NITRIFICATION RATE EFFECT ON CUMULATIVE NITROUS OXIDE EMISSION FROM SOIL

by

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ABSTRACT

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Knowledge of the relationship between rate of nitrification and nitrous oxide (N_2O) emission, and between cumulative nitrification and N_2O emission is important for developing N_2O emission mitigation strategies. Gross nitrification and N_2O from nitrification were determined using ^{15}N labelling of inorganic N. N-Serve was added to delay nitrification and results showed an increase in rate of N_2O emission with that of apparent nitrification in absence of N-Serve, but there was no relation in its presence. Same amount of cumulative N_2O was emitted for same amount of nitrogen (N) apparently nitrified, regardless of N-Serve addition. There was no relation between N_2O emission attributed to nitrification and gross nitrification with and without N-Serve. Again, same amount of cumulative N_2O was emitted for same amount of gross nitrified N, regardless of N-Serve addition. These results imply that the amount of N nitrified dictates eventual cumulative N_2O emitted, regardless of rate of nitrification.

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LIST ABBREVIATIONS

DCD	dicyandiamide
DMPP	3, 4-Dimethylpyrazole phosphate
ATU	allylthiourea
GTU	guanylthiourea
AMO	ammonium monooxygenase
ESN	environmental smart nitrogen
N	nitrogen
WFPS	water filled pore space
IRMS	isotope ratio mass spectrometer
GC	gas chromatograph
epm	effective actions per minute
rpm	revolutions per minute
M	molar
d	day
n	fractional contribution of nitrification to N_2O flux
d	fractional contribution of denitrification to N_2O flux
t	time

REG	regression
PROC	procedure
AICc	Akaike Information Criterion
GLIMMIX	Generalized Linear Mixed Model

1. INTRODUCTION

1.1 General Introduction

Nitrous oxide (N_2O) is a greenhouse gas that is very effective in decomposing ozone and has a radiative power that is 300 times more than carbon dioxide (Snyder et al. 2014). Nitrous oxide has a life span of approximately 114 years and is partly responsible for the increase in radiative forcing of the atmosphere over the past 100 years (Glenn et al. 2012; Lebender et al. 2014).

Due to the world's fast growing population and increasing demand for agricultural crops, farmers are increasing food production to provide millions of people with nutritious food, while trying at the same time reducing the environmental threats imposed by their operations (Zhu and Chen 2002). Meeting these two goals implies that farmers need to operate in a sustainable and environmental sound manner without compromising crop yield and quality (Parkin and Hatfield 2014). This will result in the use of large quantities of nitrogen (N) fertilizers (Burgos et al. 2015). The consumption of N fertilizers is estimated to have increased by 2.7% from 2000 through 2050 (Lebender et al. 2014). This will consequently increase the amount of greenhouse gas emissions, especially N_2O, since its emission to the atmosphere is related to the quantity of N fertilizer used (Parkin and Hatfield 2014).

1.2 Nitrification and Denitrification as Sources of Nitrous Oxide

Nitrification and denitrification are microbial-mediated processes widely accepted to be major contributors of N_2O emissions from soil (Clough et al. 2005; Davidson and Kanter 2014; Simpson et al. 2014; Heil et al. 2016). Some decades ago, the hole and pipe model was developed where N_2O was produced as a by-product of the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) and also during the reduction of NO_3^- by the process of denitrification (Figure 1.1) (Heil et al.

2016). Nitrous oxide can also be produced by non-enzymatic processes that also happen in soil (Manalil et al. 2014; Sander et al. 2014). Nitrite (NO_2^-) formed during nitrification and denitrification can decompose in the soil producing NO (nitric oxide) and N_2O by the process called chemo-denitrification (Maharjan and Venterea 2013). This process usually happens in acidic soils where NO_2^- exists in a protonated form (HNO_2) (Morot-Gaudry-Talarmain et al. 2002; Ma et al. 2015; Yoon et al. 2015). This product may react with soil organic matter (SOM) and some metals and usually the by-product is NO gas.

Hydroxylamine (NH_2OH) is the first intermediate produced during the first step of nitrification by autotrophic and heterotrophic organisms (Shen et al. 2003; Sabba et al. 2015; Farquharson 2016). Hydroxylamine is very reactive and decomposes very fast and its detection is difficult. Tenuta and Beauchamp (2000), in a soil microcosm study, failed to detect NH_2OH in a soil amended with ammoniacal N sources. Addition of NH_2OH resulted in recovery as N_2O and thus they concluded this intermediate in nitrification if released to soil rapidly reacts to form N_2O. More recently, using a very sensitive method to measure NH_2OH in soil, Liu et al. (2014) found this nitrification intermediate to have accumulated in soil and was highly correlated (r^2 =0.80) with N_2O emission rates. Hydroxylamine can be a source of N_2O production especially in NH_4^+ fertilized soils with high nitrification rates (Sabba et al. 2015). The oxidation of NH_4^+ to NO_3^- is thought to be mainly mediated by autotrophic bacteria (Davidson and Kanter 2014; Masaka et al. 2014; Scheer et al. 2014) but mechanisms that are involved in the production of N_2O through this process still need to be understood.

Nitrifier-denitrification is another pathway that can produce substantial amounts of N_2O (Kool et al. 2011). This process is mediated by autotrophic ammonia (NH_3) oxidizers. In this process, NH_3 is oxidized to NO_2^-, followed by subsequent reduction to N_2O and N_2 (Figure 1.1).

The enzymes involved in this process are the same as for NH_3 oxidation and denitrification (Wrage et al. 2001). Denitrification is also a major pathway that produces significant amounts of N_2O emissions (Kulkarni et al. 2014). It is a stepwise reduction of NO_3^- to N_2 via NO_2^-, NO and N_2O as intermediates and it occurs when NO_3^- is used as an electron acceptor under anaerobic conditions. During this process, N_2O can be liberated into the atmosphere if not further reduced to N_2 gas (Wrage et al. 2001).

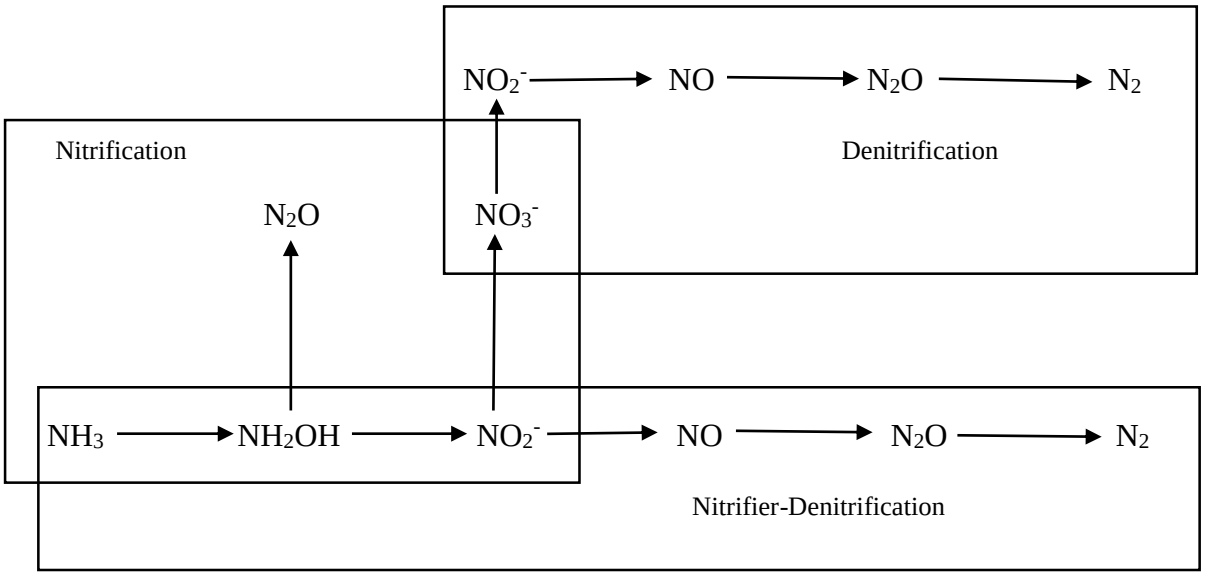

Figure 1.1 Major processes of N_2O production in soil (Wrage et al. 2001).

1.3 Methods for Determination of Nitrification and Denitrification Sources of Nitrous Oxide

A number of methods have been used to determine the contribution of nitrification and denitrification to N_2O emissions. In the late 1980s, researchers used acetylene (C_2H_2) to selectively inhibit nitrification and N_2O reduction by denitrification, respectively at concentrations of 0.1 Pa and 10 Pa (Heil et al. 2016). However, C_2H_2 selective inhibition overestimated N_2O from denitrification since not all activity of autotrophic nitrifiers was eliminated (Clough et al. 2005).

Isotope tracer (^{15}N) based methods are of great interest for partitioning the contribution different pathways to N_2O emissions, though use is restricted to few laboratories. The use of isotopic ^{15}N-labelling, offers a good opportunity to identify N_2O sources and also to quantify the relative contribution of nitrification and denitrification to N_2O emissions (Perez et al. 2006). The pool dilution method was first developed by Stevens et al. (1997). In this method, there is differential labelling of NH_4^+ and NO_3^- with ^{15}N so that nitrification from NH_4^+ and denitrification from NO_3^- may be quantified. This is achieved by periodical measuring and comparing of δ-^{15}N of N_2O, NO_3^- and NH_4^+ pools. With this information the relative importance of each process (nitrification and denitrification) can be quantified (Stevens et al. 1997; Mathieu et al. 2006).

1.4 Nitrification Rate and Nitrous Oxide Emission

Very few studies to date have reported nitrification rates and their relationship with N_2O emission. The rate at which nitrification occurs within a soil may differ due to soil conditions and management practices, for example the use of nitrification inhibitors (Wan et al. 2009). The rate of nitrification in soil is affected by many interacting factors and these include concentration of NH_4^+/NH_3, soil temperature, aeration, compaction, moisture, pH, available N (Zaman et al. 2009; Liu et al. 2013; Cheng et al. 2014; Smith et al. 2014). Tenuta and Beauchamp (2000), observed a

strong relation between apparent nitrification (NO_3^- accumulation in soil) rate and N_2O emissions of a soil treated in microcosms with increasing size of granular urea. Again, a positive (non-significant) relation between gross nitrification (NO_3^- accumulation in soil irrespective of N consumption) rates and N_2O emissions was observed in a tropical rain-forest soil (Breuer et al. 2002).

Addition of nitrification inhibitors and/or controlled release N sources (Environmentally Smart Nitrogen), results in reduced N_2O emissions (Somda et al. 1990; Wan et al. 2009; Di et al. 2014; Ruser and Schulz 2015; Sun et al. 2015). The nitrification inhibitors such as dicyandiamide (DCD), 3, 4-Dimethylpyrazole phosphate (DMPP) and nitrapyrin (2-Chloro-6-(trichloromethyl) pyridine; trade name N-Serve) applied with ammoniacal fertilizers have been found in many cases to have reduced N_2O emissions from soil (Cui et al. 2013; Liu et al. 2013). However, little is known if this reduction in emissions is due to reduced rates of nitrification or reduced total amount of N nitrified.

1.5 Nitrification Inhibitors

Nitrification inhibitors were developed to mitigate N loses through blocking the first step of nitrification (Ruser and Schulz 2015). Among the commercially available nitrification inhibitors, DCD and nitrapyrin are the most widely used N stabilizers in North America. They affect the first step of nitrification in which NH_3/NH_4^+ is oxidized to NH_2OH, and is catalyzed by the enzyme ammonia monooxygenase (AMO) (Cui et al. 2013). The AMO enzyme is bound in the membrane of autotrophic bacteria with copper as a co-factor. This enzyme is reported to have a wide range of substrates which can influence its activity.

Different mechanisms of nitrification inhibition have been described, and these include removal of co-factors by chelating compounds. Since the activity of AMO is affected by the

presence of copper, adding copper chelators inhibits the oxidation of NH_4^+ to NH_2OH, while addition of copper stimulates this reaction. Nitrapyrin and DCD have been reported to use the above mode of action (Ruser and Schulz 2015). Another mechanism is through direct binding and interaction with AMO enzyme, competitively and/or non-competitively. This group of inhibitors bind on the active site of the enzyme (competitive) or on the second site not used by the substrate (non-competitive). Non-competitive inhibition results in loss of structure of the enzyme, consequently resulting in loss of its function (Sun et al. 2015). Nitrapyrin has been reported to work as a non-competitive inhibitor (Ruser and Schulz 2015). Oxidation of reactive substrates can inactivate AMO and other enzymes, and this is referred as suicide inhibition. This mode of action usually results in an irreversible inactivation of the enzyme, and acetylene belongs to this category of inhibition. As shown earlier, nitrapyrin and DCD belong to a big group of copper chelators, and DMPP is also supposed to belong in this group. Categorizing nitrification inhibitors in one group based on their mode of action is impossible, because one inhibitor can have more than one mode of action. For example, nitrapyrin is a non-competitive inhibitor and a chelator, but it can also show a weak suicide inhibition (Ruser and Schulz 2015).

The stability of most nitrification inhibitors is affected by soil temperature (Di et al. 2014). Temperature increase in soil was observed to strongly decrease the half-life of DCD (Wan et al. 2009). At 8 °C, the half-life of DCD was nearly 120 days, but it dropped to 20 days as temperature increased to 20 °C. Doubling the application rate of DCD from 10 to 20 mg kg^{-1} increased its half-life by 45%. However, there was no difference in N_2O emission between the two rates (Ruser and Schulz 2015). Nitrapyrin and DMPP were investigated in a closed incubation study, and the authors found no significant differences between cumulative N_2O emissions between the two inhibitors (Liu et al. 2013).

Nitrapyrin has a high vapor pressure, and can volatilize to the atmosphere under field conditions; hence can result in the reduction in its efficacy. The efficacy of nitrapyrin is also speculated to be reduced by higher temperatures (Ruser and Schulz 2015). Nitrapyrin and DCD have been reported in many cases to reduce N_2O emissions in both field and laboratory studies (Wan et al. 2009; Ruser and Schulz 2015). The efficacy of these products is affected by environmental variables such as temperature, moisture and rainfall. This prompts the need for more measurements which focus on the impact of these aforementioned environmental variables on the efficacy of nitrification inhibitors.

1.6 Thesis Objectives

The objectives of this thesis were to determine using ^{15}N-labelling, if the rate of nitrification or the total amount of N nitrified determines cumulative amount of N_2O emitted from soil. Also, differentiate the relation between nitrification rate and N_2O emissions with and without inhibitor. We hypothesized that, lowering the rate of nitrification, and not the total amount of N nitrified, will results in reduced cumulative N_2O emissions from soil. Knowing the role of nitrification rates and total N nitrified has on N_2O emissions may facilitate development of mitigation measures to reduce N_2O emissions.

1.7 Thesis Structure

The structure of this thesis follows that of the "sandwich style". The thesis begins with a general introduction to present the general context and rationale of the study (Chapter 1). The results of a ^{15}N label microcosm study is given in Chapter 2 to address the objective of the thesis. The thesis concludes with a general discussion of the contribution to knowledge of completed study with recommendations for future work. The research chapter of the thesis has been prepared and formatted for submission to the Canadian Journal of Soil Science. I designed the experiment

with the guidance of Dr. Mario Tenuta. I was involved in setting up the experiment, monitoring, sampling, laboratory analyses, data processing and statistical analysis. I am the principal author of this manuscript based on this work.

