**Evaluating Nitrogen Availability and Emissions of Greenhouse Gas in Soils Amended with
Different Organic and Inorganic Fertilizers**

by

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Abstract

Laboratory and field studies were conducted to examine the effect of different amendments on the transformation of nitrogen (N) in soil and the emission of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$. Amendments included alfalfa (*Medicago sativa*), liquid cattle manure and ammonium nitrate synthetic fertilizer (AN) applied at rates typical for a dairy farm in Eastern Ontario. The highest levels of N transformations, $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ emissions occurred in plots amended with alfalfa, followed by manure, AN and control. With lower C:N ratios in the organic amendments, these results indicate the importance of carbon (C) in N cycling. Transformations and emissions of gases occurred very rapidly after application of amendments with more than half the total $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ emission occurring within the first 10 days of the field experiment. Quantities of inorganic N were highest in the plots amended with AN, followed by manure, alfalfa and control. These results have practical implications for the type of amendments and the timing of application needed to improve soil N availability while reducing greenhouse gas emissions.

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List of Symbols

Term/Symbol	Unit	Definition
N ₂ O-N	μg N ₂ O-N m ⁻² hr ⁻¹	Nitrous Oxide- Nitrogen
CO ₂ -C	mg CO ₂ -C m ⁻² hr ⁻¹	Carbon Dioxide- Carbon
AN	g	Ammonium Nitrate
β	g cm ⁻³	Bulk Density
v	g g ⁻¹	Gravimetric Water Content
θ	m m ⁻³	Volumetric Water Content
φ	%	Soil Porosity
WFPS	%	Water-filled pore space
TDN	μg N g dry soil ⁻¹	Total Dissolved Nitrogen
DOC	μg C g dry soil ⁻¹	Dissolved Organic Carbon
TOC	g m ²	Dissolved Organic Carbon + Total Organic Carbon
NH ₄ ⁺ -N	μg N g dry soil ⁻¹	Ammonium-Nitrogen
NO ₃ ⁻ -N	μg N g dry soil ⁻¹	Nitrate-Nitrogen
ANOVA		Analysis of Variance
RM ANOVA		Analysis of Variance with Repeated Measures
MANOVA		Multiple Analysis of Variance
ρ		Spearman's Rho Coefficient
ρ _s		2.65- Particle Density
g		Gravity

1. Introduction

In the past century, agriculture has become increasingly intensive in an effort to feed the growing global population. The demands placed upon the soil have increased accordingly. In an effort to deal with these demands, farmers have increased the rates at which they fertilize land to maximize production and profits. Initially, most soil nitrogen (N) additions to soil were attained from organic matter, manures, legumes and other naturally occurring sources of N. The majority of the earth's N occurs in gaseous form (di-nitrogen gas, N_2) which makes up 78% of our atmosphere. However, it was not until the early 20th century that the Haber-Bosch process enabled fixation of this abundant atmospheric N into ammonia for the manufacture of N fertilizers. Since then, the rate of N application has increased at unforeseen rates. Mengel (1996) states that industrial fixation of N is 60% higher than biological fixation. Agriculture Canada now estimates that almost 40% of the N in protein that we consume worldwide has been manufactured (Agriculture and Agri-Food Canada, 2009). Although the ease with which we now manufacture fertilizer has allowed for vast increases in crop production, there are problems associated with greater N fertilization rates.

As Robertson and Groffman (2005) point out, N occurs in nine different forms within the soil. While low soil N concentrations have the ability to limit plant growth, N also occurs in forms which are considered pollutants. Once in the soil, N is subject to many transformations, some reversible, all of which contribute to a complicated biogeochemical cycle (see Figure 1-1).

Figure 1-1: Schematic representation of the major forms of N in the terrestrial N cycle. Those processes mediated by soil microbes appear in bold. Gases appear in brackets. (From Robertson and Groffman, 2005)

In an agricultural context the two most important forms of N are NH_4^+ and NO_3^- . Of the nine possible forms, only these two are readily taken up and used by plants in the growth process. The following is a brief introduction to the key processes that govern N transformations and losses in agriculture followed by an assessment of three of the primary fertilizer options available to Eastern Ontario farmers.

An examination of N dynamics will improve our ability to recommend which of these primary fertilizer amendments may serve as the most beneficial form of fertilization on small scale farms in Eastern Ontario. More specifically, an assessment of the relative

rates of mineralization will allow us to determine which amendment is mineralized and nitrified at a rate most suitable to meet the demands of a particular crop. Furthermore, measurements of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ fluxes serve to establish which amendment loses greater quantities of N throughout the study period. GHG flux measurements also serve to gauge the rates at which microbial activity occurs. By assessing environmental variables in relation to both soil processes and GHG production we can determine with some certainty which processes are responsible for the greatest N losses in agricultural systems, and possibly where mitigation of these losses should occur.

1.1. Soil Nitrogen Processes

1.1.1. Mineralization

Mineralization consists of processes involving enzymatic breakdown of N-containing plant materials such as amino sugars, peptides and proteins resulting in the release of inorganic N in the form of NH_4^+ (Mengal, 1996). Mineralization is carried out by various micro-organisms including fungi and both aerobic and anaerobic bacteria. While these microbes consume organic matter in the form of detritus mainly as a means of acquiring C for energy, microbes must also meet nutrient demands for cellular growth (Robertson and Groffman, 2007). From this point NH_4^+ may be quickly nitrified given proper circumstances or may be immobilized once again into organic N (Figure 1-1). Often it is the balance between C consumption and the demand for nutrients that largely governs net N mineralization and immobilization. Amendments with low C:N ratios such as alfalfa (16:1) offer organisms a relatively high amount of N in relation to C. As

such it is likely that net mineralization will occur as micro-organisms release unneeded N into the soil in inorganic forms. If however an amendment such as grain straw (80:1) were added to the soil, net immobilization is likely to occur. This occurs as a result of limiting N levels and the associated need for micro-organisms to utilize what little N is available for cellular growth. Both processes occur simultaneously within the soil. In general, the mineralization process is controlled by soil temperature and water content, since these variables govern microbial activity which is responsible for mineralization. Mineralization of organic N contributes significantly to pools of inorganic N even in relation to synthetic fertilizers which do not need to be mineralized as they are already in inorganic form (Ramos, 1996). Drury et al. (2003) estimated that mineralization of soil organic matter has the ability to provide crops with anywhere between 30% and 100% of their total N requirement. Typically, N losses from the system are minimal as a result of the mineralization process. However, it should be noted that in many agricultural soils addition of amendments containing high levels of NH_4^+ like manure often result in substantial losses from volatilization (Coyne and Frye, 2005). This occurs because manure in many agricultural settings is often applied directly to the surface. As a result, manure does not have enough contact with the soil to hydrolyze completely and ammonia that does not come in contact with soil and H^+ ions is lost to the atmosphere.

