Title

A history of manure management affects nitrous oxide emission potential and the denitrification molar ratio

Abstract

The denitrification process is a significant loss pathway in the nitrogen cycle. Carbon content, often found at elevated levels in manured soils, plays a critical role in regulating nitrous oxide emissions. Nitrate content and oxygen status are the other primary drivers of emissions, yet the interaction of these three variables requires further examination to elucidate the dynamics of the denitrification process. Emissions of N_2O and N_2 were measured after the application of incrementally increasing rates of labeled-$K^{15}NO_3$ to soils historically amended either with cattle manure or with synthetic fertilizer. Significant differences were found in the N_2O emissions profiles between the two treatments with a simultaneous increasing trend in emissions with increasing fertilizer applications. Traditionally manured soils showed average N_2O emission increases of near or above an order of magnitude over non-manured soils. Additionally, the ratio of nitrous oxide to total denitrification increased significantly between the soils and as fertilizer rate increased. With respect to heavily manured fields where organic carbon content is typically high, emission potential is of significant concern where the NO_3-N pool is in abundance.

Introduction

Nitrous oxide (N_2O) losses from arable lands affect both the individual farmer and society as a whole. To the farmer, losses of nitrogen (N) mean the loss of a principal nutrient in plant growth, a potential yield deficit, and a reduction in income. To society, N loss through denitrification is costly in several ways: N_2O is extremely efficient as a heat trapping gas, and itsglobal warming potential is 310 times greater than CO_2 on a per molecule basis (IPCC, 2007). In addition, N_2O is one of several ozone-depleting substances (Crutzen, 1970) and it is now the most abundant anthropogenic emission source by an estimated factor of ten over all other sources (Ravishankara et al, 2009).

Including CO_2 offsets, the US agricultural sector is responsible for emitting an estimated 462 Tg of carbon dioxide equivalents (CO_2 eq.; EPA, 2010). Crop production accounted for 31% of these emissions, with N_2O emissions totaling 154 Tg CO_2 eq. or 79% of cropland emissions (USDA, 2011). A primary driver of N_2O emissions is the added N through synthetic and organic sources of fertilization. N_2O emissions result primarily from two often-coupled soil redox processes, nitrification and denitrification, which are tightly regulated by the availability of carbon and oxygen, as well as the soil redox state. Denitrification is often considered the predominant source of N_2O emissions (e.g., Bateman and Baggs, 2005) and is the focus of this study.

Denitrification Process

The process of denitrification closes the N cycle, returning N_2 gas to the atmosphere. This mechanism involves the sequential reduction of NO_3 to N_2 in the following steps (with the participating enzymes, Nitrate Reductase (Nar), Nitrite Reductase (Nir), Nitric Oxide Reductase (Nor) and Nitrous Oxide Reductase (Nos) listed under each; Tiedje, 1994):

$$NO_3 \text{ (ionic oxide)} \xrightarrow{\text{Nar}} NO_2 \text{ (ionic oxide)} \xrightarrow{\text{Nir}} NO \text{ (gas)} \xrightarrow{\text{Nor}} N_2O \text{ (gas)} \xrightarrow{\text{Nos}} N_2 \text{ (gas)}$$

A variety of heterotrophic microbes mediate this process and operate to a large extent in oxygen-limited environments. The participating enzymes are not extremely sensitive to O_2 concentrations with the exception of Nos. Because of its O_2 intolerance, oxygen exposure deactivates the enzyme, the only known to reduce N_2O to N_2. This generally causes an upward shift in the N2O:N2 ratio (Richardson et al, 2009).

Along with pH and temperature, the availability of nitrogen oxides and organic carbon are generally thought to be the other main regulators of denitrification and all interact in a complex fashion (Tiedje, 1988). A variety of studies have found a positive correlation between denitrification and the addition of mineral N. Even as oxygen is depleted, there appears to be a baseline level of inorganic N required for significant emissions to occur (Dobbie et al, 1999). Similarly, carbon availability has long been known to play a significant role in denitrification (Burford and Bremner, 1975). Active carbon is often supplied in manure additions. Paul and Beauchamp (1989) found that these increased levels, particularly that of volatile fatty acids (VFA), correlate with increased denitrification in waterlogged soils.

VFAs generally degrade within a few days. Thus, it might be expected that additions of manure, high in VFAs, will stimulate gas emissions. Stevens and Laughlin (2002) found that concurrent application of cattle slurry and inorganic fertilizer significantly increased overall emissions while application of inorganic fertilizer 3 to 4 days after the application of cattle slurry had no effect on emissions. Additionally, Stevens and Laughlin (2001) found that cattle slurry increased emissions when applied with synthetic fertilizer by an average of .63% of applied N.