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2. EFFECT OF LOWERING THE RATE OF NITRIFICATION USING NITRAPYRIN (N- SERVE) ON CUMULATIVE N_2O: A Microcosm ^{15}N Label Study

2.1 Abstract

Nitrification is the biological oxidation of NH_4^+ to NO_2^- under aerobic conditions and is a major source of N_2O in NH_4^+ fertilized soils. However, the relation between rates of nitrification and N_2O emissions, and between cumulative nitrification and N_2O emissions is not understood. A 41-day ^{15}N pool dilution incubation was done to examine the above relations. Microcosms of a fine sandy loam soil were amended with unlabelled ammonium sulfate with 0 and 25 mg N kg^{-1} set to 70% water-filled pore space (WFPS) to maximize N_2O emission from nitrification. Soils were incubated with and without nitrification inhibitor, N-Serve (nitrapyrin) at 10µL kg^{-1}. Enrichment of soil inorganic N was achieved by addition of $(^{15}NH_4)_2SO_4$ plus KNO_3 or $(NH_4)_2SO_4$ plus $K^{15}NO_3$ at 50 mg N kg^{-1}. Enrichment of the inorganic N pools and that of N_2O gas were determined by isotope ratio mass spectrometry (IRMS). N-Serve addition delayed complete oxidation of added NH_4^+ by two weeks, with the NH_4^+ decay rate being 0.05 and 0.161 mg N kg^{-1} d^{-1} with and without N-Serve, respectively. Apparent nitrification rate, the accumulation of NO_3^- in soil, was also slower with (0.77 mg N kg^{-1} d^{-1}) than without (2.08 mg N kg^{-1} d^{-1}) N-Serve addition over the first 17 days of the study. Nitrous oxide emission rate over the first 17-day period was also lower with than without N-Serve, being 0.02 and 0.05 mg N kg^{-1} d^{-1}, respectively. Nitrification-inefficiency, an estimate of the amount of N_2O emitted per amount of N nitrified, over the first 17 days and the whole study with N-Serve addition was 0.02 and 0.06 mg N_2O-N mg^{-1} N d^{-1}, respectively, and was not different without N-Serve addition, being 0.03 and 0.08 mg N_2O-N mg^{-1} N d^{-1}, respectively. Nitrous oxide emission increased with that for apparent nitrification in the absence of the inhibitor, but there was no relation in its presence. About the

same amount of cumulative N_2O was emitted for the same amount of N apparently nitrified, regardless of N-Serve addition. Nitrous oxide emission was partitioned to nitrification and denitrification based on the ^{15}N labels added to soil. Nitrification was the dominant source of N_2O contributing to more than 75% of the emissions, regardless of N-Serve addition. There was no relation between N_2O emission attributed to nitrification and gross nitrification rate, with and without inhibitor. About the same amount of cumulative N_2O was emitted for the same amount of gross nitrified N, regardless of N-Serve addition. These results imply that the amount of added N nitrified dictates the eventual cumulative N_2O emitted, regardless of the rate of nitrification on its own.

2.2 Introduction

Nitrogen use in agricultural soils is associated with some unintended consequences to the environment and these include nitrous oxide (N_2O) emissions to the atmosphere and nitrate (NO_3^-) leaching to subsurface waters (Van Cleemput and Samater 1995; De Antoni Migliorati et al. 2014; Lebender et al. 2014). The increase in the world's population and demand for food is resulting in the increased use of nitrogen (N) inputs in agroecosystems and this is causing more N_2O emissions from agricultural soils (Liu et al. 2014; Simpson et al. 2014). There is a need to fully understand the mechanisms and the internal N cycling to come up with strategies to reduce N_2O emissions from agricultural soils (Murphy et al. 2003; Wan et al. 2009). The major microbial processes of N_2O formation in soil (nitrification and denitrification) have received much attention in the past decades (Kulkarni et al. 2014); however, there is little information on the role of nitrification rates to N_2O emissions.

Nitrification is an aerobic biological process that involves the oxidation of ammonia/ammonium (NH_3/NH_4^+) to NO_3^- (Saggar et al. 2004; Bateman and Baggs 2005; Hu et al. 2014. This process occurs in two steps in which NH_4^+ or ammonia (NH_3) is oxidized first to nitrite (NO_2^-) via an intermediate called hydroxylamine (NH_2OH) and this reaction is affected by chemoautotrophic bacteria such as *Nitrosomonas* (Davidson et al. 2014; Lebender et al. 2014; Scheer et al. 2014). The second step oxidizes NO_2^- to NO_3^- and it is mediated by other chemoautotrophic bacteria such as *Nitrobacter* (Ahn et al. 2011). Besides producing NO_3^- that is prone to leaching and denitrification losses, nitrification also produces N_2O, a greenhouse gas that is partly involved in the increase in global warming (Deng et al. 2013). In agricultural soils, generally there are very low concentrations of NH_4^+ because nitrification occurs quickly after application of NH_4^+ producing or based fertilizers (Ambus 2005; Wan et al. 2009). In some soils, added NH_4^+ decreases and NO_3^- appears in a span of one to several weeks after application (Uchida et al. 2013; Zaman et al. 2012), depending on soil temperature and moisture. This increase in nitrifier activity following addition of NH_4^+ producing or based fertilizer is often associated with peak N_2O emissions within a crop year (Tenuta et al. 2010; Gao et al. 2013).

Nitrification and denitrification are biological processes that occur simultaneously in the soil and are both can produce N_2O (Stevens et al. 1997; Murphy et al. 2003; Mathieu et al. 2006; Kool et al. 2011). Techniques with ^{15}N labelling have been used to separate the contribution of nitrification and denitrification to N_2O emission (Wrage et al. 2004; Yang et al. 2011; Kulkarni et al. 2014). Stevens et al. (1997) found that, whatever the moisture condition, after two days of incubation, nitrification was the dominant source of N_2O emission in NH_4^+ fertilized soil. However, Mathieu et al. (2006) found denitrification was the dominant source of N_2O in a saturated soil

amended with added NH_4^+ fertilizer. Denitrification seems to be the dominant source of N_2O at >70% water-filled pore space (WFPS) (Khalil and Baggs 2005).

Nitrification rates are affected by temperature, microbial population, pH, oxygen concentration, and presence of inhibiting compounds (De Antoni Migliorati et al. 2014; Ledgard et al. 2014; Masaka et al. 2014). In general, accumulation of NO_2^- during nitrification is temporary and in very low concentrations (Akunna et al. 1993; Morot-Gaudry-Talarmain et al. 2002; Fukumoto and Inubushi 2009; Ma et al. 2015). Accumulation of NO_2^- following addition of NH_4^+ based or producing synthetic fertilizers and organic materials usually occurs in soils of neutral to alkaline pH (Maharjan and Venterea 2013). Rapid nitrification was found to result in accumulation of NO_2^- around urea granules in a soil of pH > 7.0 (Carmona et al. 1990). It seems that NO_2^- accumulation is the result of slower activity of the second step of nitrification, compared to the first because of high pH, high concentrations of NH_3/NH_4^+ and NO_2^- (Shen et al. 2013). When NO_2^- accumulates in soil and then oxygen becomes limiting, nitrifiers use NO_2^- as an alternate electron acceptor, reducing it to N_2O and N_2 in a process called nitrifier-denitrification (Bollmann and Conrad 1998; Wrage et al. 2001; Zhu et al. 2013). Nitrifier-denitrification is likely responsible for majority of N_2O emissions in soils receiving NH_4^+ based or producing synthetic fertilizers (Wrage et al. 2001).

Nitrification inhibitors are used to slow the activity of autotrophic bacteria responsible for the oxidation of NH_3/NH_4^+ to NO_2^- (Zaman et al. 2009; Wang et al. 2014). In slowing the first step of nitrification, added fertilizer N remains in the form of NH_4^+ which is subject to less leaching and denitrification losses than NO_3^- (Lan et al. 2013). Nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine) is the common name for the active ingredient in nitrogen stabilizers such as N-Serve and ENtrench. Nitrapyrin is formulated as an emulsifiable concentrate in N-Serve and as a capsule

suspension in ENtrench. N-Serve is a commercially available and commonly used nitrification inhibitor in North America. It can be applied to soil with urea, anhydrous ammonia, manure and other ammoniacal fertilizers (Migliorati et al. 2014). Other nitrification inhibitors include dicyandiamide (DCD), 3, 4-Dimethylpyrazole phosphate (DMPP), allylthiourea (ATU) and guanylthiourea (GTU) (Ruser and Schulz 2015). Nitrification inhibitors can increase crop yields by reducing N loses from leaching and denitrification prior to plant N demand (Scheer et al. 2014; Gilsanz et al. 2016). Since nitrification inhibitors reduce the rate of nitrification, they also can reduce rate of N_2O emissions from denitrification through less NO_3^- available for the denitrifiers (Cui et al. 2013). However, little work has been done to explore the relationship between nitrification rates and cumulative N_2O emissions as affected by nitrification inhibitors.

This present study was done to investigate the relationship between rates of nitrification and N_2O emission rates, and between cumulative nitrification and cumulative N_2O emissions as affected by nitrification inhibitor (N-Serve). A ^{15}N pool dilution microcosm study was thus done to address the above questions. The soil was treated with either $(^{15}NH_4)_2SO_4$ plus KNO_3 or $(NH_4)_2SO_4$ plus $K^{15}NO_3$ so that fractional contribution of nitrification and denitrification to N_2O emissions can be determined.

2.3 Material and methods

2.3.1 Soil collection

Soil was collected May 2015 from the University of Manitoba Ian. N. Morrison Research Station, Carman, Manitoba. The soil was collected from the 0-10 cm depth in a field previously planted to flax (*Linum usitatitissimum*). The soil is mapped as a Hibsin Chernozem of texture fine sandy loam and well drained with a pH of 6.5. Mean carbon, NH_4^+ and NO_3^- contents were 73.6 mg kg^{-1}, 1.23 mg N kg^{-1} and 4.5 mg N kg^{-1}, respectively. Soil was sampled from four locations

with the soils kept separate to serve as four spatially independent replicates. Each soil sample was partially air-dried for one day until it could be passed through a 4 mm mesh screen to remove stones and plant debris, then stored at 4°C.

2.3.2 Microcosm setup

2.3.2.1 Pre-incubation. Before application of treatments, soil was incubated for three weeks to allow the soil microbial communities to adjust to the incubation conditions and passing of a rewetting flush of N_2O emissions. An amount of 750 g dry weight equivalent soil was placed in 1.5 L wide-mouth sealer jars and the unit tapped to settle soil and bringing the bulky density to about 1.1 Mg m^{-3}. The pre-incubation WFPS, which is the volume of soil pores occupied by water, was set at 65% after adjusting for water (20 mL) which was to be added as N solution. The 20 mL adjusted water corresponded to 5% WFPS to attain 70% WFPS moisture content after N solution application. Water was added slowly to reach 65% WFPS moisture content using a bottle auto-dispenser. Before this, a preliminary experiment was done that established %WFPS with rapid N_2O emission (Appendix Figure 3). Literature indicates WFPS affects nitrification and denitrification N_2O emissions. These studies indicate nitrification as a dominant source of N_2O around 60-70% WFPS. In our preliminary experiment, nitrous oxide emission was most rapid at 70% WFPS which conforms to nitrification as an expected source. Thus, in the current study, 70% WFPS was chosen so as to maximize N_2O emission from nitrification.

The bulk density and %WFPS were determined in order determine the amount of water to add to reach the pre-incubation WFPS of 65% using the following equation:

65% WFPS = Volumetric water content/ Soil porosity x 100 x 0.65. (1)

In order to reduce loss of water by evaporation, all jars were covered with Parafilm (American National Can, Chicago, IL). The Parafilm was punctured twice with a pencil to assist with gas

exchange while minimizing moisture loss. The jars were arranged randomly in an incubator (Lab-Line Instruments, Chicago, IL) set at 20°C. A set of jars were weighed soon after the set up and reweighed once a week with weight lost replaced with amounts of RO water. A water reservoir was placed in the incubator to increase relative humidity and reduce evaporation from jars.

2.3.2.2 Treatment incubation. Pre-incubated soil was treated with ^{15}N as described by Stevens et al. (1997) and Mathieu et al. (2006). The treatments were: (i) soil spiked with ^{15}N-labelled KNO_3 (10% atom percent, Cambridge Isotope Laboratories, MA) and non-labelled reagent grade $(NH_4)_2SO_4$ (Sigma-Aldrich, MO), (ii) soil spiked with ^{15}N-labelled $(NH_4)_2SO_4$ (10% atom percent, Cambridge Isotope Laboratories, MA) and non-labelled reagent grade KNO_3 (EMD Chemicals Inc, NJ) and finally (iii) soil without added N. Ammonium sulfate and KNO_3 were both applied at 25 mg N kg^{-1}. The soil was either treated with a commercial grade nitrification inhibitor, N-Serve (Dow Agrosciences, Calgary, AB), or not, and it was applied at a rate of 10 µL kg^{-1} of dry soil. A set of treatment jars were used for gas sampling throughout the whole incubation period whereas other jars destructively sampled over the course of incubation. This amounted to 168 jars prepared in total, with four replicates of six treatments, one set of treatment jars for gas sampling and six sets for destructive sampling for inorganic N extraction.

Both labelled and unlabeled N sources were dissolved in 20 mL of deionized water just prior to application. This amount was adjusted from the total amount of water added in each jar to set WFPS to 70%. The inhibitor was also added to the N solution using a micro pipette. The solution was injected multiple times to distribute the N equally throughout the soil. Each injection was applied at a depth of 3 cm and approximately 2 mL of solution was released at each injection, similar to the procedure used by Murphy et al. (2003).

2.3.3 Gas and soil sampling

On each day of emission measurements, two sets of gas samples from microcosms were taken, one for N_2O analysis by gas chromatography and the other for $^{15}N_2O$ by IRMS (isotope ratio mass spectrometry). For the first set, gas samples were collected at two or three day intervals throughout the incubation period. Before closing the jar for gas sampling, the headspace of the jar was flushed with ambient laboratory atmosphere by inserting an aluminum can (355 mL) repeatedly fifteen times into the headspace of jars (Tenuta and Sparling 2011). Then the jars were sealed with screw seal jar tops fitted with a rubber septum that allowed gas sampling. Samples were taken 0 and 15 minutes after jar closure. Another preliminary experiment established suitability of the 15-minute elapsed time to determine emission rate (Appendix Figure 1). Gas samples of 10 mL were taken using a 10 mL disposable syringe (Labco, Buckinghamshire, England) fitted with a 23GL x1 (0.6 mm x 25 mm) Precision Glide needle (BD, Franklin Lakes, NJ) and injected into a 6 mL, thrice nitrogen flushed pre-evacuated vial (Exetainer, Labco, High Wycombe, England). A thin layer of silicon (GE Silicone II, Momentive Performance Materials, NC) was smeared on the surface of the septa caps to avoid gas loss from vials during storage. Gas samples for $^{15}N_2O$ analysis were sampled as described above at 0, 5, 11, 17, 20, 26, 36 and 41 days after N application. A 20 mL gas sample was taken using a 20 mL disposable syringe and injected into 12 mL pre-evacuated vial (Exetainer, Labco, High Wycombe, England). All vials were stored at room temperature in the dark before analysis. After gas sampling, the lids of the jars were removed and replaced with punctured Parafilm and returned to the incubator.

Microcosms were destructively sampled for extractable NH_4^+, NO_3^- and NO_2^- at 0, 5, 11, 17, 20, 26 and 41 days after N application. On each day of sampling, soil from the jar was placed into a plastic bag and the soil was mixed using gloved hands. About 5 g of moist soil was placed

into a 50 mL centrifuge tube (Fisher Scientific, Ottawa, ON), 25 mL of KCl solution was added and placed onto a shaker at 150 epm for 30 minutes. The solution was centrifuged at 3000 rpm for 3.5 minutes to obtain a clear supernatant. Supernatant was obtained by a 10 mL macro pipette into a 20 mL scintillation vial (Fisher Scientific, Ottawa, ON) and was stored at room temperature until analysis.

2.3.4 N_2O analysis

Concentration of N_2O in vials were determined using a gas chromatograph (Varian 3800, Mississauga, ON) fitted with an electron capture detector operated at 300 °C. A Combi-PAL auto sampler (CTC Analytics, Zwingen, Switzerland) injected 2.5 mL of vial gas into the gas chromatograph. Gases for calibration of the instrument were prepared by dilution of pure N_2O gas (Welders Suppliers, Winnipeg, MB). A vial of known concentration of N_2O from a reference standard tank (Welders Suppliers, Winnipeg, MB) was included after every 10 samples within a sample set run. When the concentrations of the reference vials were off 5% or more from the expected concentration, conditioning of the chromatograph columns or calibration was done, then the sample set analysis was repeated.