1.1.2. Denitrification

From a farmer's point of view the production of N_2O serves as a loss of valuable nutrient N from the agroecosystem. In addition, N_2O is a powerful greenhouse gas that has the ability to destroy stratospheric ozone (Intergovernmental Panel on Climate

Change (IPCC), 2007). The production of N_2O results from two separate yet equally important processes. The predominant process which leads to N_2O production under anaerobic conditions is denitrification while under aerobic conditions, it is nitrification. In situations where high soil moisture persists, N oxides are reduced to N_2O as heterotrophs search for appropriate terminal electron acceptors to replace O_2 (Martens 2005; Gillam et al. 2007). The amount of denitrification as well as N_2O production is dependent on a number of factors. As soil moisture increases, water filled pore space (WFPS) increases and leads to greater rates of denitrification. As Linn and Doran (1984) point out, denitrification is inhibited at WFPS below 60%. Further research by Drury et al. (2003) found that denitrification increased above 60% WFPS to a maximum at 95% WFPS. Studies have shown that the addition of both C substrates and NO_3^- increased the levels of denitrification (Weier et al. 1993). Increases in the availability of C enhance denitrification by increasing the energy and electron supply to denitrifiers (Gillam et al. 2007). As consumption of C substrates increase, so too does the use of O_2 , thereby increasing anaerobic conditions (Garcia-Monteil et al. 2003). Denitrification often increases as a result of the complexity of C substrates added (Gillam et al. 2007). Simpler compounds such as glucose tend to increase denitrification quickly, as opposed to C substrates composed largely of lignin which are not easily broken down and do not provide as much C for denitrification. As a result, some fertilizers such as manure are considered to have higher potentials for denitrification than synthetic fertilizers (Rochette et al. 2000; Velthof et al. 2003; Van Groenigan et al. 2004). These higher denitrification levels are related directly to the high inputs of C and inorganic N. However, as Mkhabela

et al. (2007) points out, further studies on the subject resulted in the conclusion that higher denitrification only occurred in soils where C was limiting. Other studies indicate that alfalfa is responsible for higher levels of N_2O production than both manure and AN due in part to its high C content as well as its high organic N content which is easily mineralizable after plow down (Ellert and Janzen, 2006).

1.1.3. Nitrification

Nitrification is the process responsible for both the production of N_2O and the transformation among different forms of inorganic N. During nitrification, reduced forms of N are oxidized to less reduced forms by both autotrophic and heterotrophic microbes, although autotrophic nitrification is more prevalent (Prosser, 2005). Nitrification occurs as autotrophic bacteria oxidize ammonia in order to gain as much as 440 kJ of energy (Robertson and Groffman, 2005). This process is essential as it is the main way in which NO_3^- is formed in the soil. Ammonium nitrate fertilizers contain NO_3^- and to a much lesser extent acid rain delivers NO_3^- to the soil. Autotrophic nitrifiers are obligate aerobes, and as such nitrification is inherently inhibited by high soil moisture. Oxygen level is one of the two main factors governing the levels of nitrification in soils, the second being the amount of substrate and NH_4^+ . Increased nitrification also increases the likelihood of nitrate leaching soils. Unlike NH_4^+ , NO_3^- is an anion, and is therefore subject to leaching losses. This results from the inability of NO_3^- to bond to cation exchange sites. This not only contributes to losses of N from the system but also contributes significantly to problems with water quality, including eutrophication and in some cases methemoglobinemia which can prove fatal to humans.

1.1.4. Carbon Mineralization

Like N_2O , CO_2 is an important GHG, but also serves as an indicator of microbial activity since both N transformation processes and C mineralization occur simultaneously in the soil. The production of CO_2 results from either autotrophic or heterotrophic respiration where the latter is respiration by microbes (Paul et al. 1997). Heterotrophic respiration and production of CO_2 occurs under aerobic conditions. As heterotrophic organisms break down C substrates within the soil they also consume O_2 as terminal electron acceptors (TEA) while respiring CO_2 as a bi-product (Cambardella, 2005). As heterotrophs consume C substrates, excess N is released back into the soil. Furthermore, the production of CO_2 is stimulated by fresh inputs of C as organic matter often contains high quantities of readily decomposable compounds.

1.2. Soil Fertilizer Additions

1.2.1. Manure

In Eastern Ontario where intensive dairy farming is prevalent, manure application serves both an advantage and a disadvantage to farmers. The concentration of a large number of animals in a small area has led farmers to store large quantities of manure for long periods (up to a year) in large clay-lined or concrete storage lagoons which facilitate breakdown as a result of anaerobic conditions. Others still use more conventional methods such as storing manure in large piles or spreading manure daily during both the summer and winter. Dealing with large quantities of liquid or solid waste is often time consuming and expensive. Often spreading manure takes place in either late fall after the

removal of both cereal and forage crops or in the spring prior to cultivation and planting. It is the effort involved in the movement and application of manure that serves as the major disadvantage to farmers. However, manure is advantageous in that it contains high levels of inorganic N, specifically NH_4^+ . On average dairy manure contains 1.8 g/L of usable N or 1.5 g/L of NH_4^+ assuming spring application and almost immediate incorporation into the soil (OMAFRA, 2007). Manure thus serves as a valuable replacement for expensive synthetic fertilizers. High forage, high protein diets ensure that N content in animal waste is high and beneficial for plants. Unfortunately, the advantages of manure are sometimes negated by the need for farmers to spread manure simply to make room in their storage facilities. Timing of application serves as an example of this. In both fall and spring the application of manure often occurs during periods of high rainfall since more ideal weather conditions are usually reserved for planting and harvesting. Moist conditions, especially in a humid environment such as eastern Ontario, facilitate the loss of N through nitrification and denitrification, as well as leaching losses. In addition, moist conditions facilitate soil compaction given that equipment used in the application is often extremely large. As bulk density increases as a result of field traffic, anaerobic micro-sites in the soil become more prevalent, thus further increasing the potential for denitrification. Steps have been taken to mitigate these losses, including application of manure directly into the soil; however, these processes are slower and more costly than simply broadcasting manure into the air. Furthermore, few farmers possess the necessary equipment to undertake such a process,

and as such, must hire custom operators which increases cost while further limiting the control farmers have on timing application.