Moreover, recent literature suggests that nitrous oxide emissions increase exponentially with increasing N application (Hoben et al, 2010; Ma et al, 2009; Zebarth et al, 2008; McSwiney and Robertson, 2005). However, very little has been discussed on this phenomenon with respect to increased levels of active carbon as one might expect in manured soils. Specifically, how does the addition of manure interact with increasing N additions with respect to the products of denitrification? Accordingly, the objectives of the current study were to examine the effect of a history of manure inputs on both the denitrification process and the molar ratio or partition of N_2:N_2O as a result of N fertilizer application.

Methods

Core Preparation and Incubation

The experiment was conducted using silt-loam soils from two fields with a traditional corn-soybean cropping rotation. The first field (M) was a working dairy farm with a history of manure application located in Lansing, NY, USA (42°35' N, 76°31'W). The soil at this site is an Ovid silt-loam (fine-loamy, mixed, active, mesic Aeric Endoaqualf). The second site (NM) was an un-manured soil classified as a Honeoye silt-loam (fine-loamy, mixed, mesic Glossoboric Hapludalf) located at the Cornell University research farm near Aurora, NY (46°26'□N, 76°26'□W).The soils were chosen based on their similarity in texture, both being dominant in the silt fraction. The M soil had slightly higher silt content (55% vs. 50%) while the NM soil had slightly higher clay content (38% vs. 33%). The primary difference is organic matter content with 4.1% in the M soil and 3.2% in the NM soil. The chemical and textural makeup of the soils is shown in Table 1.

Soils were sampled in bulk from the top 15 cm of the soil surface using a tile-spade one week prior to the start of the incubation. Soils were homogenized and stored at 4°C until commencement of the incubation. To maintain some structural integrity and remove excess debris and rocks, all soil material was sieved using an 8-mm mesh screen and repacked into PVC cores (5.2 cm i.d. x 13.6 cm h) to a bulk density of 1.15, which was representative of field values. The cores were incubated in the dark at 30°C and N_2O and N_2 losses were monitored for a period of 168 hours. To induce oxygen deficits and force the denitrification process, all cores were maintained at a water-filled pore space of approximately 80% throughout the duration of the experiment, which was corrected gravimetrically at each sample period.

^{15}N-Flux Methodology

Isotopically-enriched $K^{15}NO_3$ (60 atom%; Sigma Aldrich, MO, USA) was added in solution in a factorial arrangement to the manured and non-manured soils at 0, 50, 100 and 200 kg ha^{-1} with 4 replications for a total of 32 cores in a manner using similar to Panek et al. (2000). Briefly, all cores were wetted to 9 ml below the desired WFPS using DDI water. $K^{15}NO_3$ was dissolved in solution at desired rates and injected in 3 aliquots of 3 ml in different locations of the core by syringe. To achieve a uniform distribution throughout the core, the syringe was withdrawn slowly from the soil while injecting each aliquot.

Gas samples were taken on 6 separate occasions beginning at 12 hours after fertilizer injection and thereafter at 24, 36, 48, 120 and 168 hours. Sampling was conducted similar to previous studies (Millar and Baggs, 2004; Gentile et al., 2008) in which a one-hour flux is measured after cores were placed in 1-L Mason jars fitted with an airtight seal and sampling septum. Three empty jars were

incubated to serve as reference (blank) samples. Because the gas flux was found to be linear over this time period, flux can be calculated as the difference between the sample and the blank over the 1-hour period. A 15 ml sample was taken from the headspace of each jar with a syringe and injected for storage in 12 ml evacuated glass vials (Labco; Wycombe, UK).

All samples were processed at the University of California-Davis Stable Isotope Facility for N_2 and N_2O concentrations and isotopic signature with a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). After isolation and concentration of gases, each sample is transferred by a helium carrier stream N_2 gas is passed to the IRMS through a molecular sieve 5A GC column (15m x 0.53mm ID, 25°C, 3 mL/min). Simultaneously, the rest of the gas is passed through a CO_2 scrubber (Ascarite) and N_2O is trapped and concentrated. N_2O is carried by helium to the IRMS via a Poroplot Q gas chromatology column (25m x 0.53 mm, 25°C, 1.8 mL/min), which separates N_2O from residual CO_2.

The ion currents at *m/z* 44, 45 and 46 for N_2O and 28, 29 and 30 for N_2 allow for back-calculation of the enrichment of the denitrifying pool (^{15}Xn), assuming uniform mixing of added NO_3 with indigenous NO_3. Along with the molecular ratios for N_2O and N_2 (^{44}R, ^{45}R, ^{29}R and ^{30}R, respectively) in the enriched atmospheres, the fraction proportional to both gases evolved (N_2/N_2O) can be calculated (Mulvaney and Boast, 1986; Arah, 1997).