2.3.5 $^{15}N_2O$ analysis

Vials for $^{15}N_2O$ analysis were sent to University of California Davis Stable Isotope Facility. The analysis was done on an elemental analyzer (Sercon, Cheshire, UK) coupled in continuous flow mode to a stable isotope ratio mass spectrometer (IRMS) (PDZ Europa 20-20, Bremen, German). Nitrous oxide was trapped and concentrated in two liquid nitrogen cryo-traps such that the N_2O was held until the non-condensing portion of the sample gas was replaced by helium carrier, then passed to the second trap. The second trap was then warmed to ambient and N_2O was carried by helium to the IRMS via a Poroplot Q GC column (25m x 0.53mm, 25 °C, 1.8 mL/m) to

separate N_2O from residual carbon dioxide. Two reference materials were analyzed after every 10 samples. The reference materials were mixtures of N_2 and N_2O, for example 3% N_2 plus 1 ppm N_2O. The N_2 was calibrated against an Oztech N_2 standard (Oztech Trading Co). For N_2O calibration, N_2O was thermally decomposed at 800 °C to N_2 plus O_2 (oxygen). The N_2 and O_2 were then calibrated using the Oztech N_2 standard and Oztech O_2 standard, respectively. A reference N_2O peak was used to calculate isotope ratios of the sample N_2O peak. The final correct δ-^{15}N values were calculated by adjusting the provisional values for instrumental drift and changes in linearity.

2.3.6 Soil N extract determinations

During destructive sampling, soil from each jar was mixed by hand and then extracted with 2 M KCl and analyzed colorimetrically within 3 days for NH_4^+ using the Berthelot reaction, NO_2^- by azo dye formation from reaction with sulfanilamide and N-naphthylethylene-diamine dihydochloride, and NO_3^- by reduction to NO_2^- using Cu–Cd before azo dye formation using a Technicon Auto-analyzer II system (Technicon Instruments Corporation, Tarrytown, NY). The minimum reportable concentration of NO_2^- was 0.1 mg N kg^{-1} on dry soil basis. The concentration of NO_3^- (mg N kg^{-1} dry soil basis) was estimated as the difference between NO_2^- + NO_3^- (mg N kg^{-1} dry soil basis) from determination with the Cu–Cd reduction step and NO_2^- (mg N kg^{-1} dry soil basis) without the reduction step.

The δ-^{15}N NH_4^+ and NO_3^- in extracts were determined using the diffusion method of Brooks et al. (1989). Briefly, 40 mL of extract solution was added to a 104 mL specimen cup (Fisher Scientific, Ottawa, ON). The bottom of the cup lid was fitted with 5.9 cm of thin stainless steel wire (0.762 mm diameter, Acklands Grainger, Winnipeg, MB) to suspend an acidified filter disk (6 mm diameter, #3 Whatman, GE Healthcare, Buckinghamshire, UK). The acidified disk was

prepared by adding 10 µL 2.5 M $KHSO_4$ onto a 6 mm diameter filter disk using a micro pipette. Alkalization of the KCl extract was done by adding 0.2 g of magnesium oxide (Sigma-Aldrich, MO) using a plastic weighing boat (VWR, International, ON) to volatilize NH_4^+ and trapping it on the acidified disk. The cup was quickly capped and contents of the specimen cup were mixed carefully avoiding splashing the KCl on the filter disk. The cups were left at room temperature for six days before retrieval of the disks. Following trapping of NH_3 for δ-^{15}N NH_4^+ analysis, a new acidified disk was suspended over the extract solution and 0.4 g of Devarda's alloy (Sigma-Aldrich, MO) was added to reduce NO_3^- to NH_4^+ and trap volatilized NH_3 for determination of δ-^{15}N NO_3^- in the extract. The specimen cup was capped tightly, contents were mixed carefully, avoiding splashing the KCl on the filter disk. The specimen cups were left to stand closed for six days before retrieval of the disks.

Disks from the cups were dried overnight in a desiccator having an open container of liquid H_2SO_4. Each disk was placed in a tin capsule (9 x 10 mm, Costech Analytical Technologies, CA) and crimped into a tight ball using tweezers. Crimped capsules were kept in a desiccator with H_2SO_4 until shipped, and during storage at the University of California Davis Isotope Facility prior to analysis for δ-^{15}N-NH_4^+ and NO_3^-. To determine the δ-^{15}N NH_4^+ and NO_3^-, samples were first combusted at 1000 °C in a reactor with chromium oxide and silver copper oxide. After combustion, oxides were removed in a reduction reactor and helium carrier was then streamed through a water trap and an optional CO_2 trap. Di-nitrogen gas and CO_2 were separated on a Carbosieve GC column (65 °C, 65 mL/min) before entering the IRMS. Analysis was then done using a total N elemental analyzer (PDZ Europa ANCA-GSL, Sercon Ltd, Cheshire, UK) for determination of total N content with N_2 gas passed to a continuous flow stable isotope ratio mass spectrometer (IRMS) (PDZ Europa 20-20, Bremen, German) for δ-^{15}N-N_2 determinations.

2.3.7 Calculations

Daily emission (mg N_2O-N kg^{-1} d^{-1}) was calculated using the Ideal Gas Law (PV=nRT) from the increase of N_2O over the 15 minute collection period, volume of jar headspace, molecular mass of N in N_2O, incubation temperature and correction for N_2O dissolved in soil water (Moraghan and Buresh 1977). Cumulative emissions (mg N_2O N kg^{-1}) for both N treated and non-N treated treatments were calculated by summing of daily estimates of N_2O emissions obtained by linear interpolation between sampling dates over the whole incubation period. Nitrous oxide emission rate (mg N_2O-N kg^{-1} d^{-1}) for the added N was calculated by simple linear regression between cumulative emissions and day of incubation after subtracting the level of control (Table 2.1). Nitrification-inefficiency (mg N_2O-N mg^{-1} N), which is the amount of N_2O produced per amount of nitrified N was calculated as cumulative emission (mg N_2O-N kg^{-1}) from N treated treatments obtained after subtracting the control divided by NO_3^- recovered (mg N kg^{-1}) (Table 2.1).

The fractional contribution of nitrification (*n*) and denitrification (*d*) to N_2O emission over time was estimated using the model by Stevens et al. (1997). This model uses δ-^{15}N N_2O, NH_4^+ and NO_3^- from the ^{15}N-labelled NO_3^- treatment (Table 2.1). This model makes use of two emitting sources: denitrification (*d*) and nitrification (1-*d*) and this was calculated as:

$$d = \frac{am - an}{ad - an} \quad \text{(with } ad \neq an\text{)} \qquad (2)$$

where *am, ad* and *an* are the average δ-^{15}N of the N_2O, NO_3^- and NH_4^+ over time in the ^{15}N-labelled NO_3^- treatment. The $^{15}N_2O$ in the ^{15}N-labelled NH_4^+ treatment was calculated using the equations below:

$$^{45}N_2O = {}^{45}R/(1 + {}^{45}R + {}^{46}R) \qquad (3)$$

$^{46}N_2O = {}^{46}R/(1 + {}^{45}R + {}^{46}R)$ (4)

$^{15}N_2O = (({}^{45}N_2O+2*{}^{46}N_2O)/2)$ (5), where ^{45}R and ^{46}R are measured isotope ratios of N_2O with one or two ^{15}N atoms per molecule (45/44 and 46/44), respectively. Emissions due to nitrification and denitrification (mg N_2O N kg^{-1}) were calculated by multiplying the fractional contribution of nitrification (*n*) and denitrification (*d*), respectively and $^{15}N_2O$ from ^{15}N-labelled NH_4^+ treatment (Table 2.1).

Nitrate recovered from the added N (mg N kg^{-1}) was calculated by subtracting the NO_3^- level of the control from NO_3^- recovered at the end of the incubation period in the N treated soil. Apparent nitrification rate from the added N (mg NO_3^--N kg^{-1} d^{-1}), which is the accumulation of NO_3^- in soil was estimated by simple linear regression of time versus daily NO_2^-+ NO_3^- after subtracting levels of control (Table 2.1). Gross nitrification rate, which is the accumulation of NO_3^- in soil irrespective of N consumption, was calculated as by Hart et al. (1994) and Lan et al. (2013). It was calculated using the rate at which δ-^{15}N NO_3^- in the ^{15}N-NO_3^- labelled treatment declined over time and the changes in the size of the NO_3^- pool using the model below:

$$n = \frac{P0-Pt}{t} \times \frac{\log\left(\frac{P0}{Pt}\right)}{\log\left(\frac{N0}{Nt}\right)} \quad (6)$$, where *P0* is NO_3^- pool size at Day 0, *N0* is δ-^{15}N in NO_3^- pool at Day 0 above the background , *t* is time in days and *n* is the gross rate of nitrification. Nitrogen transformation rates were estimated using the ^{15}N isotope pool dilution method based on the model by (Kirkham and Bartholomew 1954). The assumptions of this method are: (i) fractionation effect is not significant (ii) uniform distribution of ^{15}N throughout the soil (iii) no recycling or remineralization of immobilized organic N, and (iv) volatilization is assumed to negligible.

Table 2.1. A summary of measures and estimates and their calculations.

Variable	Measurement	Calculation
Daily Emission rate (mg N_2O N kg^{-1} d^{-1})	GC determination of N_2O accumulation in microcosms	Slope of linear regression model of N_2O accumulation in microcosms on day of sampling.
Cumulative Emission (mg N_2O N kg^{-1})	GC determination of N_2O accumulation in microcosms	Interpolation of daily emissions after removing the control
N_2O Emission Rate (mg N_2O N kg^{-1} d^{-1})	GC determination of N_2O accumulation in microcosms	Slope of linear regression model of cumulative emission after subtracting the control.
Fractional Contribution of Denitrification (*d*) to N_2O Emission	IRMS determination of δ-^{15}N-N_2O in microcosms	$d = \frac{am - an}{ad - an}$,where am, ad and an are the average δ-^{15}N of N_2O, NO_3^- NH_4^+ over time, respectively.
Fractional Contribution of Nitrification (*n*) to N_2O Emission	IRMS determination of δ-^{15}N-N_2O in microcosms	1-*d*, where *d* is the fractional contribution of denitrification
Emissions due to Nitrification (mg N_2O N kg^{-1})	IRMS determination of δ-^{15}N-N_2O in microcosms	Fraction of N_2O flux from nitrification multiply by daily $^{15}N_2O$ fluxes from ^{15}N-labelled NH_4^+ treatment less the background from the control.
Emissions due to Denitrification (mg N_2O N kg^{-1})	IRMS determination of δ-^{15}N-N_2O in microcosms	Fraction of N_2O flux from denitrification multiply by daily $^{15}N_2O$ fluxes from ^{15}N-labelled NH_4^+ treatment less the background from the control.

NH_4^+ Decay Rate (mg NH_4^+ N kg^{-1} d^{-1})	Extraction and colorimetric analysis of NH_4^+ in soil microcosms	N (t) = N_0e^{-kt}, where N (t) is the NH_4^+ concentration at time t, N_0 is NH_4^+ concentration at Day 0, k is the decay rate and t is time in days
NO_3^- Recovered (mg N kg^{-1})	Extraction and colorimetric analysis of NO_2^-+NO_3^- in soil microcosms	NO_3^- recovered at end of study less level of control
Apparent Nitrification Rate (mg NO_3^--N kg^{-1} d^{-1})	Extraction and colorimetric analysis of NO_2^-+NO_3^- in soil microcosms	Slope of linear regression model of time and daily NO_2^-+ NO_3^- less level of control
Gross Nitrification Rate (mg N kg^{-1} d^{-1})	Extraction and colorimetric analysis of NO_2^-+NO_3^- and IRMS determination of δ-^{15}N-NO_3^- in microcosms	$n = \frac{P0-Pt}{t} \times \frac{\log(\frac{P0}{Pt})}{\log(\frac{N0}{Nt})}$, where $P0$ is NO_3^- pool size at Day 0, $N0$ is δ-^{15}N in NO_3^- pool at Day 0 above the background, t is time in days and n is the gross rate of nitrification
Nitrification-Inefficiency (mg N_2O-N mg^{-1} N)	Extraction and colorimetric analysis of NO_2^-+NO_3^- and GC determination of N_2O accumulation in microcosms	Cumulative emission (mg N_2O-N kg^{-1}) divided by NO_3^- Recovered (mg N kg^{-1}) after subtracting the control

2.3.8 Statistical analysis

The relation between cumulative nitrification and cumulative emission was determined using PROC GLIMMIX function of the Statistical Analysis Software computer program (SAS version 9.4, SAS Institute, Cary, NC, 2015). There was no need to transform the data prior to analysis since PROC GLIMMIX analyzes data that are not normally distributed by specifying the distribution to which data conforms. In this case normal distribution was used since the N_2O data conformed to the normal distribution. Mean separation was done using the Tukey method at $P<0.05$ with N-Serve as a fixed effect and time as the repeated measure. Since the experiment involved some repeated measurements, a covariant structure that gave the lowest Akaike's Information Criterion (AICc) value was used to describe the results.

Regression analysis was done using replicate data and PROC REG was used to calculate apparent nitrification rate and N_2O emission rates. Regression analysis was also used to determine the relation between rates of nitrification (apparent and gross) and daily N_2O emissions. The NH_4^+ decay constant was calculated by fitting a first order exponential decay model to NH_4^+ concentration: $N(t) = N_0e^{-kt}$, where $N(t)$ is the NH_4^+ concentration at time t, N_0 is NH_4^+ concentration at Day 0, k is the decay rate and t is time in days (Camargo et al. 2002). PROC univariate was used to test the data for normality in case of regression analysis and if data were not normal, they were transformed using the natural logarithm. The slopes of treatments on a replicate basis were compared using a t-test to determine if the slopes were different from each other at $P<0.05$.

2.4. Results

2.4.1 Extractable NH_4^+ and NO_3^- with and without N-Serve.

In the $(^{15}NH_4)_2SO_4$ plus KNO_3 treatment, approximately two hours after spiking the soil with the N solution, the NH_4^+ recovered from the KCl extracts ranged from 70 -77% of the added amount. N-Serve addition delayed complete oxidation of added NH_4^+ by two weeks. There was a greater (P<0.05) decay rate constant in the absence than in the presence of the inhibitor, being 0.161 and 0.059 mg N kg^{-1} d^{-1}, respectively (Figure 2.2 and Table 2.2). Not surprisingly, NH_4^+ concentration of the non-N treated soil remained low regardless of N-Serve addition (Figure 2.1a). As expected because of nitrification, the concentration of NO_3^- increased as NH_4^+ decreased. Nitrate increased at a faster rate without than with N-Serve addition (Figure 2.3a). There was a slight increase in NO_3^- concentration from Day 17 to the end of the incubation period in non-N treated soil regardless of N-Serve addition (Figure 2.3a). A numerically smaller (not significant) amount of added N was recovered as NO_3^- over the whole incubation period in the presence than absence of inhibitor, being 12.1 and 30.3 mg N kg^{-1}, respectively (Table 2.2). Nitrite concentrations were below the detection limit of 0.1 mg N L^{-1} throughout the incubation period.

The δ-^{15}N NH_4^+ in the $(^{15}NH_4)_2SO_4$ plus KNO_3 treatment followed the same trend as the unlabelled NH_4^+ (Figure 2.1b). N-Serve addition significantly (P<0.05) reduced complete oxidation of ^{15}N-NH_4^+. However, δ-^{15}N NH_4^+ values in non-N treated soil remained at natural abundance regardless of N-Serve addition (Figure 2.1b). Not surprisingly, the δ-^{15}N NO_3^- in the $(^{15}NH_4)_2SO_4$ plus KNO_3 treatment increased at a faster rate (P<0.05) without than with N-Serve addition in the first 20 days of incubation (Figure 2.3b). From Day 20 to 41, there was a slight decline in δ-^{15}N NO_3^- regardless of N-Serve addition (Figure 2.3b). Again not surprisingly, δ-^{15}N NO_3^- values in non-N treated soil remained at natural abundance regardless of N-Serve addition

(Figure 2.3b). As expected, δ-^{15}N NO_3^- values in the $K^{15}NO_3$ plus $(NH_4)_2SO_4$ treatment declined at a faster rate (P<0.05) without than with N-Serve addition, an effect of dilution by nitrification of unlabelled NH_4^+ on the labelled NO_3^- pool.