1.2.2. Alfalfa

In Eastern Ontario alfalfa is often used as the forage crop of choice. As a legume, alfalfa contains high levels of N due to its ability to fix N_2 gas from the atmosphere as a result of symbiotic relationships with *Rhizobia*. In turn, alfalfa is useful in producing large quantities of protein necessary for milk production. Unfortunately, the advantages of organic forms of N as a fertilizer are often overlooked in modern farming practices. This could be in part attributed to the mixed results from experiments regarding legume addition and the resulting cautious recommendations on legume amendments (OMAFRA, 2007). In general, plow down of alfalfa occurs in the fall as farmers wish to mix in manure that has been applied to the surface while also allowing the breakdown of the thick layer of sod that has built up over continuous years of forage harvesting and heavy field traffic. By plowing alfalfa under in the fall, the highly decomposable fraction of the plant, mostly comprised of leaves, may be mineralized. Further transformations may induce losses in the form of both N_2O as well as leaching losses if nitrification occurs at a level high enough to produce large quantities of NO_3^- . The more N lost throughout fall, winter and spring as a result of leaching and gaseous loss represents N not present for plant growth during the following growing season, especially in early July when crops such as corn need large quantities of N for physical growth.

1.2.3. Fertilizer

The application of synthetic fertilizers varies greatly in both method and required application rate. Additions of AN are not necessary for crops such as alfalfa or soybeans as both are legumes. Corn however requires large quantities of N which is most often applied by broadcast methods. Although exact quantities depend largely on soil nitrate contents prior to planting, recommendations for N application are often as high as 200 kg/ha. In addition to broadcast methods, side dress application of starter fertilizer at the time of planting is widely used. However, synthetic fertilizers have some disadvantages. Since AN is composed entirely of inorganic N it is often subject to high levels of loss. Potential loss of NH_4^+ results from volatilization to NH_3 and thus gaseous loss to the atmosphere. This problem is especially prevalent in fertilizers that are not incorporated immediately after application. Nitrate is subject to losses through leaching since, unlike NH_4^+ , it is an anion and therefore not bound to soil cation exchange sites. Furthermore, due to high quantities of NH_4^+ , nitrification can occur rapidly thus driving further gaseous losses in the form of N_2O . Alternate methods of applying N fertilizer include both liquid forms at the time of planting and/or cultivating prior to planting, in addition to the application of anhydrous ammonia. In some cases N is applied by knifing fertilizer into the inter-row spacing between corn, and application of small quantities of foliar N directly to plant surfaces. Like manure, application of fertilizer with methods other than broadcast and pre-plant side-dressing are far less common. Similarly, this is a result of many farmers lacking the necessary equipment needed to apply fertilizer in gaseous or liquid forms. Foliar N is easily added during the application of herbicide and pesticide;

however the relatively low quantity of N which can be absorbed by plant leaves inhibits it from being widely used.

1.2.4. Field and Incubation Experiments

The focus of the field study is to examine the short term effects of three different amendment types, AN, alfalfa, and manure on biogeochemical processes in an agricultural soil. In order to determine N dynamics within the soil, amendments were added to the soil at rates equivalent to farm conditions in eastern Ontario. Nitrogen transformations and losses were estimated by measuring the amount of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, Total Dissolved Nitrogen (TDN) and Dissolved organic Carbon (DOC) in the soil, in addition to measuring $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ emissions to account for losses from the soil. Ammonia was not measured as incorporation of amendments into the soil was believed to have minimized the likelihood of significant losses through volatilization. A complementary laboratory incubation was performed to determine how controlling environmental variables, specifically moisture and temperature influenced N transformations and losses. Furthermore, a compaction treatment was studied in the lab incubation study to determine what, if any, consequence increased bulk density had on N transformations and losses in the soil.

Chapter 2 examines the findings of the field experiment subsequent to the addition of three types of fertilizer amendments. The relationship between fertilizer additions and transformation processes, mainly mineralization and nitrification, and how the temporal dynamics of inorganic N release changes based on environmental conditions

were closely examined. In addition, TDN as well as DOC were examined to determine what other chemical characteristics may affect the transformation and subsequent loss of inorganic N. In order to determine which, if any, chemical or environmental characteristics were most influential on N loss, soil samples were collected and analyzed in addition to measuring gas fluxes using non-steady-state chambers.

Chapter 3 examines the effects of both amendment type and compaction on soil N dynamics during a lab incubation experiment. Amendment type and relative application rates were maintained from the field experiment; however, two compaction treatments, 1.0 g/cm^3 and 1.5 g/cm^3 , were used. In order to determine how bulk density affected soil N transformations and losses, $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ emissions were measured. Inorganic N was not measured since samples were contained in sealed jars and destructive sampling was not possible. Also, since soils were isolated in sealed jars, leaching losses of N were not possible such that all N and C losses could be attributed to gas emissions. In addition to controlling compaction, incubation allowed strict control over temperature and moisture conditions in the soil. This allowed us to further determine the extent to which environmental controls affect N transformations and losses.

Chapter 4 summarizes the results of both field and laboratory experiments and discusses the relevance of these findings in relation to current agricultural practices. In addition, suggestions are made regarding the potential direction for future research.

2. The Influence of Organic and Inorganic Nitrogen Amendments on Biogeochemical Processes in Eastern Ontario Soils

2.1.Introduction

The N cycle contains many “leaks” which serve as net losses of N from the system. For agriculture, an underlying focus when studying the N cycle is to close these leaks as much as possible to maximize the efficiency of N fertilizer use, and to therefore avoid the introduction of excess quantities of N which could potentially harm the economic viability of agriculture by increasing input cost. Furthermore, leaks in the N cycle also threaten the environment by increasing both levels of greenhouse gases and nitrate in groundwater. To better understand how these processes function at the farm scale, research has focused on studying not only synthetic fertilizers but also traditional forms of fertilizer such as manure and green manures including alfalfa and clover, both of which are legumes which possess the ability to fix atmospheric N. The aim of these studies is to determine ways in which fertilization techniques may be combined to better meet the requirements of the agricultural system while minimizing losses which could be both costly and environmentally damaging. The field experiment reported here had a similar goal, focusing on the typical organic and inorganic fertilizers and fertilization rates applied to soils in Eastern Ontario.

Fertilization of agricultural lands results in the alteration of the N cycle. Organic and inorganic additions increase the immediate quantities of NH_4^+ and NO_3^- available in the soil in addition to potentially increasing the levels of mineralization, immobilization,

nitrification and denitrification. Increases in these transformation processes increase the likelihood of loss of N from the soil through leaching and gaseous losses. Furthermore, fertilizer additions can alter other soil characteristics such as the quantity of soil C. Increases in soil C increase the potential for soil microorganism to consume constituents high in N, thus increasing soil N transformations and losses. Furthermore, a wide range of climatic variables including temperature and soil moisture greatly influence soil N processes.

The objective of this study was to determine the effect of different organic and inorganic amendments applied at rates typical for Eastern Ontario, on the transformation of N in the soil and emission of greenhouse gases. To determine which amendment was most effective, the levels of soil N, the amount of inorganic N were measured 16 times throughout the 133 day field experiment. In addition, gaseous losses of N and C were measured to evaluate the effect that environmental conditions play in the emission of greenhouse gases from soil.