Statistical Analyses

Due to the highly skewed nature of denitrification data, all statistical analyses were performed on log-transformed data to meet the normality assumptions of the statistical tests used herein. Analysis of variance was used to compare treatment means across time periods and simple, linear regression was used to quantify correlation between fertilization rate and emissions. Means separation was found using Tukey's Honestly Significant Difference and significance was determined at α=0.05. All statistical computing was done using the R Software package.

Results and Discussion

Trends and Variability in Emissions

Cumulative N_2O emissions were significantly higher in the traditionally manured soil material than in the non-manured soil material (Table 2). As the fertilizer rate increased, M soils produced a 53, 15.5 and 8.6-fold increases in N_2O emissions over NM soils at 50,100 and 200 kg ha^{-1}, respectively. Corresponding increases in average emissions of N_2 were 1.7, 1.7 and 1.8-fold. although these differences were not statistically significant (Table 2). Average variability between treatments was higher in M soils for N_2 emissions (CVs of 55% and 45%, respectively) with the opposite in N_2O emissions with an average CV of 64% in M soils and 93% in

NM soils.

In both M and NM soils, average N_2 emissions actually decreased between the mid and high rates of fertilizer application although a statistical change was not detected due to high variability (Fig. 1). Conversely, nitrous oxide emissions, not only showed significant increasing trends in the M versus NM effect but also within-group effects for the fertilizer factor. In M soils, an increase of fertilizer rate from the low (50 kg ha^{-1}) to mid (100 kg ha^{-1}) and low to high (200 kg ha^{-1}) produced average increases of 806 and 1821 μg N_2O-N per kg-$soil^{-1}$, respectively. The analogous increase in NM soils produced increases of 77 and 264 μg N_2O-N per kg-$soil^{-1}$ (Table 2).

High variability, particularly for denitrification data, reduces statistical power in the analysis of emissions data. Much of this variation may be attributable to a diffuse collection of active microsite "hotspots" even within re-packed cores. If O_2 consumption is relatively high, denitrification microsites may develop irrespective of matrix structure or diffusion rates (Parkin, 1987). Rover et al. (1999) found a CV of 100-200% on relatively small scale - primarily the result of a high number of randomly distributed hotspots across the sample site.

Treatment Effects on the Molar Ratio

An increasing trend with higher fertilizer rate is similarly reflected in the average molar ratio of N_2O:(N_2O+N_2) during the incubation period (Figure 3). The increase in ratio was better represented as a linear trend (R^2 = 0.89 and 0.76 for M and NM soils, respectively; Fig. 4) rather than exponential with much higher ratios found in the M soil than in the NM soil overall (Fig. 3).

As microsites develop, oxidation of easily available organic carbon triggers N_2O emissions (Parkin, 1987). This may help explain the large N_2O:(N_2O+N_2) ratio differences between the manured and non-manured soils, with organic matter contents of 4.2 and 3.1% respectively. The increased N_2O production in manured soils in this study was similar to those of other researchers. Russow et al. (2008) found that in combination with nitrate fertilizer, soils with higher organic carbon content typically produce higher N_2O rates with the primary production pathway identified as denitrification. Additionally, using soils with a history of manure application, Jagr et al. (2011) found that nitrate additions increased emissions over manure application as soil moisture increased. This effect was reversed at lower moisture. This suggests that at high moisture contents, nitrate was the limiting factor in denitrification. This hypothesis is supported in the N_2O:(N_2O+N_2) molar ratios of the current study (Fig. 1).

A high N_2O:(N_2O+N_2) molar ratio was observed in the M soil at the highest fertilization rate (Fig. 5). With moisture content maintained at 80%, there was very little limitation on N_2O production. This interaction, at higher fertilization rates, highlights the emission potential stimulated through increasing organic carbon pools. Organic matter was 0.9% higher in M soils than in NM soils (Table 1). At high fertilization rates where nitrate was essentially eliminated as a

differentiating factor, it is presumed that this carbon source was the primary driver in producing high N_2O emissions. In a similar study, Weir et al. (1993) found that at high available C levels, increasing nitrate inhibited the conversion of N_2O to N_2 with an increase in CO_2 levels, which implies a flourishing denitrifying microbial population in which N_2O is the end product.