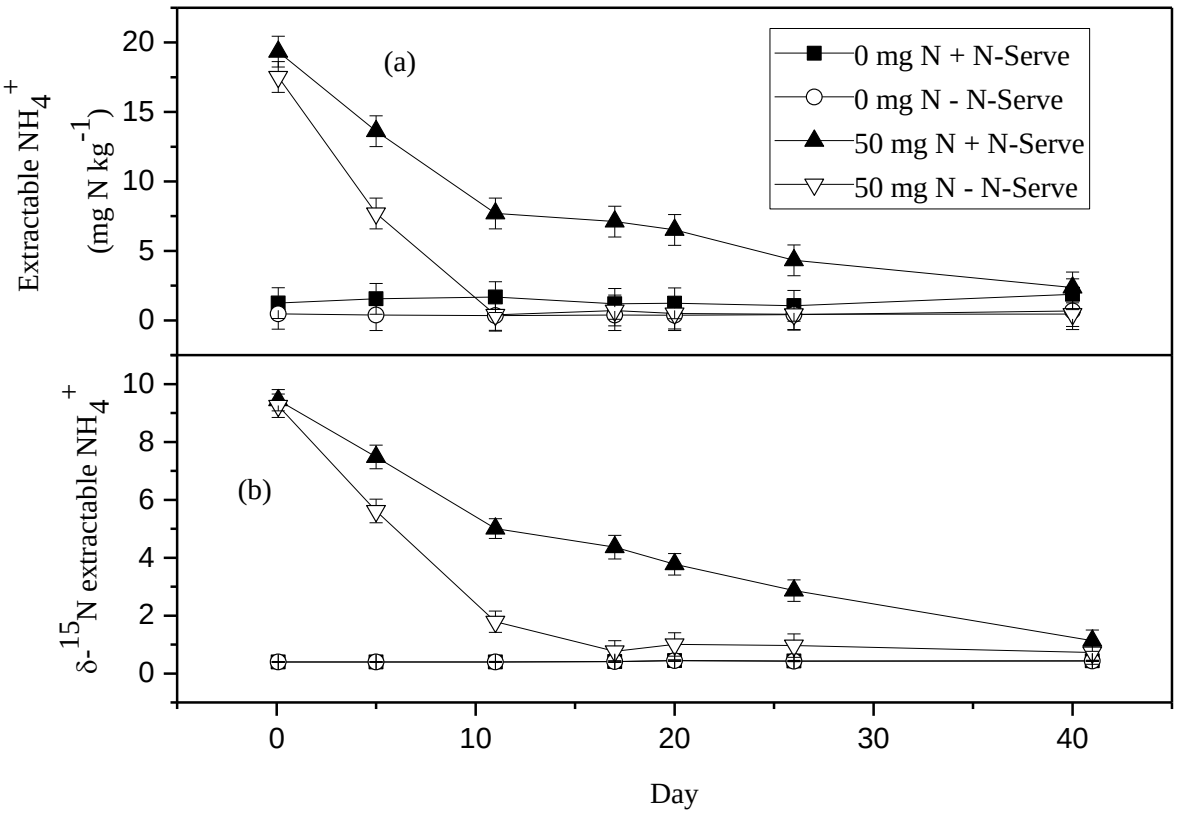

Figure 2.1 Extractable NH_4^+ concentration in soil (a) and δ-^{15}N extractable NH_4^+ (b) for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve. Error bars are ±1 standard error of the mean (n=4) or are smaller than symbols.

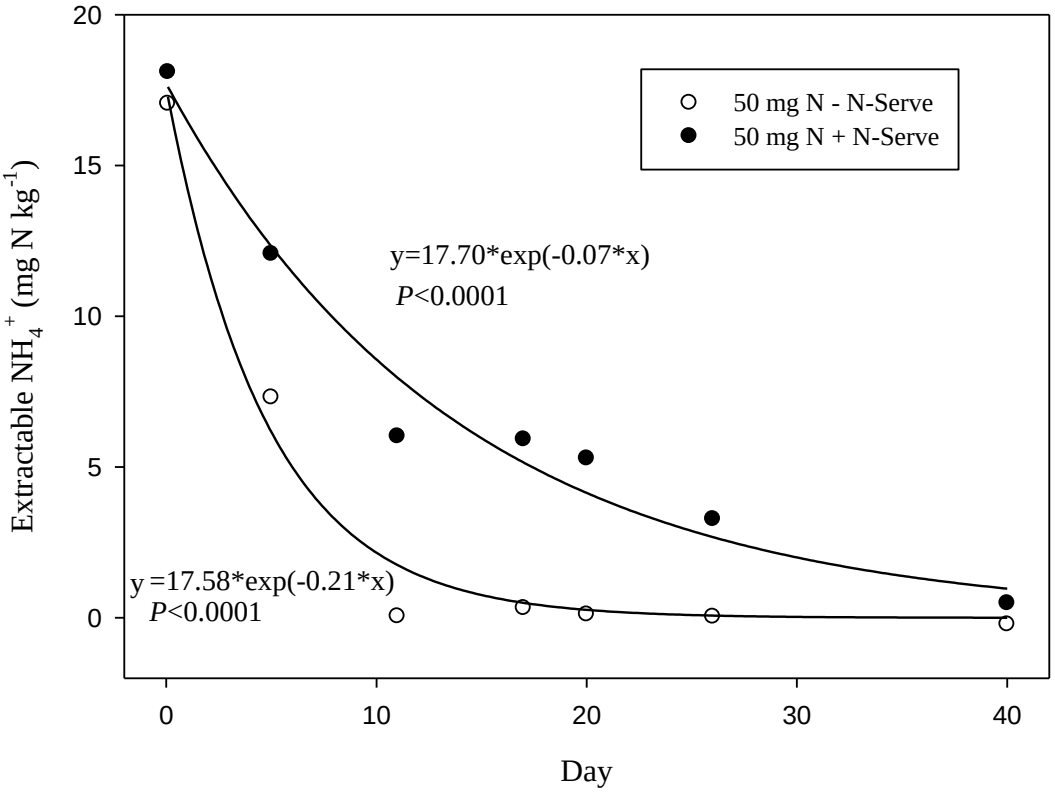

Figure 2.2 Decay in extractable NH_4^+ in soil as described by the first order exponential decay model for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve.

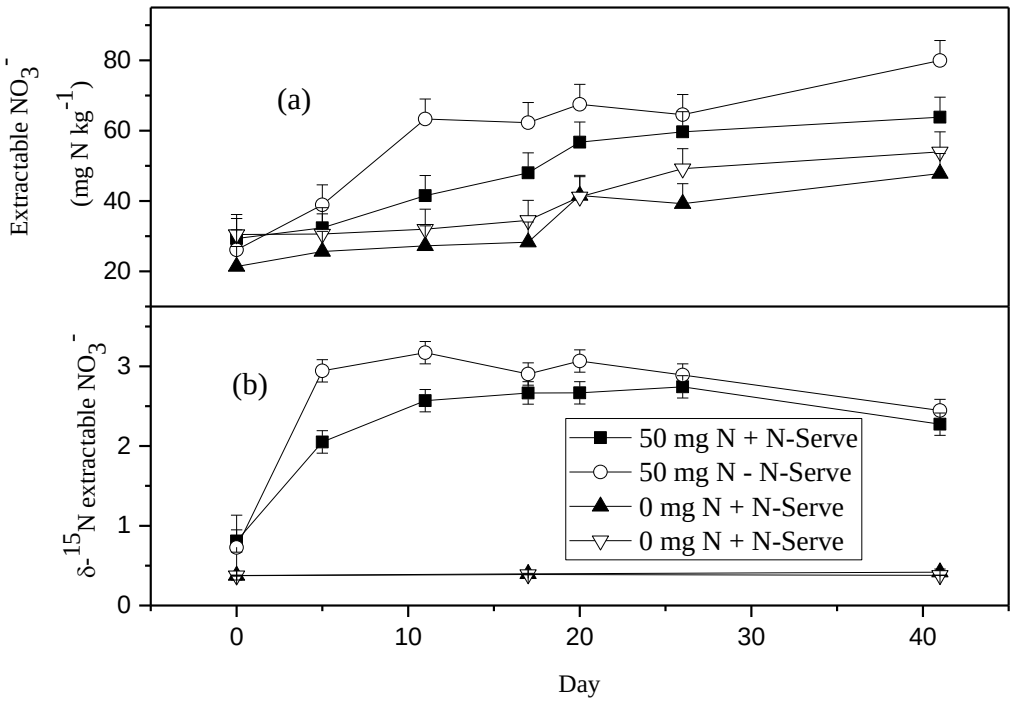

Figure 2.3 Extractable NO_3^- concentration in soil (a) and δ-^{15}N extractable NO_3^- (b) for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve. Error bars are ±1 standard error of the mean (n=4) or are smaller than the symbols.

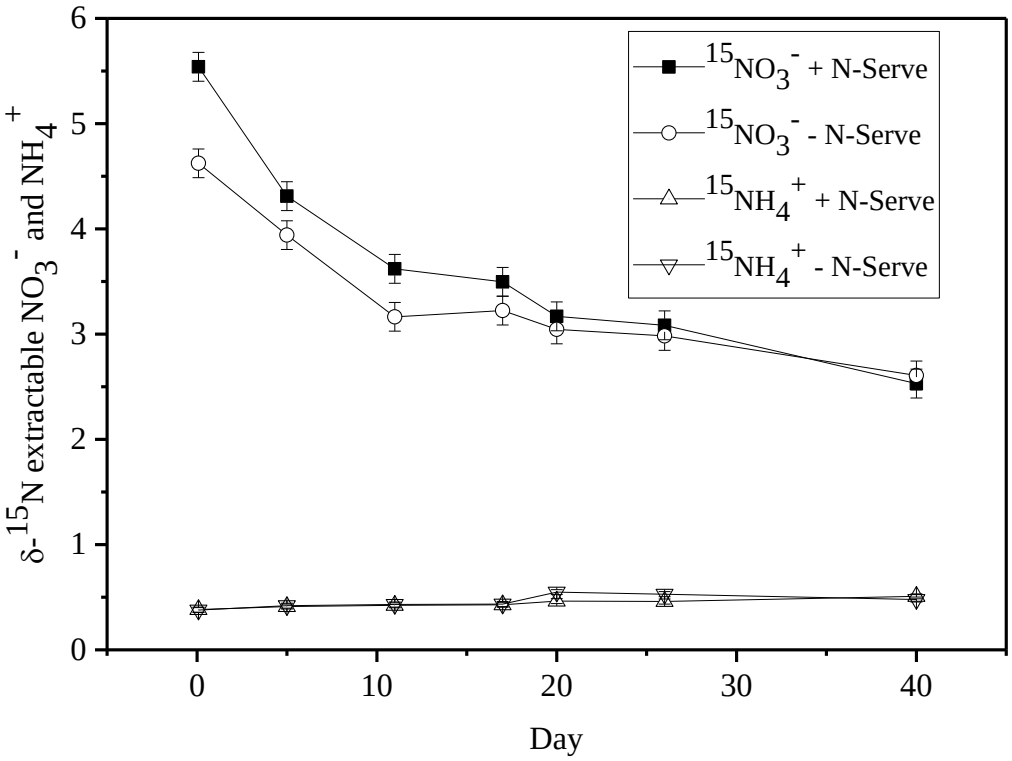

Figure 2.4 Extractable δ-^{15}N NO_3^- and NH_4^+ in soil for microcosms treated with combinations of $K^{15}NO_3$ and N-Serve. Error bars are ±1 standard error of the mean (n=4) or are smaller than the symbols.

2.4.2 Changes in apparent and gross nitrification rates with and without N-Serve.

Apparent nitrification rate over the first 17 days obtained by simple linear regression was greater (P<0.05) with than without N-Serve addition, being 0.77 and 2.08 mg N kg^{-1} d^{-1}, respectively (Figure 2.5). There was a linear relation between apparent nitrification and day with coefficients of determination (r^2) of 0.30 and 0.56 with and without N-Serve, respectively (Figure 2.5). Gross nitrification rates were greater (P<0.05) without than with N-Serve in the first 20 days of incubation (Figure 2.6). From Day 26 to 41, gross nitrification rates without N-Serve declined and rates were similar to those with N-Serve addition (Figure 2.6).

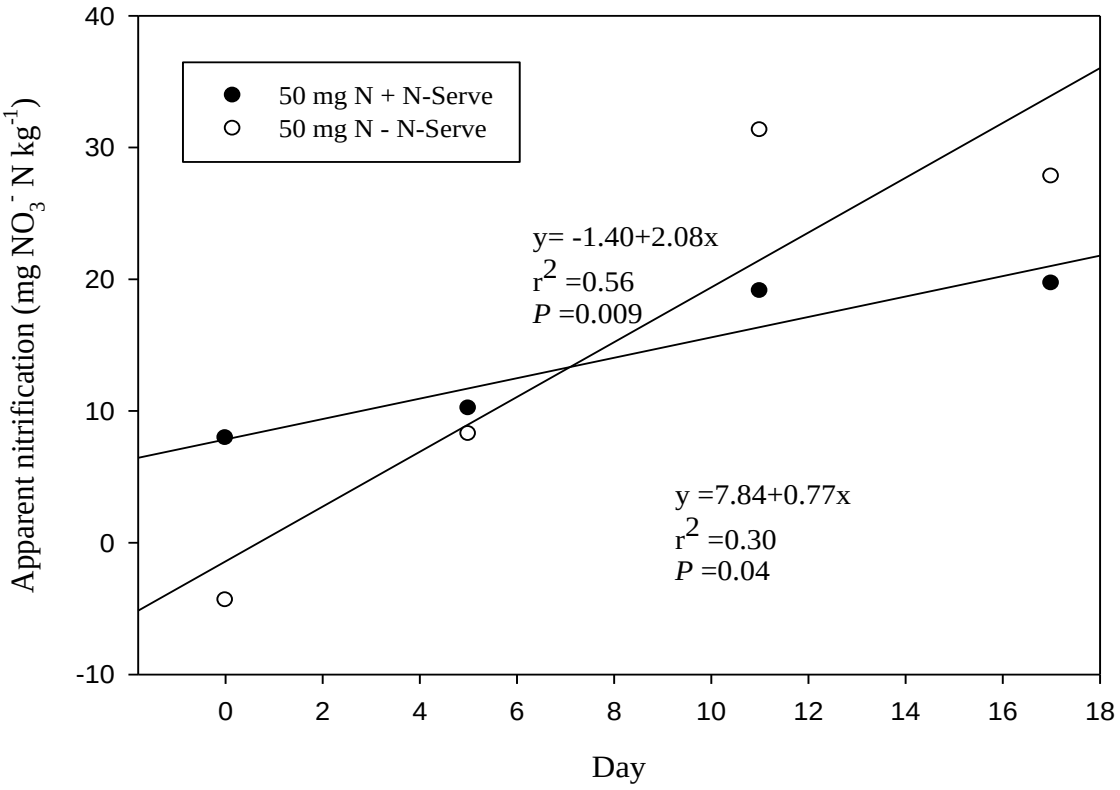

Figure 2.5 Apparent nitrification for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve for the first 17 days of the study.

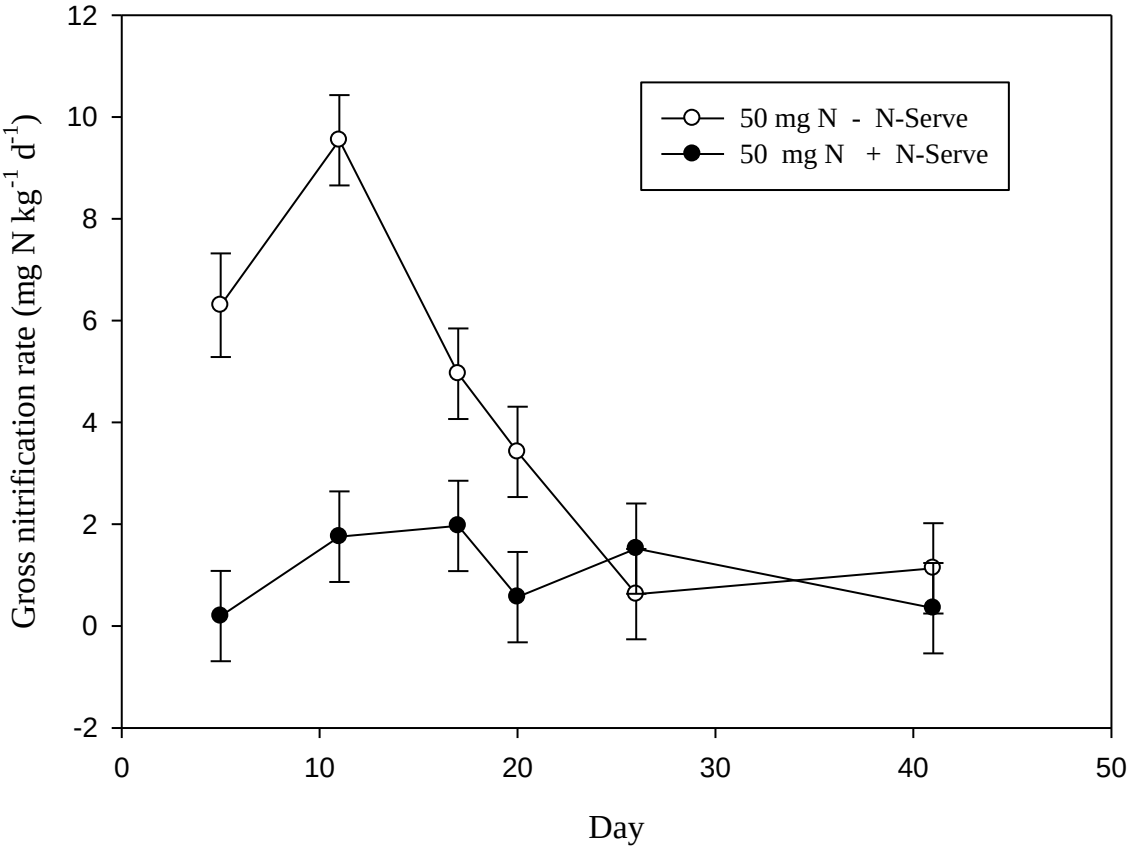

Figure 2.6 Gross nitrification rate for microcosms treated with combinations of $K^{15}NO_3$ and N-Serve. Error bars are ±1 standard error of the mean (n=4).