2.2.Materials and Methods

2.2.1. Soils, Experimental Site and Sample Preparation

A 133 day field study was undertaken at the Eastern Cereal and Oilseed Research Centre (ECORC) of Agriculture and Agri-Food Canada located in Ottawa, ON Canada (45 22' N, 75 43' W). The soil type is a Melanic Brunisol (Canadian Soil Classification System) with a fine sandy loam texture (67% sand, 23% silt, 10% clay) and a pH of 6.6. Experimental plots were not planted with crops to reduce the likelihood of interference

from nutrient uptake as well as micro-meteorological disturbances to temperature and moisture dynamics. Through N uptake, contributions of root exudates influencing microbial activity, and other influences, the presence of plants would have increased the difficulty of assessing the predominant processes responsible for N transformation and losses. Gas sampling and mineralization plots were arranged in a randomized block design with additions of alfalfa, liquid cattle manure and ammonium nitrate fertilizer (AN) in addition to control plots. Gas sampling chambers and mineralization plots were located 1 m from the north edge of the experimental site and were placed 0.75 m apart (Figure 2-1). Prior to application of amendments and insertion of gas sampling collars the soil was roto-tilled to ensure homogenization of the soil. In addition, disturbance of the soil reduced bulk density that had occurred in previous field seasons as a result of consolidation, while also aiding in the destruction of weeds and other vegetation. Fluxes of both $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ were measured using non-flow-through non-steady-state chambers (Rochette and Hutchinson 2005). Twenty 55 cm x 55 cm clear acrylic collars were inserted approximately 0.10 m into the soil. The organization and placement of the collars can be seen in (Figure 2-1).

Figure 2-1: Experimental plot map for field study, TT indicates location of Thermocouples

Upon insertion of the collars, soil from within the 10 cm depth inside the collar was excavated out and placed into a large Rubbermaid container. Amendments were added

and mixed using hand tools. Complete removal of the soil allowed for a more thorough homogenization of the soil than could have been provided using other mixing techniques. This technique also made it easier to ensure that there was no cross-contamination between amendment types. Separate containers were used for each amendment and tools were cleaned prior to the addition of a new amendment type. After mixing, the soil was placed back into its respective collar. Soil was then levelled and packed lightly. After allowing one day settling, depth measurements from the soil surface to the edge of the collar were taken at 16 points in each of the twenty collars. These depth measurements were then used to determine the total headspace during gas sampling.

Headspace measurements were taken two more times throughout the sampling season to correct for any consolidation that may have occurred. Each treatment consisted of 5 replicates. Application rates were typical for small scale dairy operations in Eastern Ontario. Ammonium-nitrate was applied at approximately 112 kg ha^{-1} , manure was applied at approximately 30000 L ha^{-1} , and alfalfa was applied at approximately 3320 kg ha^{-1} . In this case the farm of choice was a small commercial farm located in Sarsfield ON, Canada, approximately 40 km east of the experimental site. Alfalfa was collected from fields at this location, as was liquid dairy cattle manure. Samples were analysed for total N using the Kjeldahl method on a TRAACS auto-analyzer (Bran-Luebbe, Analyzing Technologies, Elmsford, NY, USA) (Bremnar, 1965). Ammonium nitrate fertilizer was not collected from this site but was applied at a rate equivalent to that used at this location during spring seeding of corn. Amendments were manually applied to the soil on June 9,

2008, day of year (DOY) 161. Table 2-1 is a description of the amendment characteristics

Table 2-1 Description of the amendments used in the experimental site for dry matter (DM) ammonium N ($\text{NH}_4^+\text{-N}$), nitrate N ($\text{NO}_3^-\text{-N}$), total N (TN), total organic carbon (TOC).

	Application (g N m ⁻²)	DM (g m ⁻²)	NH ₄ ⁺ -N (g N m ²)	NO ₃ ⁻ -N (g N m ²)	TN (μg N g ⁻¹)	TOC (g m ²)	C:N Ratio
Alfalfa	10.6	363	-	-	102.6	145.2	14:1
Manure	9.23	165	8.4	.80	89.7	74.1	7.5:1
AN	11.7	-	5.9	5.9	109.7	-	-

2.2.2. Soil Surface N₂O-N and CO₂-C fluxes

Nitrous oxide and CO₂-C fluxes were measured 16 times throughout the season. Sampling was initially intensive, occurring approximately 2-3 days apart depending on weather conditions as well as to capture larger fluxes soon after application, whereas less frequent sampling took place later in the season when large quantities of N₂O-N and CO₂-C had been emitted from the soil. Prior to placement of the chamber upon the collar at the time of sampling, any plants growing inside the chambers were removed to ensure that no interference with GHG production occurred. For collection of gas samples the collars in the soils were covered with a 0.14 m high plexi-glass chamber (vented and insulated) which covered the same area as the collars. Chambers were also fitted with closed cell foam on the edges to ensure an air tight seal. During gas sampling bricks were used to hold the sampling chamber tightly to the collar to ensure no leaks occurred between the edges (Figure 2-2).

Figure 2-2: Gas sampling collars capped with vented and insulated collars during gas sampling period. Bricks were used to hold chamber tightly to ensure no leaks occurred around edges.

Gas samples of 25 mL were taken from the chamber headspace using a 30 mL syringe fitted with a 26 gauge needle. The gas sample was then injected into a 12 mL pre-evacuated exetainer (Labco, UK) containing approximately 3mg of $\text{Mg}(\text{ClO}_4)_2$. Nitrous oxide was analyzed using a gas chromatograph (Varian 3800, Varian Inc, Walnut Creek, CA) equipped with an electron capture detector (ECD) and a headspace autoinjector (Combi Pal, CTC Analytics, Zurich, Switzerland). The ECD was operated at 390 °C using an Ar/CH_4 carrier gas at 70 mL min^{-1} . Gas separation was obtained using a Haysep N 80/100 pre-column (0.32 cm diam. x 50 cm length) and Haysep D 80/100 mesh

analytical columns (0.32 cm diam. x 200 cm length) in column oven maintained at 65 °C (Gregorich et al. 2007). Carbon dioxide was measured using a flame ionization detector (FID) which was operated at 250 °C using a He carrier gas at 15 mL min⁻¹. At least 3 replicates of four to six standards were included in every gas sampling event to maintain quality control. In addition two He filled standards were used in each run to ensure proper evacuation and storage of vials. The concentration of N₂O (ppmv) was estimated based on a quadratic calibration equation, while CO₂-C (ppmv) was estimated based on a linear calibration. Soil N₂O-N and CO₂-C gas fluxes (µg N₂O-N m⁻² h⁻¹) were calculated using the following:

$$Emission\ of\ N_2O = \frac{\left(\frac{\Delta N}{\Delta t} * P\right) \left(\frac{R}{T}\right) * 60 * \left(\frac{V}{A}\right) M}{t}$$

Where change in the concentration of N₂O (µL N₂O-N L⁻¹ min⁻¹) was estimated based on a quadratic equation fitted to chamber [N₂O] in ppmv vs. time in minutes; P is atmospheric pressure at the study site; R= Ideal gas constant= 0.082057 µL atm K⁻¹ µMol⁻¹; T= In situ air temperature (K); 60 = minutes per hour; V= chamber headspace volume (L); A= chamber area m²; M= molar conversion, 28 g N/mol N₂O and 12 g C/mol CO₂.