A rich and diverse microbial population (quality and quantity) itself potentially provides a feedback mechanism for further stimulation of the denitrification process (Cavigelli and Robertson, 2001). In examining differences in microbial communities between soils receiving manure versus mineral fertilizer, Parham et al. (2003) found differences particularly between the rapidly reproducing r-strategist abundance with high populations in manure-amended soils. The authors postulated that manure inputs provide for proliferation of both r- and K-strategists because manure offers a more balanced ratio of inorganic nutrients to organic C. Conversely, inorganic fertilizers (NPK) only supply inorganic nutrients leaving organic carbon as the limiting factor, thus reducing the r-strategist population. Under controlled conditions, Cavigelli and Robertson (2000) found that these differences can have an effect on Nos gene expression, thus shifting the molar ratio toward N_2 production with Nos enzymes more active in successional (uncultivated) fields rather than in agricultural fields. A microbial basis for the change in reductase activity suggests that the microbial population existing in the M fields has less capability for N_2O reduction despite the availability of reductant and oxidant. Microbial population was not enumerated in this study, however, and further study is necessary to ascertain how respective populations might have changed between these two fields and how these changes affect emissions factors.

The current yearly emissions factor recommended by the IPCC is 1% of N supplied either through synthetic or organic sources (IPCC, 2006). The N_2O emissions factor for this study were approximately 3% and .3% for M and NM soils respectively. While these values do not have any practical application, they do highlight the differences in emission potential and the difficulty in deriving a static emissions factor for yearly emissions as the N_2O:(N_2O+N_2) molar ratio is the primary variable in this equation. Looking at interactive effects, Stevens and Laughlin (2002) calculated an emissions factor of 2.9% when adding manure slurry simultaneously with mineral fertilizer.

Additionally, the role that carbon plays in determining this ratio remains unclear, particularly in sustaining a high ratio throughout the study period. As Fig. 4 shows, the N_2O:(N_2O+N_2) ratio increased with increasing KNO_3 for both M and NM. At the beginning of the incubation in nearly all factorial combinations, the N_2O:(N_2O+N_2) molar ratio tends toward a higher emission of N_2O, where the mechanism is assumed to be a lag between the development of Nar and Nos production at the onset of favorable denitrifying conditions (Letey et al., 1980). Using a single N rate and three incremental C rates, Stevens and Laughlin (1998) found the total denitrification to be similar between the mid and high C rates but the mid rate had a higher N_2O production rate (higher molar ratio),

which they attributed to a nitrate limitation in the high C rate. In the M soils particularly, this study corroborates this finding as a higher N_2O:(N_2O+N_2) molar ratio was maintained where N was presumably not limiting (200 kg ha-^{1}) during the entire incubation while at lower rates, the molar ratio declines with time and in the lower carbon content of NM (Fig. 5). Initial carbon content can be assumed to be approximately equal within each treatment, which further implies that nitrate content controlled the trend in the N_2O:(N_2O+N_2) molar ratio.

Additionally, Mathieu et al. (2006) found a moderately significant, positive relationship between CO_2 and N_2 production, concluding that C (with CO_2 as a proxy for C availability) promotes conversion to inert N_2. These seemingly incongruous conclusions can be rectified by varying and limiting NO_3 contents, however. It appears that high N_2O losses may be maintained as long as both factors are found in abundance and will tend toward N_2 production particularly as NO_3 becomes limiting.

This latter study was confounded by the persistence of cores maintaining a high N_2O rate during the incubation. The authors postulated that, again, microorganismal differences exist with variable ability/sensitivity to reduce N_2O. In examining the longitudinal N_2O:(N_2O+N_2) data (Fig. 5), anomalies exist with similar implications: At high N rates for both N and NM soils, a high molar ratio was maintained and generally increased with time. This change was a result of decreasing N_2 production while N_2O production remained fairly stable. Carbon quality, particularly in the NM soil, may have affected this as hotspots were created by an expanding microbial population and new, water-soluble carbon sources became bioavailable toward the end of the incubation (Boyer and Groffman, 1996), while NO_3 levels remained non-limiting.

Conclusion

Based on the results herein, the manured soil had a general propensity for N_2O production through denitrification given sufficient oxygen deficiencies producing a nearly 25-fold increase in N_2O emissions when averaged across fertilizer application rates. Under increasing fertilizer N rates, N_2O emissions increased by over 800 μg N_2O-N per kg-soil^{-1} between 50 kg ha^{-1} and 100 kg ha^{-1} over 1800 μg N_2O-N per kg-soil^{-1} between 50 kg ha^{-1} and 200 kg ha^{-1} in the M soil. Increases in the NM soil were 75 and 260 μg N_2O-N per kg-soil^{-1}. The IPCC currently suggests a static emissions factor of applied fertilizer N (IPCC, 2006).