2.4.3 Nitrous oxide emissions with and without N-Serve.

Cumulative emissions from N treatments increased from Day 0 up to 41 without N-Serve while they increase at a slower rate from Day 0 to 41 with N-Serve addition (Figure 2.7). The total amount of N_2O from N treatments for the whole incubation period was higher ($P<0.05$) in the absence than in the presence of N-Serve, being 1.89 and 0.73 mg N_2O N kg^{-1}, respectively (Table 2.2). As expected, cumulative emissions increased slightly over the study in the non-N treated soils but were not different ($P>0.05$) regardless of N-Serve addition (Figure 2.7).

Nitrous oxide emission rate from the N treatments for the first 17 days as determined by simple linear regression was greater ($P<0.05$) without than with N-Serve addition, being 0.05 and 0.02 mg N_2O N kg^{-1} d^{-1}, respectively (Figure 2.8 and Table 2.2). There was a strong linear association for cumulative emissions and day with coefficients of determination (r^2) of 0.91 and 0.99 with and without N-Serve, respectively.

The δ-^{15}N-N_2O values above the background were determined on gas evolved from the $(^{15}NH_4)_2SO_4$ plus KNO_3 and also $(NH_4)_2SO_4$ plus $K^{15}NO_3$ treatment. For $(^{15}NH_4)_2SO_4$ plus KNO_3 treatment, the δ-^{15}N-N_2O values remained higher without than with N-Serve (Figure 2.9a). For $(NH_4)_2SO_4$ plus $K^{15}NO_3$ treatment, the δ-^{15}N-N_2O values were not different ($P>0.05$) regardless of N-Serve addition. The δ-^{15}N-N_2O values for $(NH_4)_2SO_4$ plus $K^{15}NO_3$ treatment were high at the start and gradually declined to the end of the incubation period (Figure 2.9b).

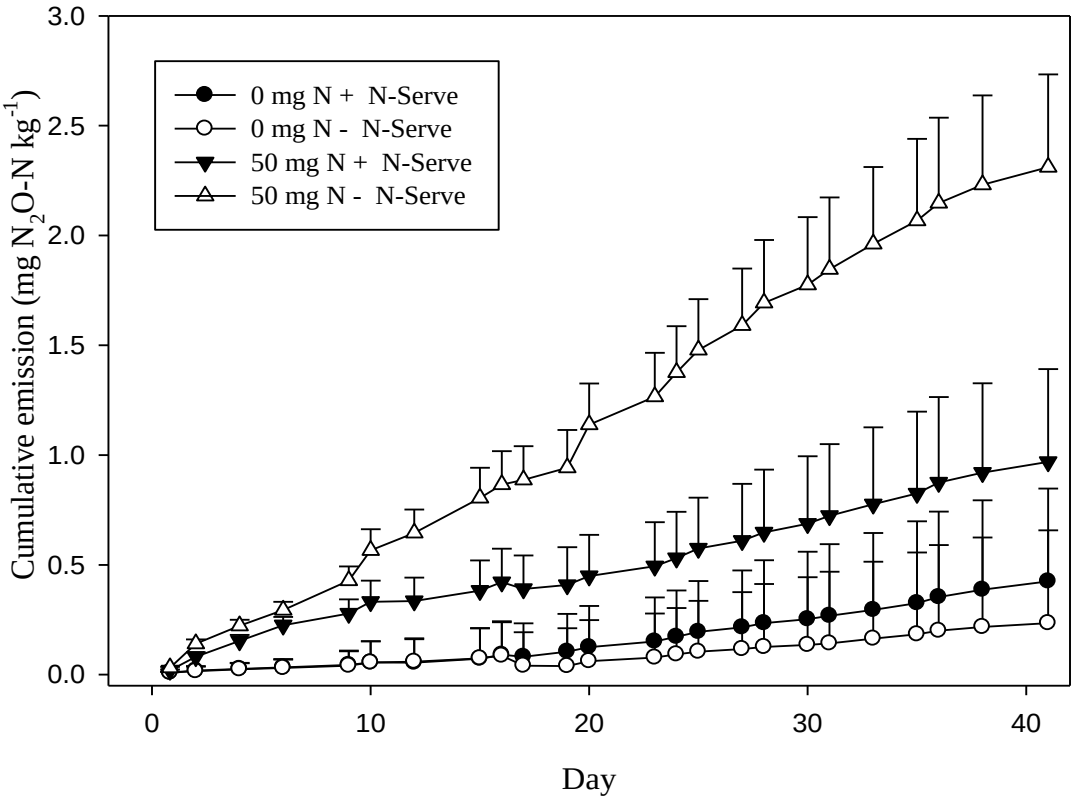

Figure 2.7 Cumulative N_2O emission for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve. Error bars are ±1 standard error of the mean (n=4).

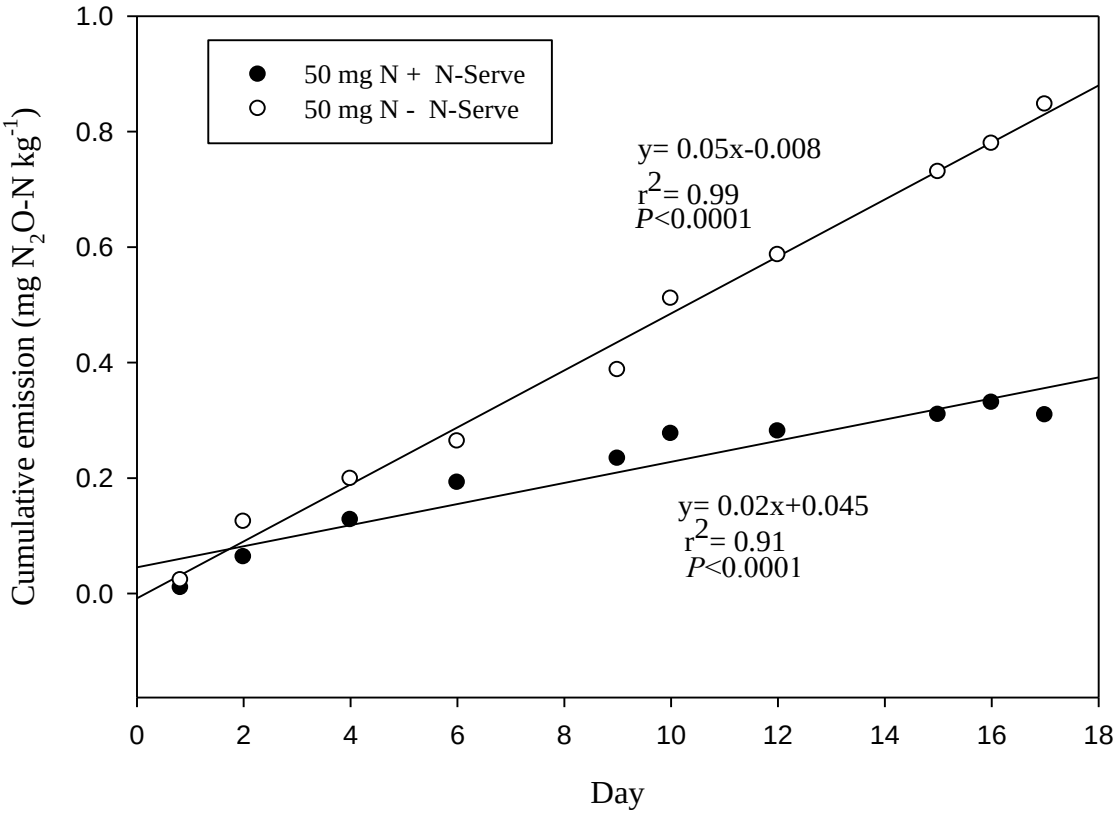

Figure 2.8 Relation of cumulative N_2O emission and day for the first 17 days of incubation for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve.

Table 2.2. Effect of N-Serve on NH_4^+ decay, apparent nitrification rate, NO_3^- recovery, N_2O emission rate, cumulative N_2O emission and nitrification-inefficiency for added N treatments minus the control.

N-Serve addition	NH_4^+ decay constant (mg N kg^{-1} d^{-1})	Apparent nitrification rate (mg N kg^{-1} d^{-1}) for the first 17 days	NO_3^- recovered (mg N kg^{-1}) for the whole period	N_2O Emission rate (mg N kg^{-1} d^{-1}) for the first 17 days	Cumulative emission (mg N_2O-N kg^{-1}) for the whole period	Nitrification-Inefficiency (mg N_2O-N mg^{-1} accumulated NO_3^--N) for the first 17 days	Nitrification-Inefficiency (mg N_2O-N mg^{-1} accumulated NO_3^--N) for the whole period
with	0.059±0.01a†	0.77±0.202a	12.1±8.7a	0.02±0.0092a	0.73±0.153a	0.02±0.006a	0.06±0.019a
without	0.161±0.02b	2.08±0.202b	30.3±8.7a	0.05±0.0039b	1.89±0.468b	0.03±0.014a	0.08±0.019a

†Values shown are mean values of four independent replicates ± 1 standard error of the mean.

a, b Values followed by different letters in a column are significantly different at $P<0.05$.

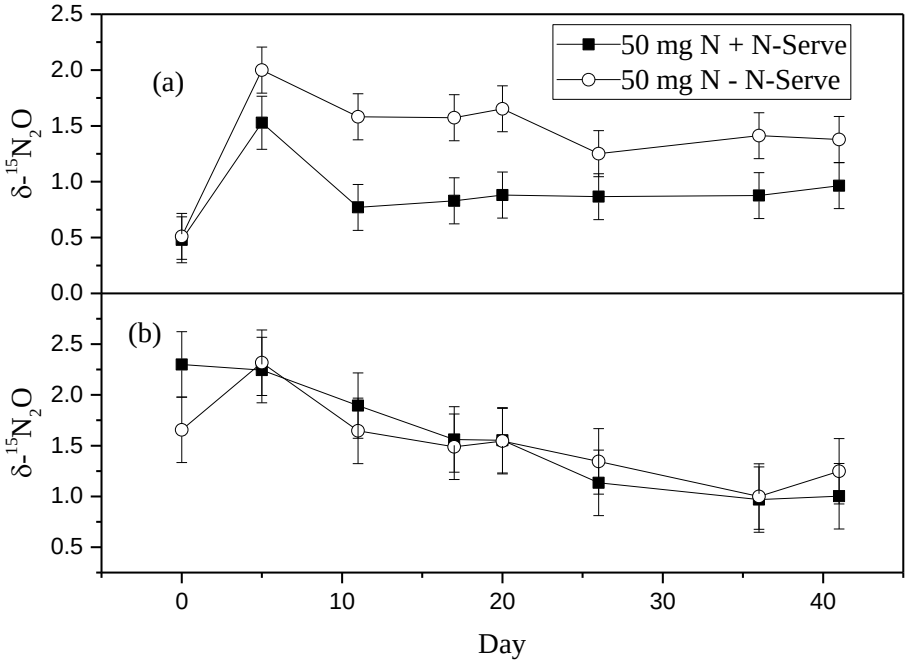

Figure 2.9 The δ-^{15}N-N_2O of evolved gas for microcosms treated with $(^{15}NH_4)_2SO_4$) plus KNO_3 (a) and $(NH_4SO_4)_2$ plus $K^{15}NO_3$ (b) with and without N-Serve. Error bars are ±1 standard error of the mean (n=4).

2.4.4 Contribution of nitrification and denitrification to N_2O flux.

The fractional contribution of denitrification and nitrification to N_2O flux was quantified from the $(NH_4)_2SO_4$ plus $K^{15}NO_3$ treatment (Figure 2.10). Both nitrification and denitrification contributed to N_2O flux but nitrification was the dominant source throughout the incubation period. The fraction of N_2O from nitrification did not differ (P>0.05) with N-Serve additions and it was also the case for denitrification. The fractional contribution of nitrification started lower but higher than denitrification from the first day of incubation regardless of N-Serve addition and it gradually increased to the end of the incubation period (Figure 2.10). Contrarily, the fractional contribution of denitrification started higher but lower than nitrification from the first day of incubation regardless of N-Serve addition and it gradually declined to the end of the incubation period (Figure 2.10).

For the whole incubation period, nitrification was responsible for 79 and 78% of N_2O flux while 21 and 22% came from denitrification with and without N-Serve, respectively. There were higher daily N_2O fluxes due to nitrification in the absence than in the presence of inhibitor (Figure 2.11). A higher (P<0.05) cumulative N_2O was emitted due to nitrification without than with N-Serve (Figure 2.12b). Nitrification-inefficiency over the first 17 days and the whole period was 0.02 and 0.06 mg N_2O-N mg^{-1} N d^{-1}, respectively with N-Serve and 0.03 and 0.08 mg N_2O-N mg^{-1} N d^{-1}, respectively without N-Serve. Nitrification-inefficiency over the 17 day and whole incubation period was no effected by inhibitor addition (P>0.05) probably due high variability among spatially independent individual replicates.

From the progress of cumulative apparent nitrification, it took eight days in the absence of N-Serve and seventeen days in its presence to produce 20 mg NO_3^- N kg^{-1}. Given this same amount of N nitrified, there was similar cumulative N_2O at Day 17 (0.36 mg N_2O-N kg^{-1}) in the presence

of N-Serve as at Day 8 (0.34 mg N_2O-N kg^{-1}) in its absence (Appendix Figure 4). Again, from the progress of cumulative gross nitrification, it took four days without N-Serve and twenty days with N-Serve to produce 5 mg NO_3^- N kg^{-1} (Figure 2.12a). Given the above amount of N nitrified, there was similar cumulative N_2O at Day 20 (0.10 µg N_2O-N kg^{-1}) in the presence of inhibitor as at Day 4 (0.085 µg N_2O-N kg^{-1}) in its absence (Figure 2.12b).