In all cases cumulative emissions of both N₂O-N and CO₂-C were estimated based on linear interpolation between sampling dates. This method assumes that values measured on each sampling date were representative of the mean daily values. Daily air

temperature, precipitation, relative humidity, and air pressure were obtained from the Central Experimental Farm Weather Station within 400 m of the study site.

2.2.3. Soil Mineralization and Lab analysis

Mineralization plots were excavated and mixed in the same manner as the gas plots; these plots were twice the size of gas plots to ensure there would be enough soil for prolonged sampling. Subsequent to replacement of the soil back into the mineralization plots one brass core was placed in each plot. Cores were left in the soil until the next sampling period, at which point they were removed and the soil was placed into pre-labelled plastic bags and sealed. Subsequent to the removal of each core, new cores were placed in the soil where they were left until the next sampling period. Like gas sampling chambers, mineralization plots were picked clean of any growing plants at each sampling date. Soil moisture was measured using a time domain reflectrometry Soil Moisture Meter (Spectrum Technologies Inc, Plainfield IL). Soil temperature was recorded using thermocouples (Labcor Products Inc, Fredrick MD). A total of 5 thermocouples were buried at 5 cm in the 0.75 m space between the gas sampling chambers and the mineralization plots at five locations throughout the experimental site (see Figure 2-1). In the lab each soil sample was weighed before having a 10 g subsample removed and placed in a small tin and oven dried at 110 °C for a period no less than 1 day. Samples were subsequently removed and weighed to attain measurements for bulk density. Bulk density (β , g m⁻³) was calculated using the following: $\beta = \text{oven dry soil weight} / \text{total core volume}$. In addition, this procedure allowed us to calculate gravimetric water content (v), volumetric water content (θ), soil porosity (ϕ) and water filled pore space (WFSP) as

follows: $v = ((\text{Wet weight of soil} - \text{dry weight of soil}) / \text{dry weight of soil})$; $\theta = v \times \beta / \rho_w$ where ρ_w is the density of water (g m^{-3}); $\phi = (1 - \beta) / \rho_s$ where ρ_s is soil particle density = 2.65 g m^{-3} ; $\text{WFPS} = (\% \theta) / (1 - (\beta / \rho_s))$. For NH_4^+ and NO_3^- analysis, subsamples from each core weighing approximately 10 g were placed into 250 ml wide mouth, flat bottom Centrifuge bottles (Labcor Products Inc, Fredrick MD). Each sample was then mixed with 100 ml of 2 M KCl, sealed and shaken for 1 hour. After shaking was complete, samples were filtered through Whatman #42 filter paper and sealed in 100 ml plastic containers. Since immediate analysis was not possible, samples were frozen until analysis. Samples were analysed for total N using the Kjeldahl method on a TRAACS auto-analyzer (Bran-Luebbe, Analyzing Technologies, Elmsford, NY, USA) (Bremnar, 1965). Extracts were also collected for determination of TDN and DOC. Fifteen gram subsamples from each core were mixed with 30 ml of 0.5 mM CaCl_2 . Extracts were centrifuged at 3295 g for 20 min. The resulting supernatant was then filtered under vacuum through a $0.45 \mu\text{m}$ cellulose nitrate membrane filter (Kalbitz et al., 2003). Filtrates were frozen and stored until analysis. Total dissolved nitrogen and DOC extracts were measured using a Shimadzu 5050 analyser (Shimadzu, Columbia, MD).

2.2.4. Statistical Analysis

Multiple statistical analyses were undertaken to determine significant relationships between treatments and response variables as well as to test for correlations between independent environmental variables. Statistical tests started at a broad scale and became more specific depending on the time period of interest. At the largest scale a single Multivariate Analysis of Variance (MANOVA) was performed to determine if

there was a significant overall effect of amendment addition and the response of several dependent variables, in this case $\text{N}_2\text{O-N}$, $\text{CO}_2\text{-C}$, TDN, DOC, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ production. The use of a MANOVA allowed for one test on all variables thus avoiding the likelihood of a Type 1 error when using numerous individual tests on the same hypothesis. This and all following tests were held to a 0.05 significance level. Subsequent to the completion of this first broad MANOVA, 6 individual ANOVAs using repeated measures were done to determine the effect that time had on the dependent variables, excluding the last 3 sampling dates when response variables had returned to baseline values. Upon completion of the repeated measures ANOVA, two different series of Analysis of Variance (ANOVAs) were run. The first was used to determine the significance of each treatment in relation to the dependent variables. Four separate ANOVAs were then done on the blocks of data which had been divided into 4 periods: „early’ (DOY 162-172, June 11th to June 21st), „middle’ (DOY 176-186, June 25th to July 5th), „late’ (190-207, July 9th to July 26th) and lastly „fall’ (DOY 225-294, August 13th to October 19th) periods. Division of the data was based on observed trends in both the environmental and response data. Tukey’s Honestly Significant Difference (HSD) post-hoc tests were used to establish differences between individual amendment types and the response variables. In all cases, a Bonferroni correction was applied to all ANOVAs to decrease the likelihood of creating a Type I error as a result of repeated testing of the same hypothesis. Using the Bonferroni correction, significance was reduced thus forcing more stringent limitations on the p value for each variable. In all cases significance was reduced by $0.05/n$, where n is the number of ANOVAs produced. In all statistical

analyses, the response variables were either log or –log transformed to attain as closely as possible normality and equality of variance, both assumptions necessary for parametric statistics. To ascertain where correlations occurred between independent variables, a non-parametric Spearman's Rho (ρ) test was done on the four blocked sampling periods (early, middle, late and fall). This analysis was done on non-transformed data since Spearman's rho is a non-parametric test, and as such normality and equality of variance are not required assumptions of the test. All statistical analysis was done using JMP 7 Statistical Software (SAS Institute).