A non-linear trend would imply that as the N rate increases, the discrepancy between a fixed emissions factor and observed emissions will increase with applied N (Hoben et al., 2010). While a non-linear change point was not determined, further work is necessary outside of the parameters of the present data set. The results in this study suggest that not only does increasing N affect the N_2O:(N_2O+N_2) ratio, there is also an interaction between carbon levels as highlighted by the significantly increased molar ratios in the M soil over the NM soil at corresponding N rates. Thus, in the manured soil with higher carbon content, additional N_2O emissions may be expected at equivalent fertilizer rates

due to protracted N_2O emissions under oxygen-deficient conditions without necessarily increasing overall denitrification (Fig. 1).

Under conditions where high organic C content is likely, as is often the case for soils in animal-based agricultural systems (Wander et al., 1994), management practices aimed at controlling the nitrate pool (more precise N fertilizer rates, timing and formulations) are likely to produce more rapid and sustained results in lowering N_2O emissions.This would intervene at the beginning of the denitrification process as opposed to the alternative of forcing a sufficiently low redox potential to complete reduction to gaseous N_2. Additionally, there may be an increased probability of N_2 production (as opposed to N_2O) of the remaining, denitrifying NO_3 pool when it is the limiting factor. In general, manure-based systems may exacerbate the increase in N_2O emissions with increasing fertilizer rate, making accounting of nitrous oxide and overall emissions in these systems extremely complex.

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Table 1. Physical and chemical characteristics of soils evaluated in the study.

Treatment	Sand	Silt	Clay	pH	Organic Matter
	g kg^{-1} soil				%
Manure	330	550	120	7.1	4.1

Non-Manure	380	500	120	7.9	3.2

Table 2. Means (geometric) and ANOVA statistics including Tukey's HSD for treatment effects. Significance is determined at $p < .1$ due to high variability. Additionally, comparisons are included as differences in fertilizer rate within management practices (inter-management; M:M and NM:NM) and between management practices (intra-management (M:NM) where factor levels with corresponding letters are not significantly different.

Management	Fertilizer Rate	Mean N_2O Emission †	Tukey HSD		95% CI	
	kg ha^{-1}	μg kg-$soil^{-1}$	Inter-management $p < .1$	Intra-management $p < .1$	Upper Bound	Lower Bound
M	50	539.45	a	a	1658.81	175.43
M	100	1345.45	ab	b	2882.51	628.01
M	200	2359.95	b	c	5995.13	928.98
NM	50	10.18	c	d	39.53	2.62
NM	100	87.00	d	e	328.82	23.02
NM	200	273.68	d	a	1110.35	67.46

Management	Fertilizer Rate	Mean N_2 Emission †	Tukey HSD		95% CI	
	kg ha^{-1}	μg kg-$soil^{-1}$	Inter-management $p < .1$	Intra-management $p < .1$	Upper Bound	Lower Bound
M	50	2561.78	a	a	9068.25	723.70
M	100	4053.81	a	a	6466.59	2541.28
M	200	3084.08	a	a	7885.57	1206.20
NM	50	1476.67	b	a	2972.99	733.46
NM	100	2413.03	b	a	5368.09	1084.69
NM	200	1672.27	b	a	3628.05	770.79