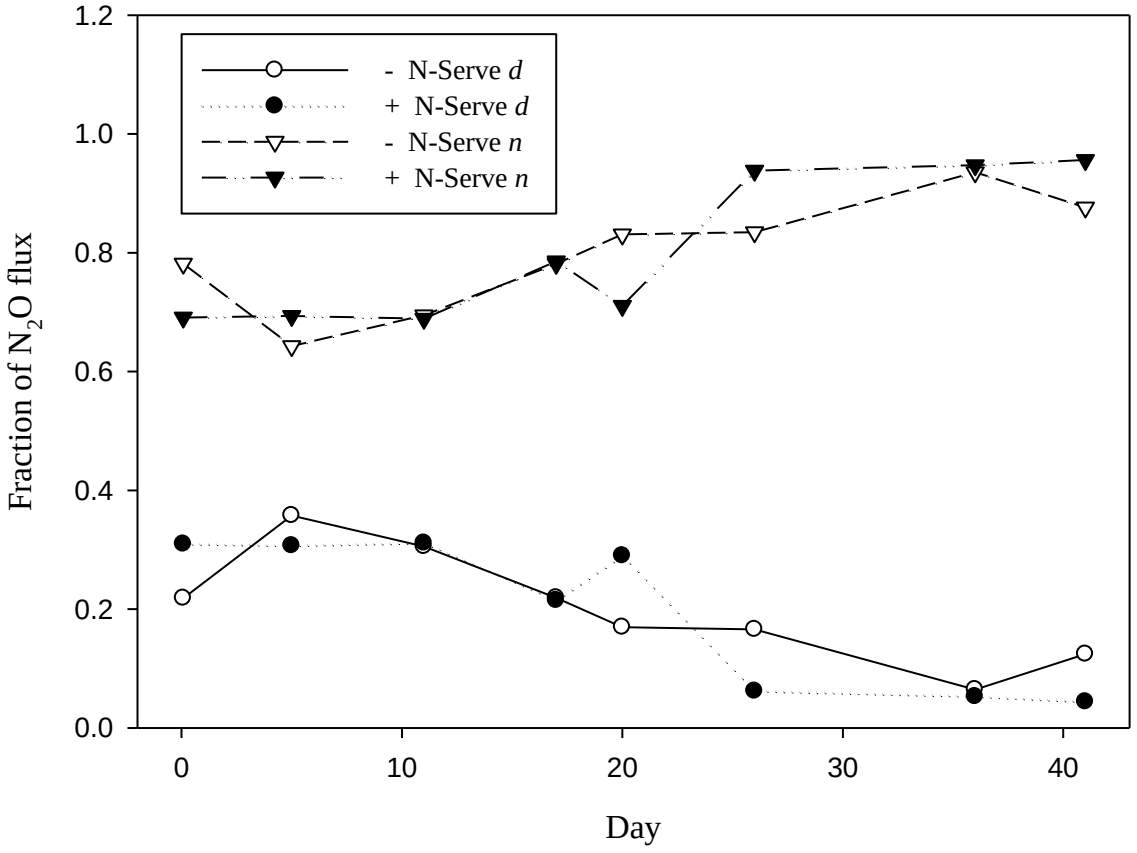

Figure 2.10 Fraction of N_2O flux due to nitrification (*n*) and denitrification (*d*) for microcosms treated with combinations of $K^{15}NO_3$ and N-Serve.

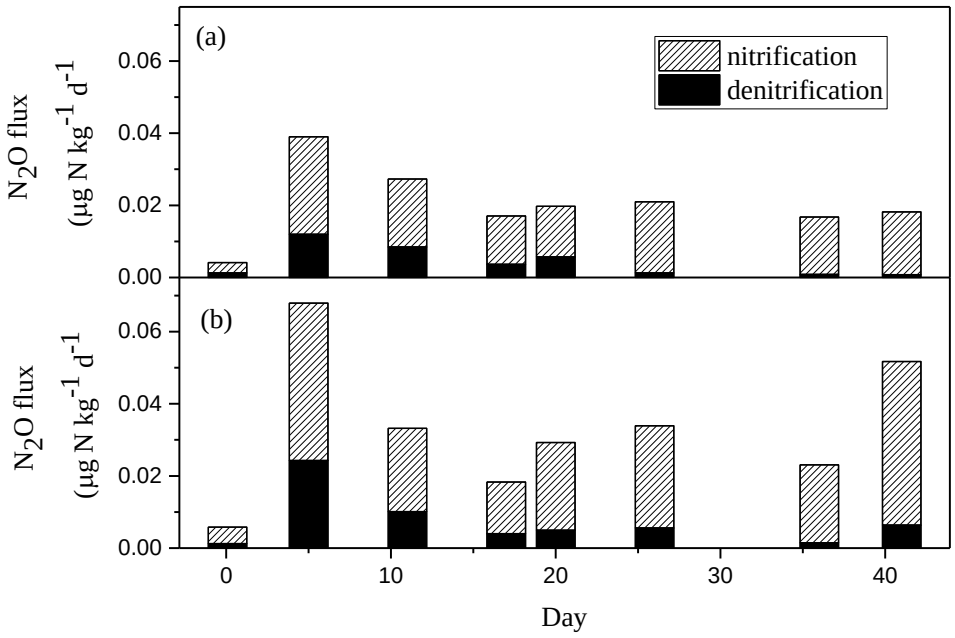

Figure 2.11 Nitrous oxide flux due to nitrification and denitrification (on each sampling day) for microcosms treated with $(^{15}NH_4)_2SO_4$ with (a) and without (b) N-Serve.

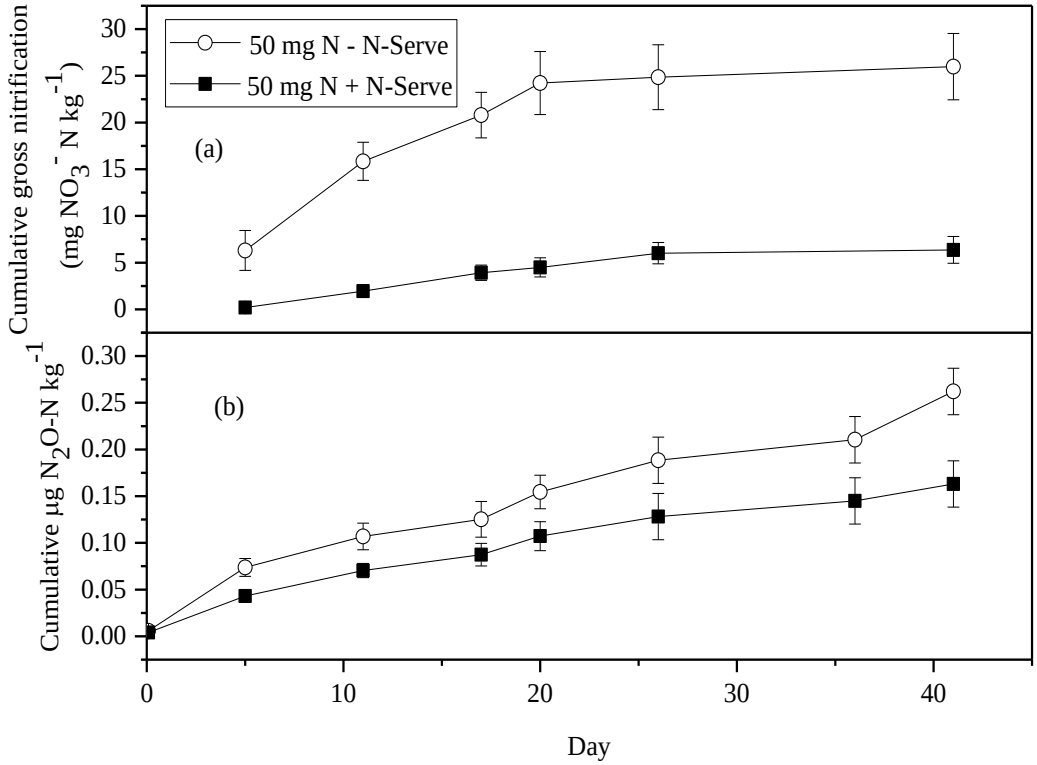

Figure 2.12 Cumulative gross nitrification for microcosms treated with $(NH_4)_2SO_4$ plus $K^{15}NO_3$ (a) and cumulative N_2O emission due to nitrification for microcosms treated with $(^{15}NH_4)_2SO_4$ plus KNO_3 (b), with and without N-Serve. Error bars are ±1 standard error of the mean (n=4) or are smaller than the symbol.

2.4.5 Relation of gross and apparent nitrification rates with N_2O emission rates.

Simple linear regression was used to determine the effect of N-Serve on N_2O emission and apparent nitrification over the first 17 days of the study (Figure 2.13). Nitrous oxide emission increased in relation to apparent nitrification in the absence of inhibitor, while there was no relation in its presence. The coefficient of determination (r^2) and the regression slope in the absence of inhibitor was 0.98 and 0.0017, respectively (Figure 2.13).

Linear regression was also used to determine the effect of the nitrification inhibitor on gross nitrification and N_2O emission due to nitrification over the whole incubation period (Figure 2.14). There was no relation between gross nitrification rate and N_2O emission due to nitrification, regardless of inhibitor addition (Figure 2.14).

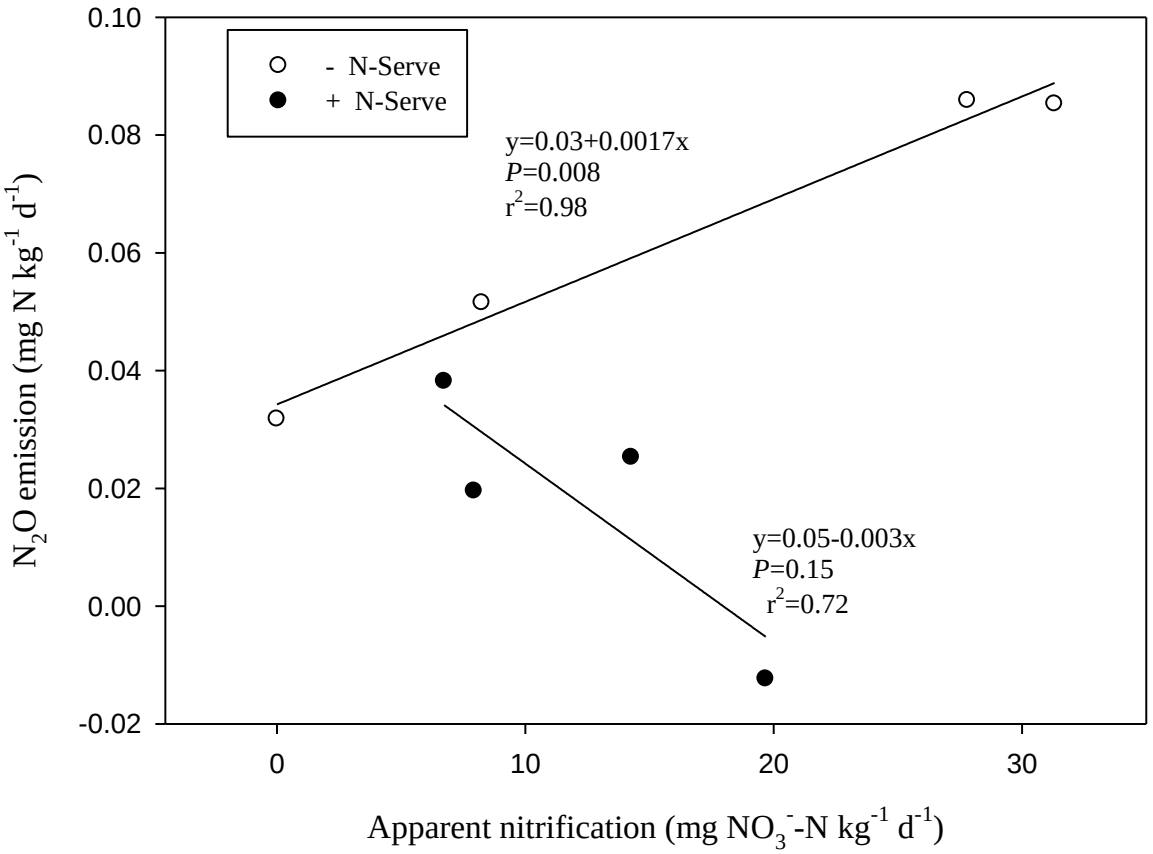

Figure 2.13 Relation between apparent nitrification rates and N_2O emission rates for the first 17 days for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve.

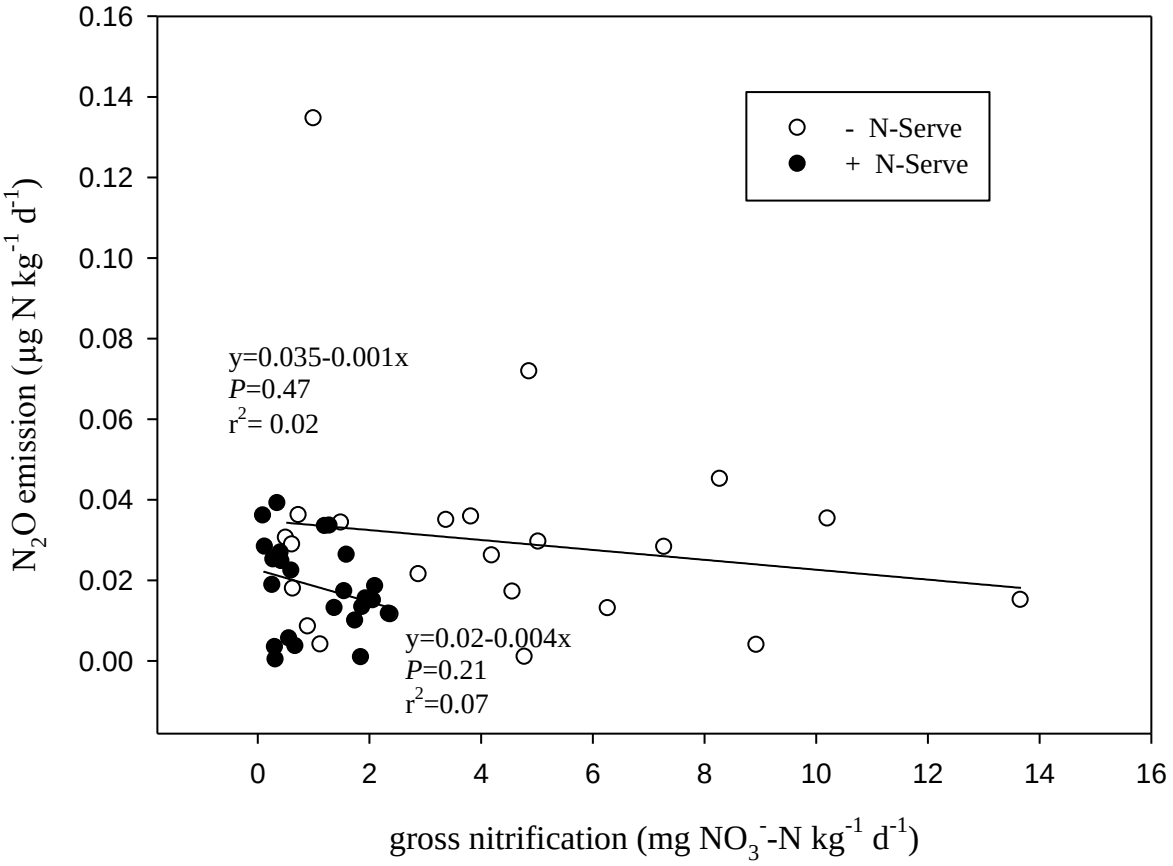

Figure 2.14 Relation between gross nitrification rates and N_2O emission rates due to nitrification for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve.

2.5 Discussion

2.5.1 Nitrification in treatments.

Complete NH_4^+ oxidation was delayed by two weeks with N-Serve addition as shown by a lower decay rate constant in the presence than in the absence of inhibitor. Nitrate appeared more slowly in the presence than in the absence of inhibitor. This indicated that the inhibitor was able to delay the oxidation of NH_4^+ to NO_3^- (Liu et al. 2013; Ruser and Schulz 2015). The observed increase in NO_3^- in the control with and without N-Serve suggests that mineralization and nitrification occurred in the soil. Since there was no observed increase in NH_4^+ concentration in the control, this suggests that the mineralized NH_4^+ was quickly converted into NO_3^- by nitrifiers. Nitrite was below detection limit throughout the incubation period regardless of N-Serve addition and we assumed that this intermediate was quickly oxidized to NO_3^- or was quickly reduced to N_2O and NO by the action of nitrifiers through the process of nitrifier-denitrification (Wrage et al. 2004; Kool et al. 2011).

The same trend for unlabelled NH_4^+ was also observed for δ-^{15}N NH_4^+. These results agreed with the observed increase in gross nitrification rates without N-Serve in the first two weeks of incubation. However, gross nitrification rates remained low with N-Serve and this was indicated by the persistence of added NH_4^+ beyond two weeks. These results concur with the findings by Barraclough and Puri (1995) who found a delay in the oxidation of added NH_4^+ with N-Serve in both labelled and unlabelled NH_4^+ pools. Lan et al. (2013) found a similar trend after adding DCD and NH_4^+ to a paddy soil. Results indicate that N-Serve was able to inhibit the first step of nitrification.