2.3.Results

2.3.1. Environmental Variables

Precipitation was frequent throughout the study period occurring almost every day during the early, middle and late sampling periods (Figure 2-3). Day 186-192 was the longest period without rain until late August, which was long after the initial intensive sampling. Rainfall events were often intense and involved large volumes of precipitation. (e.g. Day 167 received over 27 mm of rain). Examination of the climate normals from 1971 to 2000 indicates that rainfall during the experimental period was not substantially higher than an average year. However, June did receive approximately 20% more rainfall than normal. This meant that conditions were wetter on a whole than normal for this particular month. Climate data indicates that as much rain was received after application of amendments on June 9th as was normal for the entire month of June. Given that amendments had recently been added to the soil at this time it is likely that increased

quantities of precipitation contributed to losses associated with high soil moisture (Environment Canada, 2009). Cumulative rainfall during the sampling season was 289 mm. More important than the cumulative patterns however is the timing with which rainfall was delivered. The largest cumulative rainfall was received in the fall period, in which 129.8 mm fell; this accounted for 44% of the total sampling season precipitation, however, this period was 86 days long. When compared to other periods, specifically the early period which received 65.6 mm of rain in only 10 days, which accounted for 22% of the cumulative rainfall, followed by the middle period with 13% total cumulative rainfall in 10 days and the late period with 16% cumulative rainfall in 16 days. Furthermore, large volumes of rain occurred during periods in which high levels of N were present in the soil. This could have contributed to substantial losses of N via leaching.

Figure 2-3: Daily total precipitation throughout the 133 day study period

Air temperature data collected from the Central Experimental Farm Weather Station showed that air temperatures were relatively consistent throughout the sampling season (Figure 2-4). In addition, the daily average soil temperature collected from the thermocouples at the site followed the trend observed in air temperature. The greatest variations in soil temperature were observed between the late summer period and the fall sampling period at which time there was a nearly 10 °C drop in soil temperature (Figure 2-4). However, while the early, middle and late periods did have similar temperatures,

there were large variations in individual sampling days. For instance, there was a 7 °C difference between the first and last day of the early period. Climate data indicated that temperatures were not above normal during the sampling season (Environment Canada, 2009).

Figure 2-4: Daily average soil temperature (n = 5) and air temperature throughout the 133 day study period

Soil temperature, WFPS, and VWC were significantly correlated with each other for the duration of the experiment indicating the interdependence of environmental variables.

The effects of these large rainfall events can be seen when examining both VWC and WFPS. Both are dependent on rainfall but also have significant effects on nitrification and denitrification. Increased WFPS and VWC occur in direct accordance with rainfall events (Figure 2-5). More importantly, large rainfall events seen specifically on DOY 167 drive WFPS to levels nearing the threshold of 60%, a point at which denitrification begins to occur at increased rates. It is likely that both nitrification and denitrification processes are responsible for losses of N from the soil by N_2O -N production.

Figure 2-5: Dynamics of water filled pore space (WFPS) and volumetric water content (VWC) during the study period

The use of the MANOVA and ANOVAs with repeated measures (henceforth referred to as RM ANOVA) were able to provide a broader view of the treatment effects than the ANOVAs and Spearman's Rho tests of correlation, however, they give little insight regarding short term dynamics during study periods. Overall, there was a significant treatment effect ($F = 5.45$, $df = 851.84$, $p < 0.001$). The RM ANOVA was

not conducted on the entire data set. Instead, the last three sampling dates which accounted for the entire fall sampling period were excluded. These sampling dates were excluded due to the high number of zero and null values as the gas fluxes returned to levels below instrument detection levels. Excluding these days decreased the likelihood of making type II errors in rejecting time $\times$ treatment interactions as not significant. The results of the RM ANOVA are summarized in Table 2-2 below.

Table 2-2: Results of the repeated measures Analysis of Variance for all response variables on all data. Degrees of freedom for the test examining the interaction between time and treatment are 39 and 12.59. Degrees of freedom for treatment for the test examining treatment effect are 3 and 16.

	Exact F	All between	Approx. F	Time x Treatment	Approx. F	Time
CO ₂ -C	58.13	***	3.49	*	138.26	***
N ₂ O-N	10.39	**	1.55	NS	19.65	**
TDN	61.50	***	3.02	*	307.43	***
DOC	6.33	**	1.21	NS	39.88	**
NO ₃ ⁻	30.40	***	3.43	*	89.23	**
NH ₄ ⁺	14.56	***	3.83	**	30.40	**

*** P < 0.001, ** P < 0.01, * P < 0.05, NS- Not Significant

Table 2-2 shows the effect of treatment on the response variables were usually highly significant (P<0.0001). Furthermore, time was significant in all treatments. However, the time $\times$ treatment interaction was not significant in all cases. Results of the RM ANOVA indicate that N₂O-N and DOC did not show a significant time $\times$ treatment interaction, while all other variables showed significance at P<0.05.

2.3.2. Nitrous Oxide Fluxes

Figure 2-6 shows the seasonal fluxes of N₂O-N emissions for the duration of the study period. For each treatment the N₂O-N fluxes peaked on DOY 168 with the exception of AN which peaked 4 days later on DOY 172. The highest N₂O-N fluxes were seen in the manure treatment with 151.4 µg N₂O-N m⁻² hr⁻¹ followed by alfalfa with 143.8 µg N₂O-N m⁻² hr⁻¹, AN with 73.2 µg N₂O-N m⁻² hr⁻¹ and control plots with 37.0 µg N₂O-N m⁻² hr⁻¹. These high fluxes reflect the chemical nature of the amendment. Manure contained high levels of NH₄⁺-N which most likely had sufficient time to nitrify before heavy rainfall on DOY 167 induced high WFPS which increased the rate of nitrification while initiating conditions conducive to denitrification. Addition of alfalfa was similar but did not release equal quantities of N₂O-N compared to the manure treatment since alfalfa contained only organic forms of N at time of application and therefore had less NH₄⁺-N available for immediate nitrification and denitrification. Furthermore, statistical analysis indicated that alfalfa and manure were not statistically different regarding emissions during DOY 168. Ammonium nitrate and control plot N₂O-N production was lower, most likely as a result of low levels of C in the case of AN or N and C in the case of the control limiting microbial activity. Nitrous oxide emissions slowly decreased after DOY 168, however there were short term fluctuations in N₂O-N emissions, most notably between DOY 182 and 186. Slightly drier, warmer conditions between DOY 177 and 180 could have increased nitrification which increased N₂O-N fluxes. Heavy rains on DOY 181 increased both WFPS and VWC which could have increased both nitrification and denitrification, both of which would have increased N₂O-

N production. Day 198 also showed a slight increase in N₂O-N fluxes; however, this was only strongly visible in the alfalfa plots. A decrease in the levels of DOC in the soil at this point in manure, AN and control treatments may have made decreased microbial activity responsible for N₂O-N production. This indicates that, while N₂O-N emissions were slower initially, alfalfa is capable of releasing equivalent, if not higher, quantities of N₂O-N than other amendments given sufficient time and proper conditions. In all treatments N₂O-N emissions declined by about DOY 225 as soil temperature decreased.

Figure 2-6: N₂O-N fluxes during the experimental season. Bars located above the graph denote early, middle, late and fall sampling periods.