† Geometric Mean

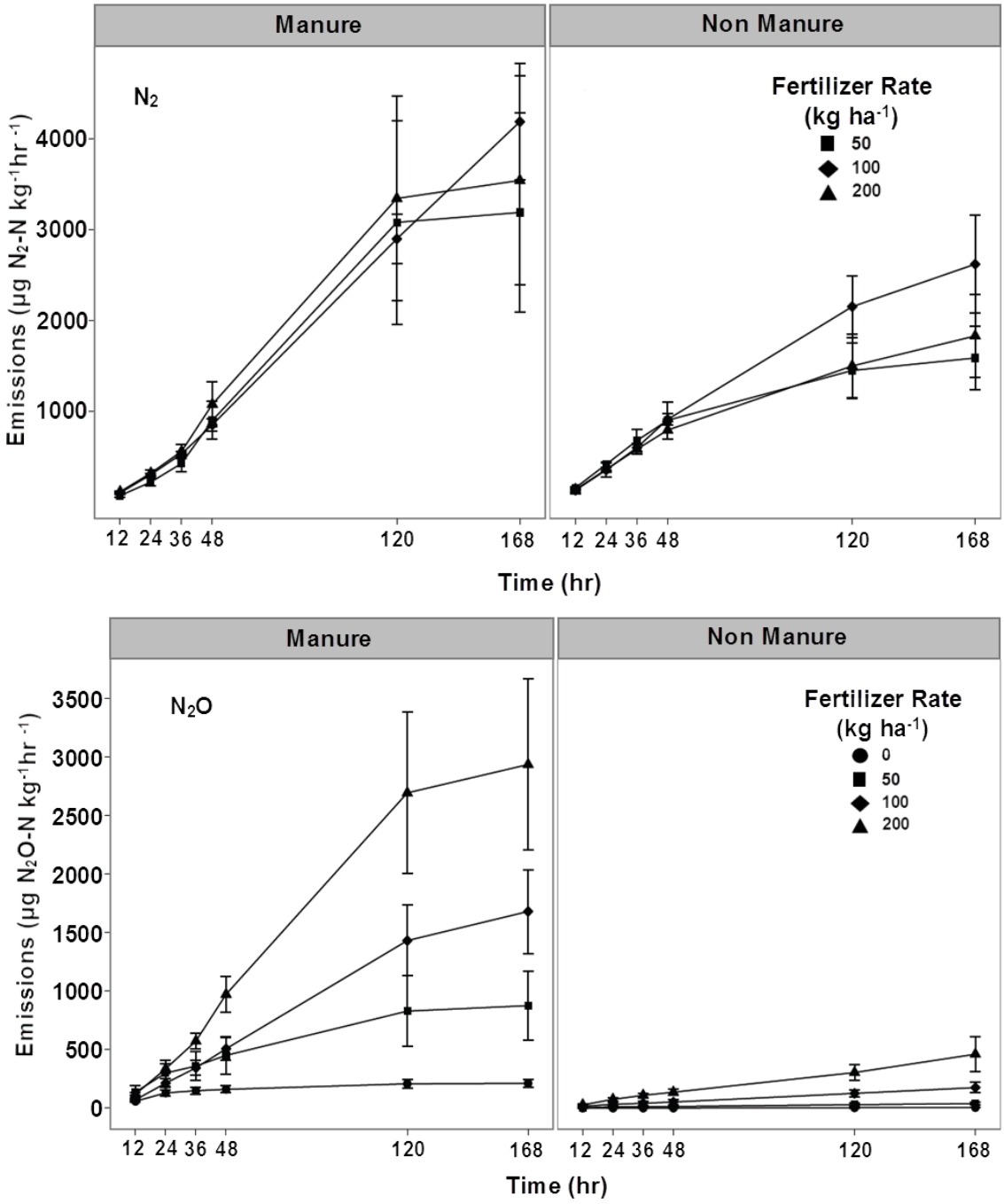

Figure 1. Cumulative total emissions of N_2O (A) and labeled N_2 (B) from 0 to 168 hours of incubated M and NM soils. Error bars represent ± one SEM.