The δ-^{15}N NO_3^- in the $(^{15}NH_4)_2SO_4$ plus KNO_3 treatment increased at a faster rate in the absence than in the presence of inhibitor up to Day 20 and thereafter there was a decline in δ-^{15}N

NO_3^- regardless of N-Serve addition. This decline in the δ-^{15}N NO_3^- could be attributed to NO_3^- immobilization or denitrification that could have taken place in the soil. Another reason might be as a result of dilution of labelled NO_3^- from unlabelled mineralized N and this can be supported by an observed increase in the NO_3^- concentration in the unfertilized soil from Day 20 onwards. The δ-^{15}N NO_3^- in the $(NH_4)_2SO_4$ plus $K^{15}NO_3$ treatment declined with time as it was being diluted by NO_3^- from nitrification of unlabelled NH_4^+ (Shunfeng et al., 2015). The dilution was faster without than with N-Serve and this indicated the ability of N-Serve to lower the rate of nitrification. The δ-^{15}N NH_4^+ values in this treatment remained low at their natural abundance. This indicated that there was no remineralization of immobilized NO_3^- back into the NH_4^+ pool (Stevens et al., 1997) since this is one of the assumptions that must be met in as far as pool dilution experiments are concerned.

Although there is a big difference between field and laboratory conditions, studies like this are vital in providing information on the mechanisms involved in N transformation processes. The ability of N-Serve to reduce the rate of nitrification observed in this study helps in reducing NO_3^- loses through leaching and denitrification (Alonso-Ayuso et al. 2016). The latter process is involved in the production N_2O, a greenhouse gas, while the former results in ground water pollution (Di et al. 2014).

2.5.2 Nitrous oxide emissions and nitrification rates with and without N-Serve.

Nitrous oxide emissions and nitrification rates in both unlabelled and labelled treatments were effectively reduced by the application of N-Serve. These results concur with many studies which demonstrated the effectiveness of nitrification inhibitors in decreasing N_2O emissions in NH_4^+ fertilized soils (Zaman et al. 2009; Shen et al. 2013; Wang et al. 2014; Ruser and Schulz 2015). It is clear in this study that N-Serve can mitigate N_2O emissions by slowing the rate of

nitrification, a process that is involved in the production of N_2O (Akiyama et al. 2010). To date, it is not clear whether there are other mechanisms by which N-Serve mitigate N_2O emissions besides the above mechanism (Cui et al. 2013).

Apparent nitrification over the first 17 day period was greater in the absence than in the presence of N-Serve and this corresponded with greater cumulative emissions. Farquharson, (2016) found nitrous oxide emissions were related to potential nitrification rates in several soils. In our study, gross nitrification rates were greater without than with N-Serve in the first twenty days of incubation and this corresponded with some daily emission spikes from nitrification. However, discrepancies between treatments were observed on some days and these might be attributed to the variability in emissions between individual, spatially independent replicates. These observations indicated daily emissions were dependent on the rates of nitrification, since nitrification was the dominant process of N_2O emission throughout this study. Since N-Serve blocks the activity of ammonia monooxygenase (AMO), it is possible that the addition of N-Serve resulted in a reduced accumulation of NH_2OH in the soil, hence reduced N_2O emissions (Sabba et al. 2015). Accumulation and decomposition of NH_2OH results in the release of a substantial amounts N_2O as a by-product (Tenuta and Beauchamp 2000).

Although we did not measure the presence of NH_2OH, it could be the reason why more cumulative N_2O was produced without than with N-Serve. Accumulation of NH_2OH in the soil may result in the amount of N_2O produced per given amount of N nitrified to be high, hence more cumulative N_2O could be produced in soils with higher rates of nitrification (Sabba et al. 2015). Lower apparent and gross nitrification rates in the presence of inhibitor resulted in less NO_3^- recovered than in the absence of inhibitor, although differences were not significant. Large cumulative emissions in the absence of N-Serve might be attributed to a larger amount of N

nitrified and not necessarily due only to differences in nitrification rates. Chen et al. (2010) compared N-Serve and DMPP on N_2O emissions and they found more N_2O emissions in treatments without inhibitor which also corresponded to larger amounts of NO_3^- recovered. This indicates that the differences in emissions observed in their study might also be due to differences in the amount of N nitrified with and without inhibitor.

2.5.3 Contribution of nitrification and denitrification to N_2O flux.

Cumulative apparent and gross nitrification were compared to cumulative emission to determine the amount of N_2O emitted per mg of N nitrified, with and without inhibitor addition. From the progress of cumulative apparent nitrification, a similar amount of cumulative N_2O was emitted with the same amount of N nitrified, with and without N-Serve. Based on cumulative gross nitrification and cumulative emission due to nitrification, there was again a similar amount of cumulative N_2O emitted with the same amount of gross nitrified N, with and without inhibitor. These findings indicate that, as far as nitrification-related N_2O is concerned, the total amount of N nitrified is more important in determining cumulative emissions than the rate of nitrification. These results also show that cumulative emissions may not be reduced by lowering the rate of nitrification but they are lowered by reducing total added N nitrified. These results concur with the findings by Di and Cameron (2014), who found a reduction in cumulative N_2O with DCD in a simulated grazed grassland. The reduced N_2O emissions were accompanied by a 76% reduction in the NO_3^- recovered in the leachate in the presence DCD. The reduced N_2O emissions in their study was due to a reduced amount of N nitrified in the presence of DCD and not due to differences in rates of nitrification, alone.

In the present study, nitrification was the main source of N_2O emission regardless of N-Serve addition. Nitrification was responsible for more than 75% of N_2O emissions, regardless of

N-Serve addition. However, it must be noted that, not all of the N_2O was necessarily as a result of nitrification since N_2O could have been formed through the reduction of NO_2^- by nitrifier-denitrification. Mixing of NO_2^- produced from nitrification and denitrification is highly unlikely since in most cases the rates of NO_2^- consumption exceed the rates of its diffusion (Wan et al. 2009). Taking this into account, the findings from this study suggest that nitrification and nitrifier-denitrification were the major processes that contributed significantly to the N_2O emission.

Denitrification was responsible for less than 25% of the emissions regardless of N-Serve addition. This indicates that N_2O production in this study was produced by two processes which were occurring simultaneously in the soil (Webster and Hopkins 1996; Bateman and Baggs 2005; Carter 2007; Uchida et al. 2013; Smith et al. 2014). However, since the incubation conditions favoured nitrification, it is not clear whether the occurrence of denitrification in this experiment was due to nitrifier-denitrification or due to the development of anaerobic microsites in the soil (Mathieu et al. 2006). These results agree with other studies which also found the dominance of nitrification emission of N_2O under aerobic conditions (Wrage et al. 2004; Sutka et al. 2006; Carter 2007; Cheng et al. 2014; Smith et al. 2014; Farquharson 2016). However, in other studies, for example, Stevens et al. (1997), found that, whatever the moisture content of the soil, nitrification and denitrification contributed to N_2O flux in NH_4^+ fertilized soils.

Nitrification was lower in the first days of incubation regardless of inhibitor addition but increased to the end of the incubation period. This observation could have been attributed to the increase in soil moisture soon after N solution application which might have resulted in a partial decrease in oxygen concentration in the soil. Denitrification was higher in the first ten days of incubation regardless of the presence of the inhibitor and it decreased to the end of the incubation period. This could be attributed to the presence of carbon during the early days of incubation and

could have resulted in intense denitrification since carbon is needed as an energy source by denitrifiers (Stevens et al. 1997; Wan et al. 2009). The reduction in the contribution of denitrification in the subsequent days could be due to the depletion of the carbon source by the microbes. The fractional contribution of nitrification and denitrification was not affected by the addition of N-Serve throughout the incubation period. This suggests that, the contribution of denitrification and nitrification is largely dependent on the soil conditions for example moisture and not on the presence of the nitrification inhibitor. These results agree with the findings by Lan et al. (2013) who also observed no differences between the fractional contribution of nitrification to N_2O emissions with and without DCD in a paddy soil.

2.5.4 Relation of gross and apparent nitrification rates with N_2O emission rates.

A regression analysis was performed to see the relation between gross nitrification and daily emissions due to nitrification, and apparent nitrification and daily emissions with and without N-Serve. Nitrous oxide emission increased in relation to apparent nitrification in the absence of inhibitor, while there was no relation in its presence. There was again no relation between gross nitrification rate and N_2O emission due to nitrification, with and without inhibitor addition. Although there was no relation between N_2O emission and nitrification rate with N-Serve addition, a decreasing trend was observed instead. This indicated that there was no increase in N_2O emissions with increase in nitrification rates with N-Serve addition. The ratio of N_2O: gross nitrification was lower at the start of the incubation period since less N_2O was produced despite observed high gross nitrification rates. This observation could be attributed to the increase in soil moisture soon after N solution application. This might have resulted in a partial decrease in oxygen concentration resulting in more N_2O reduced to N_2 either by the action of nitrifiers or denitrifiers (Wrage et al. 2004).

The ratio of N_2O: gross nitrification is expected to change under changing environmental conditions but the control of this is not yet well understood (Yang et al. 2011). This ratio became higher later after solution application and this might be due to the stabilization of the soil moisture. The stabilization of soil moisture might have resulted in an increased oxygen concentration in the soil resulting in much of the N nitrified to be emitted as N_2O leading to a higher N_2O: gross nitrification ratio. Also the high concentration of carbon at the start of the incubation could have resulted in the growth of denitrifiers despite the prevailed aerobic conditions. The growth of denitrifiers was found to be dependent on carbon concentration rather than oxygen concentration (Yang et al. 2011). However, there is a possibility of N-Serve inducing some unknown mechanisms that reduce N_2O: gross nitrification ratio or that enhance N_2O consumption. Although it is not well proven, N-Serve was once reported to promote denitrification (Bremner and Yeomans 1986; Somda et al. 1990) and could be a reason for this decreasing trend. Although N-Serve may not affect denitrification as a process, it may affect certain steps of N_2O consumption.

2.6 Conclusions

Based on cumulative nitrification (apparent and gross) and cumulative emissions, almost the same amount of N_2O was emitted with and without inhibitor given the same amount of N nitrified over time. These results imply that, as far as nitrification-related N_2O is concerned, the amount of added N that is nitrified dictates the eventual cumulative N_2O emitted regardless of the rate of nitrification. Inhibitors may reduce cumulative emissions by reducing cumulative nitrification. The reduction in cumulative nitrification also lowers NO_3^- leaching and denitrification related N_2O emissions. The numerically larger although not significantly larger amount of NO_3^- recovered over the whole incubation period in the absence than in the presence of inhibitor, might be the reason of a higher cumulative N_2O emitted without than with N-Serve

addition. Nitrification-inefficiency was not affected by the addition of nitrification inhibitor. This indicated that, there was no difference in N_2O emitted per unit of added N nitrified, with and without inhibitor.

Nitrification and denitrification often occur simultaneously despite the prevailing soil moisture conditions making the use of ^{15}N isotope tracer necessary so as to distinguish N_2O emissions due to nitrification and denitrification. This is because anaerobic microsites often exist in the soil even if the soil moisture conditions favor nitrification. There was no relation between gross nitrification rate and N_2O emission due to nitrification, with and without inhibitor addition. Nitrous oxide emission increased in relation to apparent nitrification in the absence of inhibitor, while there was no relation in its presence. The decreasing trend, although not statistically significant, between nitrification rates (apparent and gross) observed in the presence of the inhibitor, may indicate the probable N_2O consumption in the presence of inhibitor. The strong positive relation between apparent nitrification and daily emissions in the absence of inhibitor, indicates the dependence of N_2O emission to nitrification rates.

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3. GENERAL SYNTHESIS

3.1 Important findings of significance to farmers and society.

The addition of N-Serve resulted in lower daily emissions and eventual cumulative emissions over the 41-day incubation period. Application of inhibitor to soil also reduced the amount of NO_3^- recovered over the whole incubation period. The reduced accumulation of NO_3^- results in reduced risk of N losses by leaching and denitrification, thereby reducing ground water pollution and N_2O emission. However, a similar amount of N_2O was emitted with and without inhibitor for the same amount of N nitrified. These results imply that, as far as nitrification-related N_2O is concerned, the amount of added N nitrified determines the eventual cumulative N_2O emissions, regardless of the rate of nitrification, alone. Reducing total N nitrified by any means can be useful in lowering cumulative N_2O emissions and NO_3^- accumulation. Less NO_3^- in the soil reduces NO_3^- leaching and denitrification related N losses.

The decreasing trend, although not significant, between daily emissions and nitrification rates (apparent and gross) observed in the presence of inhibitor may indicate the occurrence of N_2O consumption in the soil. The strong positive relation between apparent nitrification and daily emissions, indicates the dependence of N_2O emission to nitrification rates in the absence of inhibitor. Nitrification inhibitors target the first step of nitrification (Zaman et al. 2009) and can reduce accumulation of intermediates such as NH_2OH and NO_2^- (Venterea et al. 2015). The former can decompose to produce N_2O, while the latter can be further reduced to N_2O when oxygen becomes limiting, through a process called nitrifier-denitrification (Sabba et al. 2015).

Nitrification and denitrification were occurring at the same time in the soil. However, nitrification was the dominant process of N_2O production throughout the incubation period, accounting for more than 75% of the emissions. Farmers should know that, in aerobic, NH_4^+

fertilized soils, nitrification will be the major source of N_2O emission; hence, it may be beneficial to invest in nitrification inhibitors to reduce N_2O emissions. These results agree with other studies, which showed that nitrification was the dominant process of N_2O emission under well aerated conditions (Stevens et al. 1997; Bateman and Baggs 2005; Mathieu et al. 2006). In the past, the contribution of nitrification to N_2O emissions was undermined, but with the use of ^{15}N isotope tracer, it is now known that nitrification is also a major source of N_2O in aerobic, NH_4^+ fertilized soils.

Denitrification was greater in the early than in the later days of incubation. This might be as a result of availability of carbon in the early days which could have promoted the growth of denitrifiers (Wan et al. 2009). Some studies have observed the growth of denitrifiers to be mostly affected by soil carbon concentration and not oxygen status of the soil (Weier et al. 1993). This can imply that, in soils with high amounts of decomposable carbon, denitrification can be a major source of N_2O even in NH_4^+ fertilized soils.

3.2 Unexpected findings

Nitrous oxide emission increased in relation to apparent nitrification in the absence of inhibitor, while there was no relation in its presence. There was again no relation between gross nitrification rate and N_2O emission due to nitrification, with and without inhibitor addition. Some studies have reported a positive relation between gross nitrification rates and daily emissions (Wan et al. 2009; Ambus 2005; Lan et al. 2013). Although there was no relation between N_2O emission and rates of nitrification (apparent and gross) with N-Serve addition, a decreasing (non-significant) trend was observed instead. It is not clear whether this decreasing, non significant trend was due to the effect of inhibitor or other soil factors. It is recommended to test N-Serve for mechanisms that could have resulted in this phenomenon.

Nitrite was not detectable throughout the experiment even in the days when rates of nitrification were higher. In most studies, NO_2^- is detected when nitrification rates are high, especially in calcareous soils (Ma et al. 2015). This finding suggests that NO_2^- was being quickly converted before accumulation in the soil. In addition, nitrification is known to cause acidification of the soil and this might have augmented the acidity of the soil, since the soil was slightly acidic (pH 6.5) at the start of the experiment. This could be the reason that prevented NO_2^- to accumulate since it rarely accumulates in acidic conditions. However, the pH of the soil must have been measured at all sampling dates to prove this assertion.

3.3 Challenges

The use of ^{15}N pool dilution techniques in studying gross N transformations is based on a couple of assumptions. Challenges with the use of this technique arise when one or more of the assumptions are not met. These assumptions must be met to minimize potential sources of errors associated with the use of this technique. In this technique, there should be no significant amount of isotopic discrimination within the soil (Hart et al. 1994). The assumption assumes that all microbial processes which consume N must not discriminate between the ^{14}N and the ^{15}N isotopes. However, significant discrimination of isotopes may occur within the soil if incubation periods are too long and if the enriched N pool is not several times above the natural abundance. However, errors associated with this assumption can be counteracted by having short incubation periods and making sure that the enriched N pool is much greater than the natural abundance (Murphy et al. 2003; Stevens et al. 1997; Mathieu et al. 2006).

