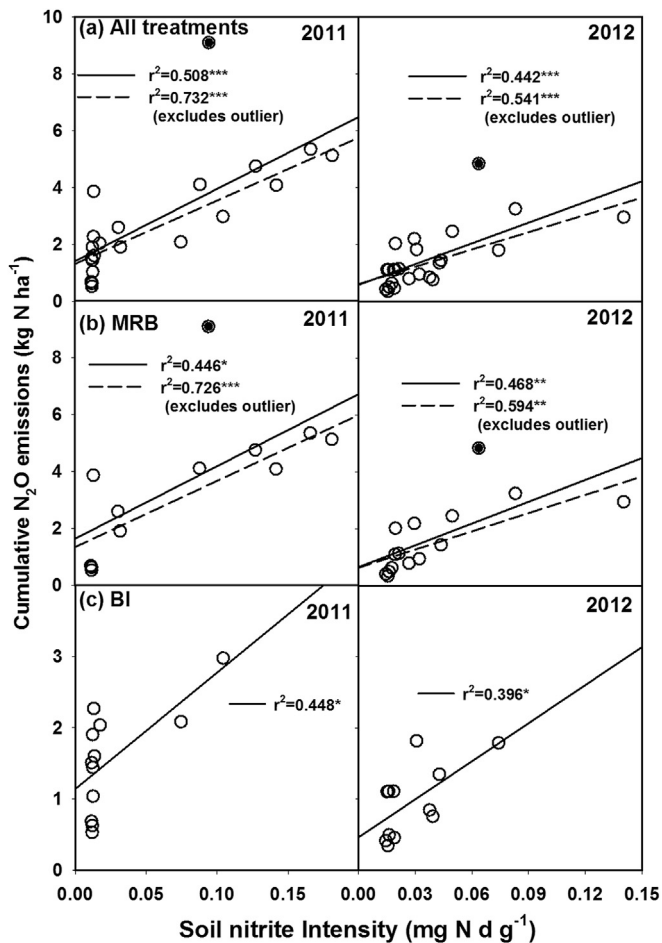


Fig. 5. Single factor linear regression results for cumulative area-based growing season N₂O emissions (ca-N₂O) versus nitrite intensity (NO2I) using data from (a) all treatments, (b) mid-row banding (MRB) treatments, and (c) broadcast incorporated (BI) treatments. Also shown in (a) and (b) are results from separate analyses that excluded one (solid) data point each year from one of the MRB replicates that received urea as the N source. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

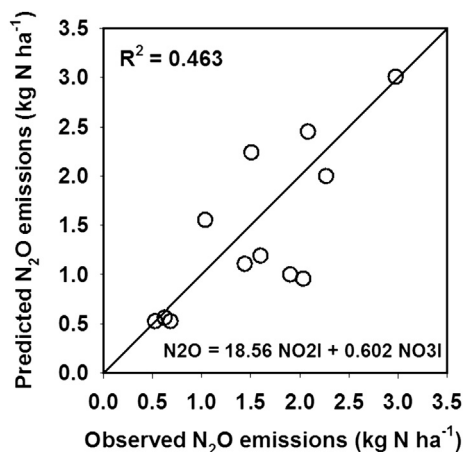


Fig. 6. Multiple linear regression results for cumulative area-based growing season N₂O emissions (ca-N₂O) versus (NO2I) and nitrate (NO3I) intensities as separate independent variables for 2011 data from broadcast incorporated (BI) treatments with 1:1 line.

of their relative importance under different conditions (e.g. with respect to pH) remains a subject of research (Venterea, 2007). Thus, because NO₂⁻ is a key substrate for at least three N₂O producing processes, measurement of NO₂⁻ dynamics alone or together with WFPS does not necessarily allow for determination of the most predominant process.