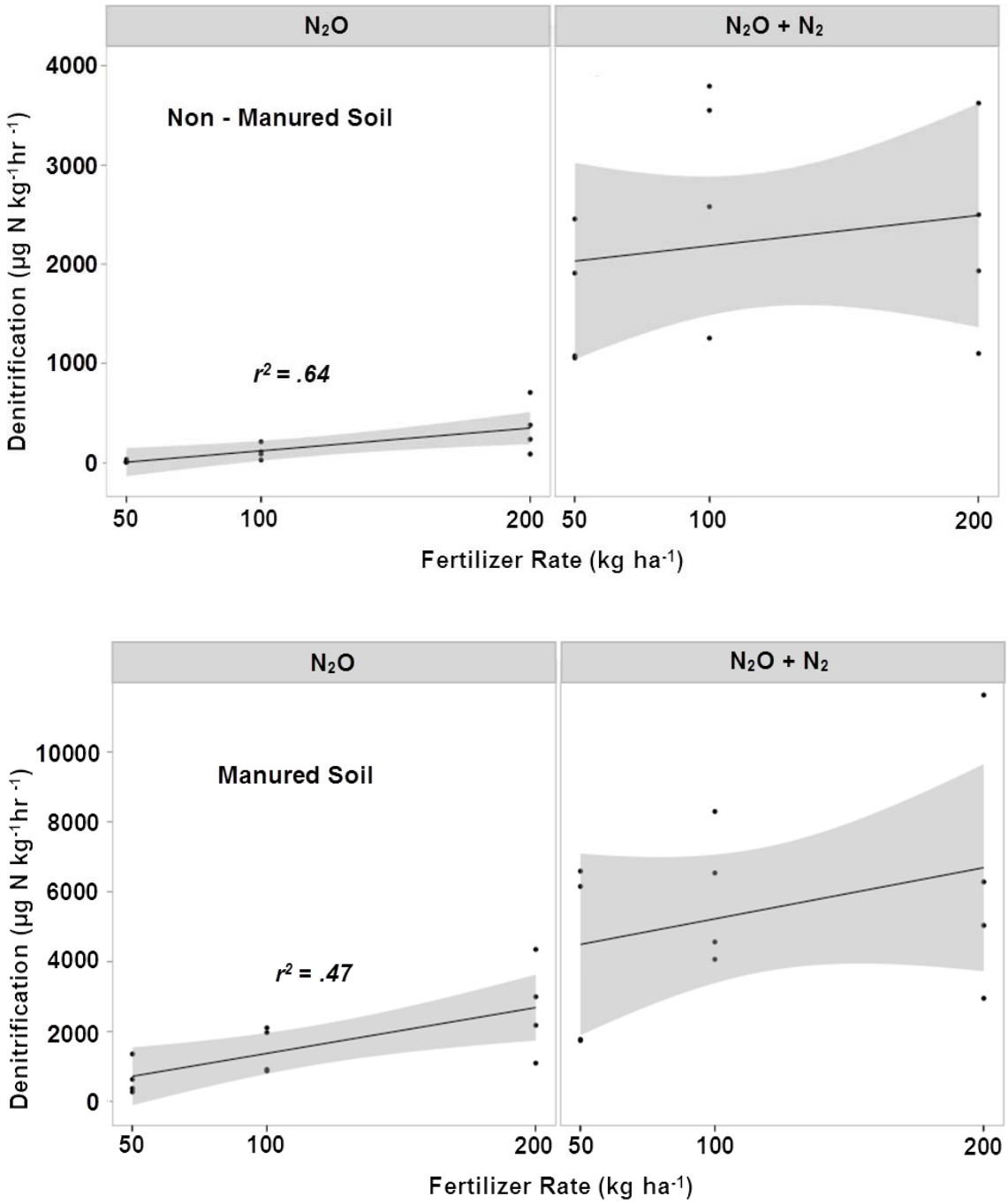

Figure 2. Linear regression of N_2O and total denitrification derived from enriched fertilizer on fertilizer rate for NM (A) and M (B) soils. Shaded areas show .95 confidence intervals for standard error. Fertilizer rate was a significant predictor for N_2O only at α=0.05.

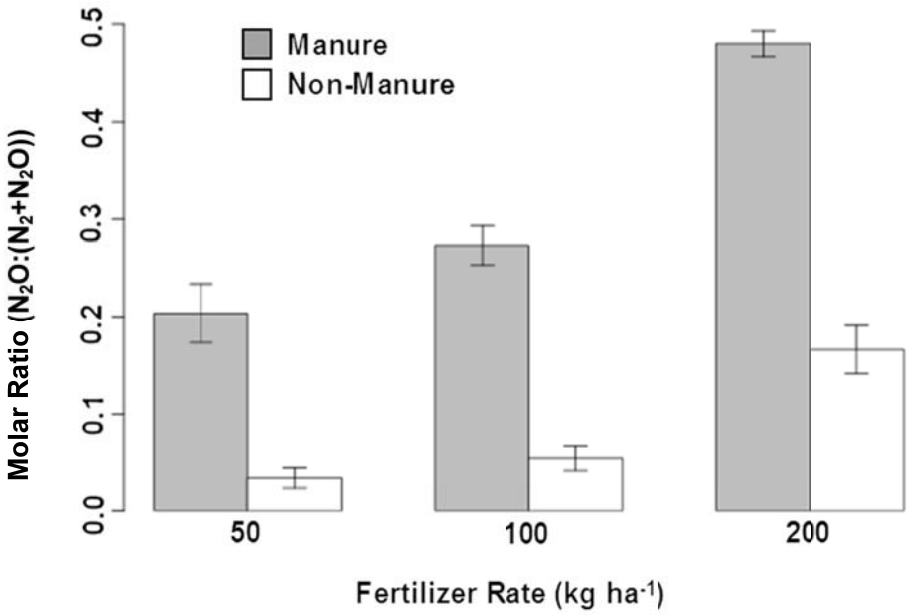

Figure 3. Average mole ratio of N_2O to total emissions (N_2O:(N_2+N_2O)) derived from labeled KNO_3 during the 168-hour incubation period. Error bars represent ± one SEM.