There should be uniform distribution of ^{15}N within the soil. This is one of the prerequisites in as far as ^{15}N pool dilutions are concerned. However, attaining a complete uniform distribution of ^{15}N within the soil can be a challenge. Some studies have approximated errors of about 10%

when less than 70% of the microsites received the ^{15}N (Davidson et al. 1991). Errors can be large if the ^{15}N is not well distributed within the soil matrix. In addition to the above conditions, there should be a state of equilibrium between the added and the indigenous N pools. This implies that the added ^{15}N should be at the same chemical state and location as the indigenous N. However, this can be a challenge to achieve given the heterogeneity of the soil and the soil microbes. It is difficult for the applied N to be in an immediate state of equilibrium with the already existing N.

It is well known that heterotrophic nitrification can be a major source of N_2O emissions especially in acidic soils fertilized with NH_4^+ based or producing fertilizers. The enzyme for the first step of heterotrophic nitrification is different from the one involved autotrophic nitrification. The use of nitrification inhibitors such as N-Serve to lower the rate of nitrification can be a challenge where heterotrophic nitrification is dominant. The use of polymer coated urea fertilizers such as ESN might be of use in these soils. To date there is no labelled ESN on the market so differentiating between nitrification and denitrification using ESN fertilizer is not yet possible.

3.4 Recommendations to farmers, society, scientists and for future work

Reducing the total amount of N nitrified by any means is a good strategy that can be used by farmers to reduce cumulative N_2O emissions in agricultural soils fertilized with NH_4^+ based or producing fertilizers. The total amount of N nitrified is more important for determining cumulative N_2O emissions than the rate of nitrification on its own. The use of slow release fertilizers such as ESN and urease inhibitors can result in reduced cumulative nitrification and cumulative N_2O emissions. This can be useful especially to farmers who apply their NH_4^+ based or producing fertilizers in fall or apply their fertilizer without split application. The use of NO_3^- based fertilizers instead of NH_4^+ or NH_4^+ producing fertilizers in soils with high nitrification potential may be useful to reduce N_2O emission due to nitrrification.

However, these results may not represent what may happens under field conditions so there is a need for field studies. Also, these results might not be representative of what may happen in all soil types, so different soil types may be worthwhile testing to determine the applicability of these findings to different soil types. Also incubations of greater than 40 days to completely nitrify added NH_4^+ with N-Serve are recommended to fully validate these results. If this study is done in acidic soils, heterotrophic nitrification might contribute a substantial amount of N_2O since it is not affected by nitrification inhibitors; hence it might be necessary to separate its contribution to N_2O emissions.

3.5 Conclusions

N-Serve was effective in reducing apparent and gross nitrification rates in the investigated soil over the 41-day incubation period. The inhibitor reduced NO_3^- recovered and cumulative emissions, making it an effective strategy to mitigate NO_3^- leaching and N_2O emissions in agricultural soils. However, a similar amount of N_2O was emitted with and without inhibitor, for the same amount of N nitrified. The non-significant differences in nitrification-inefficiency observed also indicate that there was no difference in the amount of N_2O emitted per unit of added N nitrified, regardless of inhibitor addition. This again indicates that inhibitors reduce cumulative N_2O emissions by reducing the total amount of nitrified N. The amount of added N nitrified determines the eventual cumulative N_2O emissions, regardless of nitrification rate on its own.

Nitrification was found to be the major process responsible for N_2O emissions, contributing to more than 75% of the total N_2O emitted. Nitrification can be a major contributor to N_2O emissions in well aerated soils fertilized with NH_4^+ based and/or producing fertilizers. Nitrification and denitrification can occur simultaneously even if the soil is well aerated to favor nitrification. This is due to the development of anaerobic microsites particularly within soil aggregates. The decreasing, although non-significant trend between nitrification rates and daily emission with N-

Serve, indicated the possibility of N_2O consumption in the presence of inhibitor. The positive relation between N_2O emission and apparent nitrification rate without inhibitor, indicated the dependency of N_2O emissions to nitrification rates.

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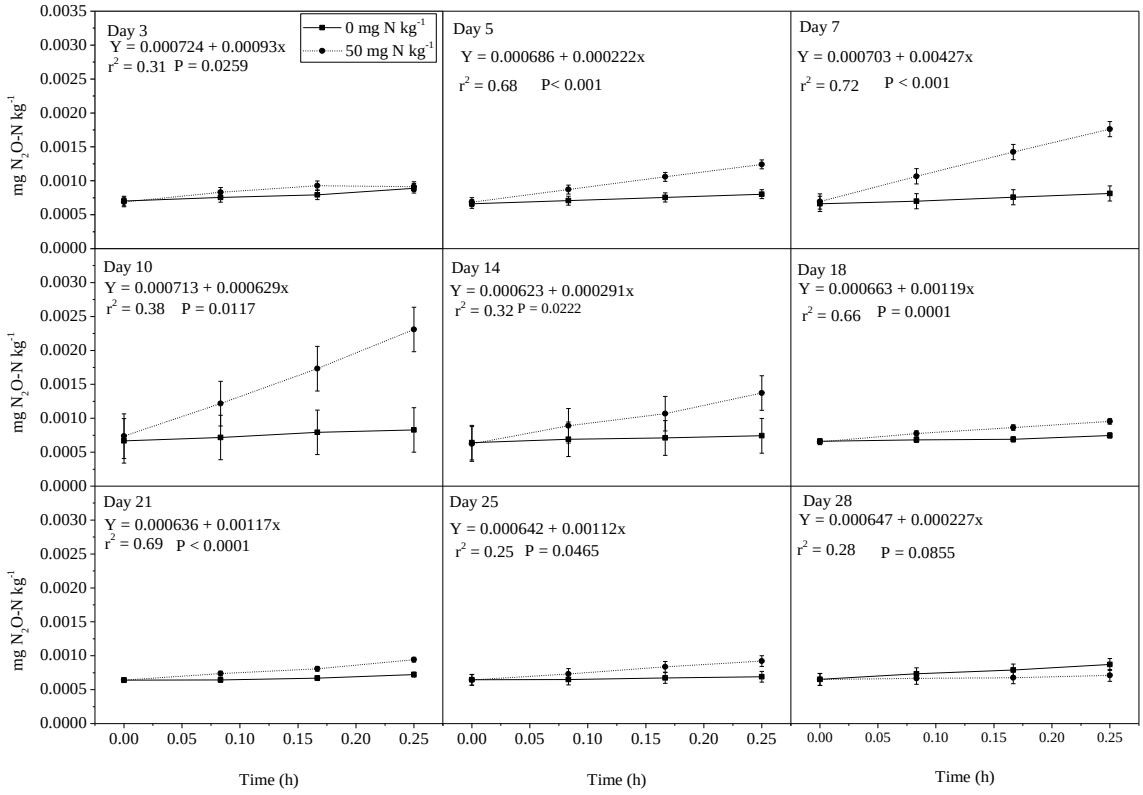
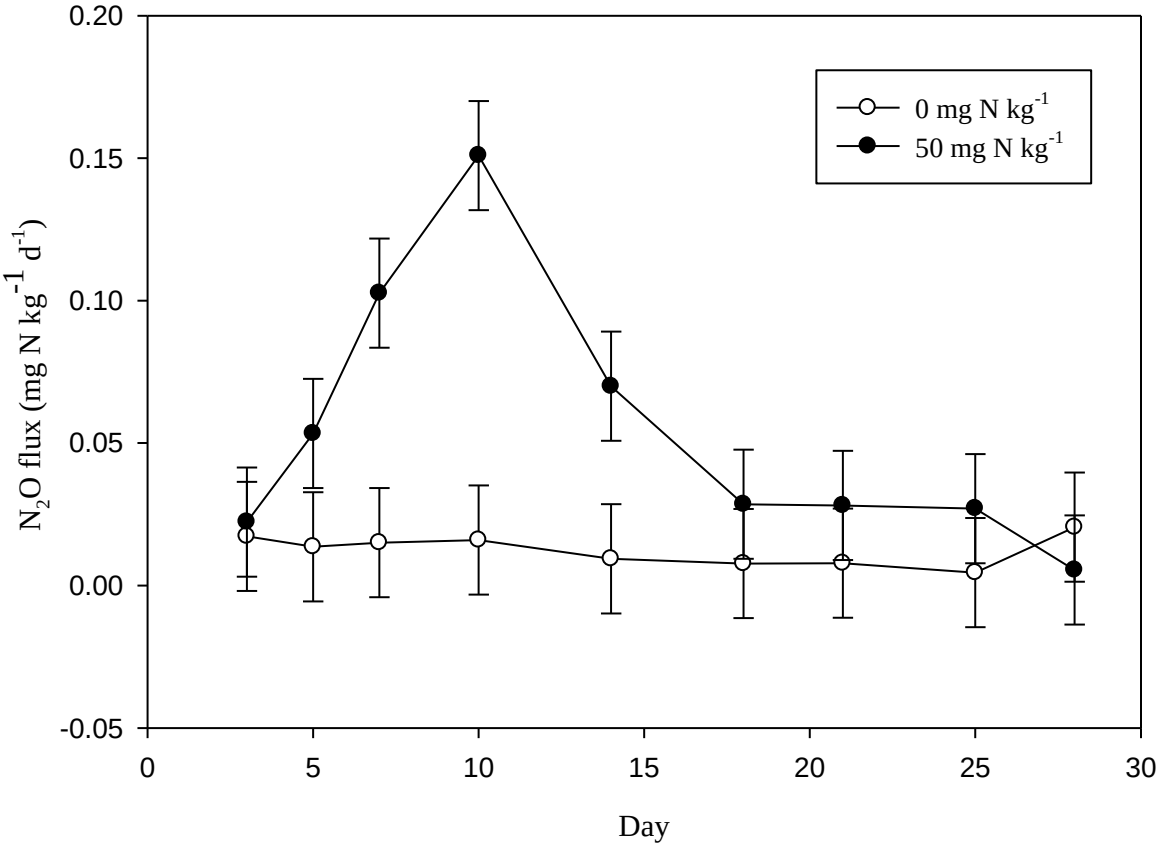
4. APPENDIX

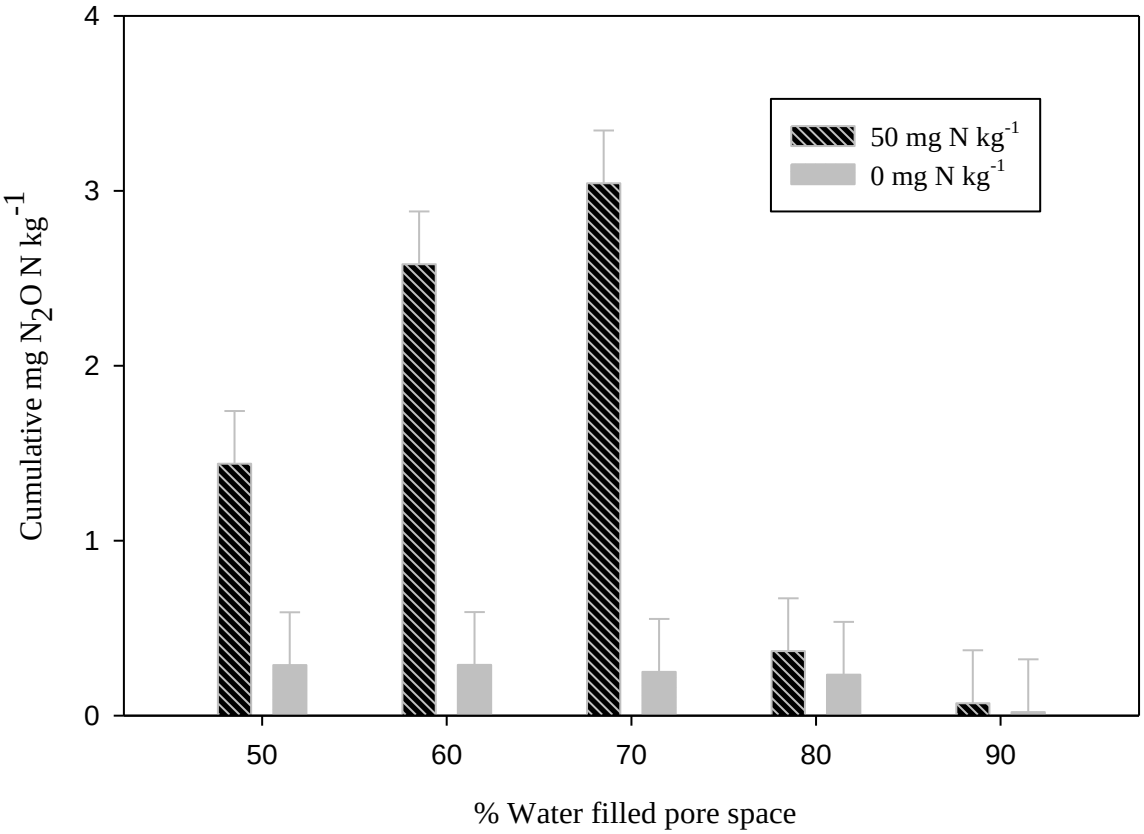
Appendix A: Determining the linearity of headspace accumulation of N_2O, suitable time of sampling after closing the incubation vessel and the % WFPS that produces the highest amount of N_2O after ammonium sulfate amendment.



Appendix Figure 1. Linearity of head space accumulation of N_2O after closing incubation vessels for microcosms treated with urea in a preliminary experiment. Error bars are ±1 standard error of the mean (n=4).

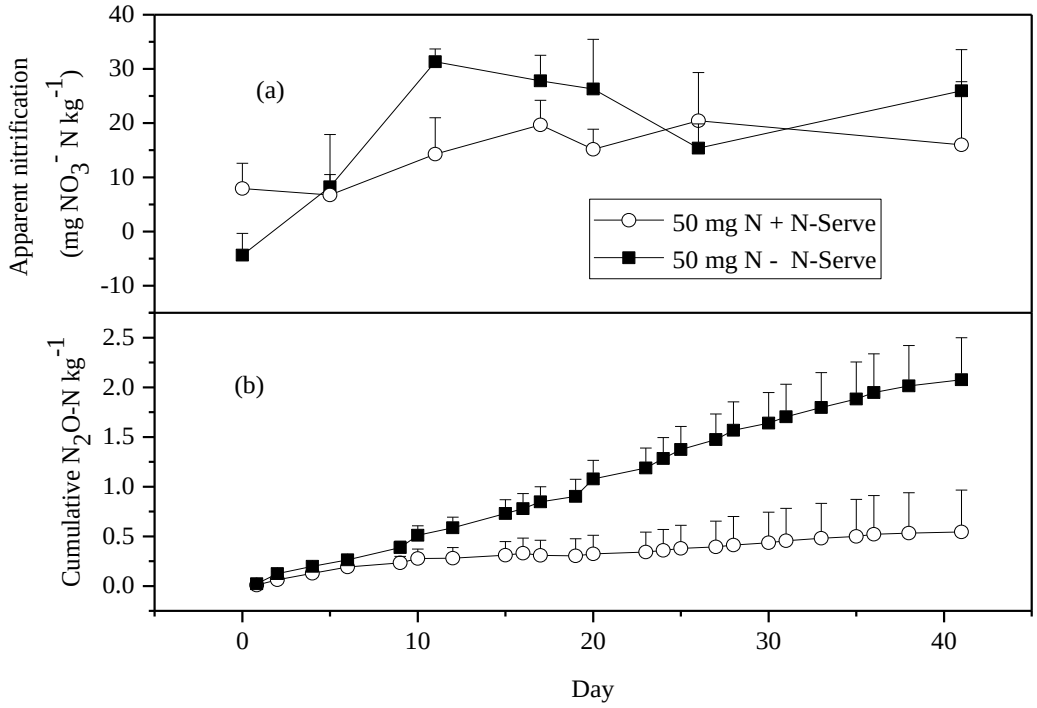


Appendix Figure 2. Temporal pattern of nitrous oxide emission rate for microcosms treated with urea in a preliminary experiment. Error bars are ±1 standard error of the mean (n=4).



Appendix Figure 3**.** Cumulative N_2O emissions as affected by water filled pore space (% WFPS) for microcosms treated with $(NH_4)_2SO_4$ in a preliminary experiment. Error bars are ±1 standard error of the mean (n=4).

Appendix B: Relationship between cumulative apparent nitrification and N_2O emissions with and without N-Serve.



Appendix Figure 4. Apparent nitrification (a) and cumulative emissions (b) for microcosms treated with combinations of $(^{15}NH_4)_2SO_4$ and N-Serve. Error bars are ±1 standard error of the mean (n=4).