Free NH₃ toxicity is often cited as the cause of NO₂⁻ accumulation following addition of alkalizing fertilizers (e.g. anhydrous ammonia [AA] and U) (e.g. Van Cleemput and Samatar, 1996), although a study by Hawkins et al. (2010) suggested that elevated soil pH itself may have an important inhibiting effect on NOs. Conditions within a band of AA or U are likely to generate NH₃ toxicity due to high levels of NH₄⁺ combined with elevated pH resulting from hydrolysis of AA or U which shifts the NH₄⁺/NH₃ equilibrium towards NH₃. In the current study, soil NH₄⁺ levels near the location of fertilizer placement in the MRB treatments exceeded 1000 μg N g⁻¹ and were 15–200 times greater than in the BI treatments even several weeks after N was applied. Limited measurements in 2012 (unpublished data) found that soil pH (in 1 M KCl) in the center sampling position in the MRB treatments increased from 6.1 to as high as 8.5 the day after N application, while increasing more slowly to a maximum of 6.9 in the BI treatments 7 d after application before returning to baseline. Thus, NH₃ toxicity appears to explain the greater and more persistent NO₂⁻ accumulation in the MRB treatments in this study.

Banding has been previously shown to increase N₂O compared to broadcast application (Halvorson and Del Grosso, 2013; Cheng et al., 2006). The current results, consistent with Engel et al. (2010), suggest that increased N₂O emissions with banding results from increased NO₂⁻ accumulation. Some studies have found no increase in N₂O with banding compared to broadcast (e.g. Burton et al., 2008a) but with no measurement of NO₂⁻ dynamics. Banding has potential benefits, as it can slow nitrification and reduce NO₃⁻ leaching, decrease NH₃ volatilization losses, and increase yields and/or NUE (reviewed by Malhi et al., 2001). Even though agronomic benefits of banding were not shown in the current study, our results suggest that banded application of IU could achieve other benefits of banding without the unintended consequences of increased N₂O. In separate measurements at the same site, banded IU reduced NH₃ volatilization by 67 and 96% compared to banded and broadcast U, respectively (unpublished data), consistent with other results (e.g. Sommer et al., 2004). Halvorson and Del Grosso (2013) compared N source and placement effects on N₂O emissions and evaluated the very same PCU and IU products used here. Similar to our results, Halvorson and Del Grosso (2013) found that the increase in N₂O emissions due to banding compared to uniform broadcast application occurred to the smallest extent with IU as the N source compared to PCU and U in each of 3 study-years. These results are consistent with the intended effects of the microbial inhibitors present in the IU product; i.e., NBPT is intended to slow urea hydrolysis thereby reducing pH elevation and NH₄⁺ production, both of which could reduce NH₃ toxicity effects on NOs; and DCD is intended to slow NH₄⁺ oxidation to NO₂⁻ which could allow NOs to utilize NO₂⁻ at a rate more closely matched to its production rate. The current results showing that a banded 50/50 mix of IU/U reduced both N₂O and NO2I suggest that a mixed N source may provide a more economical N₂O mitigation strategy compared to using 100% of the IU product which generally has higher cost than conventional U. Reduction in N₂O emissions using band-applied PCU compared to banded-U was significant only during the drier year (2012). Sistani et al. (2011) measured greater N₂O emissions with PCU than U in the wetter of two years. Our NO2I and N₂O data suggest that intra-annual differences in PCU performance were partly due to increased NO2I in the wetter year, which may have resulted from enhanced urea release through the porous polymer coating during

wetter periods followed by more rapid hydrolysis and nitrification that may have increased NH_3 toxicity effects on NO_3^- .

The regulation of NO_2^- dynamics has been well-studied in wastewater systems, and kinetic models have been developed that consider the toxicity of HNO_2 and NO_3^- as well as NH_3 on AO and NO activity (Hunik et al., 1993; Park and Bae, 2009). Development of comparable models for soils would need to evaluate potential effects of soil properties and processes that might influence NO_2^- dynamics. For example, differences in nitrifier community composition among soils might affect how NO_2^- dynamics respond to differing NH_4^+ input rates (Verhamme et al., 2011). Another potentially important process is nitrosation which provides an abiotic pathway for NO_2^- to become incorporated into soil organic matter (SOM) (Stevenson and Swaby, 1964). Nitrosation reactions may in effect compete with other processes (e.g., ND) for available NO_2^- . Experiments by Azhar et al. (1986) using a mineral soil having organic C content similar to that in the current study (4%) indicated that a substantial fraction of the NO_2^- produced during nitrification underwent nitrosation. In theory, nitrosation is promoted at lower pH (Stevenson and Swaby, 1964). Thus, it is possible that pH elevation resulting from urea hydrolysis may have at once promoted NH_3 toxicity and inhibited nitrosation, thereby making more of the NO_2^- available to be reduced to N_2O . However, nitrosation itself can also produce N_2O , but controls over its occurrence and composition of by-products in soils are poorly understood. Smith and Chalk (1986) found wide variation among five soils in amounts of added $^{15}\text{NO}_2^-$ recovered in SOM but the extent of incorporation was not correlated with pH or total C or N content. Better understanding of how nitrosation is influenced by pH and SOM content and composition are needed before these factors can be incorporated into N_2O emissions models.