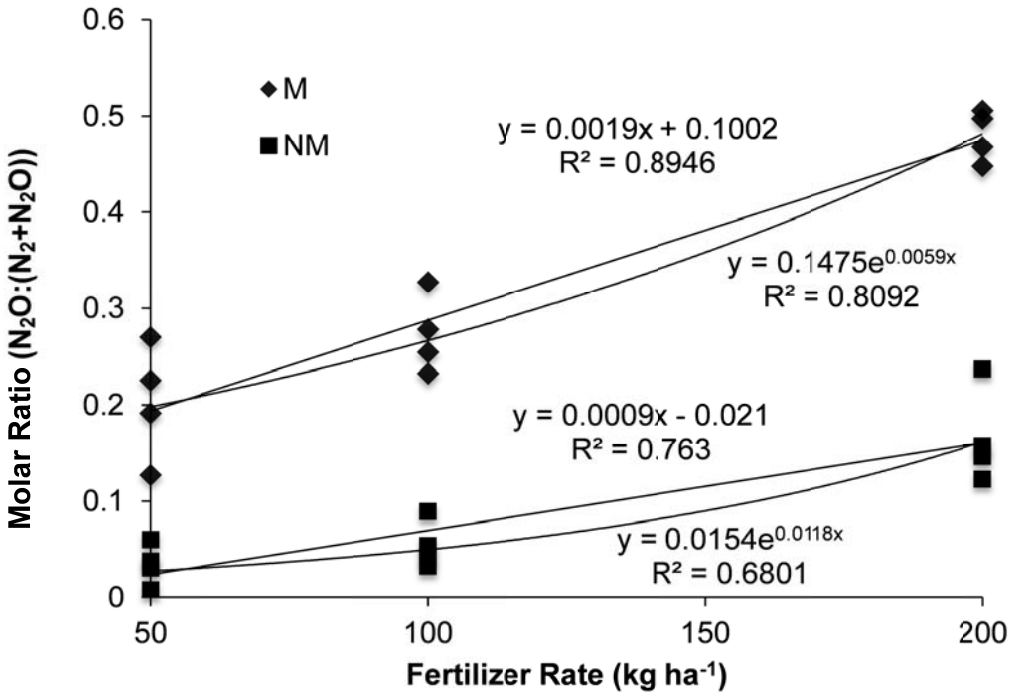

Figure 4. Linear and non-linear regressions of the average N_2O:(N_2+N_2O) molar ratio as a function of fertilizer rate.

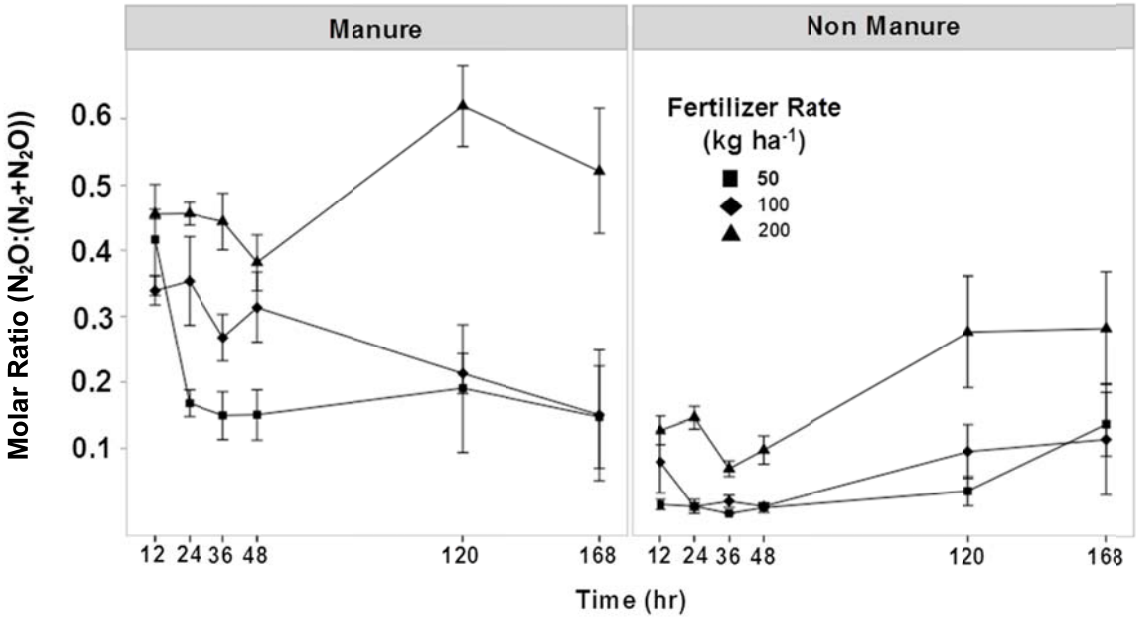

Figure 5. Time series of the average molar ratio ($N_2O/N_2 + N_2O$) for M and NM soils. Error bars represent ± one SEM.