For each of the sampling periods the cumulative N₂O-N was calculated and is shown in Table 2-3. Cumulative N₂O-N was estimated by linearly interpolating data between sampling periods. The total N₂O-N emitted was substantially higher during the early period. Sharp decreases in the quantity of N₂O-N produced occurred as the sampling season progressed. Cumulative release of N₂O-N within the first 10 days accounted for 47% of the total release over 133 days in alfalfa plots, 51% in manure plots, 37% in AN

plots and 34% in control plots. Results of the Tukey's Kramer HSD test (Table 2-3) indicate that significant differences between amendment types occurred throughout the experimental season. Cumulative totals indicate that alfalfa was significantly different than control, but manure and AN were not. Furthermore, cumulative production of N₂O-N fluxes was not significantly different between manure and AN plots when compared to control plots.

Table 2-3: Cumulative N₂O-N emissions for the different periods during the study. Values in brackets indicate the percent total N₂O-N emitted since DOY 162. Different letters indicate significant differences as determined by Tukey's Kramer HSD.

Cumulative N ₂ O-N ($\mu\text{g N}_2\text{O-N g dry soil}^{-1}$)					
Treatment	Early Season (DOY 162-172)	Middle Season (DOY 176-186)	Late Season (DOY 190-207)	Fall (DOY 225-294)	Total
Control	0.06(34)a	0.03(18)a	0.03(17)a	0.05(31)a	0.18b
Alfalfa	0.24(47)c	0.10(19)c	0.08(16)a	0.09(18)a	0.51a
Manure	0.16(51)bc	0.06(17)b	0.05(15)a	0.05(17)a	0.32ab
AN	0.12(37)b	0.08(26)c	0.07(23)a	0.04(14)a	0.31ab

Table 2-4 summarizes the ANOVA results on all 6 response variables including all sampling dates. In contrast to the results where the last 3 sampling dates are excluded (Table 2-4), some treatment effects are no longer significant. However, treatment effects on N₂O-N were statistically significant for the full data set even though fluxes were not significantly different among treatments during the fall period. Temporal variations in N₂O-N fluxes in the alfalfa treatment were found to be significantly correlated to CO₂-C, DOC in the early sampling period ($\rho = -0.43$ and $\rho = 0.42$ respectively) (Appendix). All

treatments showed a significant correlation to WFPS during the first sampling period (alfalfa $\rho = 0.63$, control $\rho = 0.70$, AN $\rho = 0.55$ and manure $\rho = 0.60$) (Appendix).

Table 2-4: Results of Analysis of Variance for total data set and selected sampling periods

DOY	df	CO ₂ -C	N ₂ O	DOC	TDN	NO ₃ ⁻	NH ₄ ⁺
All days	339	***	***	NS	NS	***	NS
162-172	99	***	***	NS	**	***	***
176-186	99	***	***	NS	***	***	NS
190-225	79	NS	NS	NS	**	**	NS
225-294	59	NS	NS	NS	NS	NS	NS

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS- Not Significant

2.3.3. Carbon Dioxide Fluxes

Emissions of CO₂-C were highest at the beginning of the experiment for the alfalfa and manure treatments (Figure 2-7). CO₂-C flux was highest in the alfalfa treatments peaking at 562 mg CO₂-C m⁻² hr⁻¹ on DOY 162 followed by manure at 185 mg CO₂-C m⁻² hr⁻¹ on the same date. In contrast, CO₂-C fluxes reached their maximum later in the study period for the AN treatment (137 mg CO₂-C m⁻² hr⁻¹ on DOY 198) and the control treatment (125 mg CO₂-C m⁻² hr⁻¹) on DOY 198.

Figure 2-7: CO₂-C fluxes during the experimental season. Bars located above the graph denote early, middle, late and fall sampling periods.

This highlights the role of C in N₂O-N production under certain circumstances. Microbial activity responsible for denitrification depends strongly on C as a substrate, and as such, high CO₂-C emissions are due to greater rates of microbial activity. Results of the ANOVA (Table 2-4) show that CO₂-C emissions differed significantly between treatments in the first two sampling periods up to the point at which labile C had been consumed. Results of the Tukey's Kramer HSD test (Table 2-5) indicate that alfalfa was

significantly different from manure, which was significantly different from AN and control plots. Control and AN plots were not significantly different in CO₂-C production during the early period. In the middle sampling period alfalfa and manure were no longer significantly different from one another but were both significantly different from AN and control plots. By the fall sampling periods no significant differences existed. Carbon dioxide increased in the late sampling period to levels higher than that seen in the middle and fall sampling period. Significant differences existed between control and alfalfa plots. Inspection of the precipitation data shows what appeared to be a strong relationship between CO₂-C flux and rainfall events. Water filled pore space was strongly correlated to CO₂-C fluxes in all 4 treatments during the early period (alfalfa $\rho = -0.67$, control $\rho = -0.67$, AN $\rho = -0.75$ and manure $\rho = -0.53$) (Appendix). Soil temperature also showed significant correlation to CO₂-C production indicating that microbial activity is dependent on soil temperature (alfalfa $\rho = 0.81$, control $\rho = 0.78$, AN $\rho = 0.81$ and manure $\rho = 0.72$) (Appendix).

Despite determining that CO₂-C was significantly related to soil temperature, VWC, WFPS and DOC for the early period of sampling (Appendix), none of these relationships were still significant during the middle period, however some of these relationships did become significant again in the late period and continued to be so into the fall. In all, CO₂-C showed a greater number of significant relationships to environmental variables than N₂O-N. Nitrous oxide emissions had more significant relationships with amendment. This indicates that amendment type may have played less

of a role than environmental drivers in influencing CO₂-C while the opposite was true for N₂O-N production.

Table 2-5: Cumulative CO₂-C emissions for the different periods during the study. Values in brackets indicate the percent total CO₂-C emitted since DOY 162. Different letters indicate significant differences as determined by Tukey's Kramer HSD.

Cumulative CO ₂ -C (µg CO ₂ -C g dry soil ⁻¹)					
Treatment	Early Season (DOY 162-172)	Middle Season (DOY 176-186)	Late Season (DOY 190-207)	Fall (DOY 225-294)	Total
Control	0.20(11)a	0.19(10)a	0.42(23)a	0.93(55)a	1.70a
Alfalfa	0.26(29)c	0.26(10)b	0.52(20)b	0.94(41)a	2.48c
Manure	0.31(19)b	0.26(12)b	0.45(23)ab	0.81(49)a	1.83bc
AN	0.21(11)a	0.19(10)a	0.47(25)ab	0.89(54)a	1.75ab

Table 2-5 is a summary of the cumulative CO₂-C emissions throughout the sampling season. Unlike N₂O-N, the release of CO₂-C was more prolonged. This occurred because CO₂-C production is dependent on microbial activity under aerobic conditions while N₂O-N production can occur under both aerobic and anaerobic conditions. In alfalfa plots, approximately 29% of the total CO₂-C was released during the early season sample, while manure released 19%, AN 11 % and control 11%. Alfalfa contains large quantities of C and is therefore capable of maintaining a larger and more active microbial population which should be capable of producing higher quantities of CO₂-C than other treatments.