While measurement of soil NO_2^- may lead to enhanced understanding of controls over N_2O emissions and assist in developing mitigation practices, it also presents some challenges. The chemical instability of NO_2^- requires special precautions including an additional extraction to determine NO_2^- separately from NO_3^- using pH-adjusted extraction solutions and shorter storage periods than required for NO_3^- (Stevens and Laughlin, 1995). In addition, it is likely that soil NO_2^- concentrations will vary substantially at scales of cm or less and to be greatest within fertilizer bands or close to dissolving urea granules (Bezdicsek et al., 1971). Correlations between *in situ* soil N concentrations and N_2O fluxes may be confounded because of differing spatial scales over which each quantity varies and/or is measured. Better collocation of measurements could be achieved by intensive soil sampling from beneath chambers following each measurement (Venterea and Rolston, 2000b). However, this approach requires moving chambers between each event and results in substantial soil disturbance and large samples numbers or masses of soil requiring analysis. High temporal dynamics of soil N levels and N_2O fluxes also contribute to the challenge. Sampling limitations were likely important in the current study even though more than 2100 soil N and 1400 N_2O flux measurements were made over two growing seasons. Even weekly soil sampling at two positions and two depth intervals may not have sufficiently captured soil N dynamics. For example, the one apparently outlying replicate with the greatest ca- N_2O in the U-MRB treatment each year may have resulted from not capturing a peak in soil NO_2^- occurring between sampling events. Alternatively, factors other than NO_2^- (e.g. NO_3^- or O_2 availability) could have affected N_2O emissions to a greater extent for this replicate. Limitations related to the temporal frequency of soil sampling may also have been important in the BI treatments where elevated NO_2^- levels were shorter-lived than in the MRB treatments. Thus, even more intensive sampling regimes than used here may be needed to better understand management effects on soil N and N_2O dynamics. Cumulative soil N intensity and ca- N_2O

were more highly correlated ($r^2 = 0.44\text{--}0.51$) than daily soil N concentrations and daily N_2O flux ($r^2 = 0.22\text{--}0.42$) on dates each year when both quantities were measured. These results suggest that cumulative indices may serve to dampen spatial and temporal variations in daily quantities but more robust evaluation is needed in this regard. The lower correlations with daily measurements may have resulted from spatial variations in soil N dynamics and other soil properties (e.g., water content, temperature, soluble C) between soil sampling locations (which varied on each event) and flux chamber locations (which were fixed), differences (at the hourly scale) in the timing of soil and gas sample collection, and/or a lag between N_2O production in soil and its emission due to time required for partitioning of N_2O between soil-water and gas phases and diffusion to the soil surface.

5. Conclusions

Our results clearly imply that management practices which reduce NO_2^- accumulation have potential to also reduce N_2O emissions. Compared with NO_3^- , NO_2^- has higher rates of reactivity in producing N_2O , a broader range of conditions under which it is susceptible to reduction to N_2O , and a more proximal position with respect to N_2O in all known N_2O -producing mechanisms in soil including heterotrophic denitrification. Thus, the role of soil NO_2^- dynamics in regulating N_2O emissions, as shown here, is likely to be important under a broad range of conditions. This is not intended to imply that denitrification of NO_3^- is not also a key driver of N_2O ; for example, the role of NO_3^- is implied by our results in the BI treatments in the wetter year. However, our results do indicate that separate consideration of NO_3^- and NO_2^- dynamics can provide more insight than measurement of their combined concentrations as typically done.

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