2.3.4. Ammonium Production

Levels of NH₄⁺-N were significantly affected by the application of amendments to the soil. Ammonium production was highest in the AN treatments at 25.6 µg NH₄⁺-N g

dry soil⁻¹ on DOY 163 (Figure 2-8). Manure had the second highest quantity of NH₄⁺-N with 11.1 µg NH₄⁺-N g dry soil⁻¹ on DOY 162. Alfalfa slightly lagged behind other treatments reaching a maximum on DOY 165 with 7.4 µg NH₄⁺-N g dry soil⁻¹. Control plots showed very little production, with highest levels reaching 1.6 µg NH₄⁺-N g dry soil⁻¹ on DOY 168. Highest average levels of NH₄⁺-N were recorded in AN treatments, followed by manure, alfalfa and control (Table 2-6). Despite differences, results of the Tukey's HSD test show that AN, manure and alfalfa treatments were not significantly different from one another in the early period, but were significantly different from the control. Results of the Tukey's Kramer HSD indicate that over the total duration of the experiment, average NH₄⁺-N in AN plots was significantly different from alfalfa, manure and control (Table 2-6).

Figure 2-8: $\text{NH}_4^+\text{-N}$ quantities in the soil during the experimental season. Bars located above the graph denote early, middle, late and fall sampling periods. Arrows indicate increases in $\text{NH}_4^+\text{-N}$ quantities in the soil on DOY 179-182 and DOY 193-198.

Unlike cumulative gas fluxes, average quantities of inorganic N (both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) were not linearly interpolated but were instead derived by summing the average values for each sampling period. Quantities of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ could not be interpolated due to the inability to account for losses from leaching which would have contributed to cumulative quantities.

Table 2-6: Average $\text{NH}_4^+\text{-N}$ production for different periods of time throughout the experimental season. Values in brackets indicate cumulative amount of $\text{NH}_4^+\text{-N}$ produced.

Treatment	Average $\text{NH}_4^+\text{-N}$ ($\mu\text{g NH}_4^+\text{-N g dry soil}^{-1}$)				
	Early Season (DOY 162-172)	Middle Season (DOY 176-186)	Late Season (DOY 190-207)	Fall (DOY 225-294)	Total
Control	0.86(36.6)a	0.72(31.2)a	0.60(20.4)a	0.46(11.8)a	2.64a
Alfalfa	4.28(71.1)b	0.87(14.5)a	0.75(10.0)a	0.43(4.4)a	6.35a
Manure	5.20(76.2)b	0.81(11.9)a	0.67(7.9)a	0.46(4.0)a	7.14a
AN	11.13(89.2)b	0.75(6.0)a	0.52(3.4)a	0.29(1.4)a	12.70b

Over the duration of the experiment, AN plots had approximately double the average amount of $\text{NH}_4^+\text{-N}$ when compared with manure and alfalfa (Table 2-6). Ammonium nitrate contains large quantities of $\text{NH}_4^+\text{-N}$, as does manure, while alfalfa contains only organic N which must be mineralized to $\text{NH}_4^+\text{-N}$. Ammonium-nitrate and manure treatments therefore add large quantities of $\text{NH}_4^+\text{-N}$ immediately upon addition. Although AN and manure added large amounts of $\text{NH}_4^+\text{-N}$ to the soil, transformation processes, mainly mineralization, were not necessary. Alfalfa surpassed manure in production of $\text{NH}_4^+\text{-N}$ after the second sampling date which indicates that intense mineralization occurred rapidly and liberated substantial quantities of $\text{NH}_4^+\text{-N}$. Unlike initial quantities of $\text{NH}_4^+\text{-N}$ in the soil from AN and manure treatments, this $\text{NH}_4^+\text{-N}$ was mineralized from organic N, not simply added. Under field conditions nitrification most likely occurred quickly, decreasing levels of $\text{NH}_4^+\text{-N}$, which explains why high levels of $\text{NH}_4^+\text{-N}$ occur only one sampling day before the highest levels of $\text{N}_2\text{O-N}$ (Figure 2-8). Aside from the high initial production of $\text{NH}_4^+\text{-N}$ there was little variation in production,

with the exception of a brief period between DOY 180 and 190 indicating increased levels of mineralization.

Over the duration of the experiment, only the AN plot had a significantly greater NH_4^+ -N production than the other treatments and control plots (Table 2-6). In contrast, in the first sampling period fertilizer, manure and alfalfa were significantly different from the control. Presumably, by the end of the early sampling period, the majority of organic N had been mineralized. As such, only low levels of NH_4^+ -N remained as most had probably already been nitrified within a short time following mineralization. Only in the early sampling period was there a substantial number of significant relationships between NH_4^+ -N and other response variables. During the early period, all treatments, with the exception of the control, showed significant correlations with VWC (alfalfa $\rho = -0.48$, AN $\rho = -0.62$ and manure $\rho = -0.69$), WFPS (alfalfa $\rho = -0.56$, AN $\rho = -0.59$ and manure $\rho = -0.60$), N_2O -N (AN $\rho = -0.58$ and manure $\rho = -0.40$), TDN (alfalfa $\rho = 0.54$, AN $\rho = 0.62$ and manure $\rho = 0.47$), and NO_3^- (alfalfa $\rho = 0.63$, AN $\rho = 0.81$ and manure $\rho = 0.60$) (Appendix). This is not surprising since soil temperature and aeration govern the mineralization process, while the quantities of NH_4^+ -N control the amount of nitrification which ultimately contribute to the levels of N_2O -N produced.

2.3.5. Nitrate Production

Like NH_4^+ -N, NO_3^- -N showed a strong response to the addition of a treatment to the soil. The highest initial quantities of NO_3^- -N occurred in AN plots with $57 \mu\text{g NO}_3^- \text{N g dry soil}^{-1}$ on DOY 163, only one day after application (Figure 2-9). Manure and

alfalfa levels were considerably lower with $16 \mu\text{g NO}_3^- \text{-N g dry soil}^{-1}$ and $13 \mu\text{g NO}_3^- \text{-N g dry soil}^{-1}$ respectively. Control plots remained low at $5.5 \mu\text{g NO}_3^- \text{-N g dry soil}^{-1}$ (Figure 2-9). Increases in $\text{NO}_3^- \text{-N}$ levels were not instantaneous; rather they lagged slightly depending on the treatment. In AN plots, $\text{NO}_3^- \text{-N}$ levels reached a maximum only 1 day after application, compared to manure which did not reach a maximum until 4 days after application and alfalfa 7 days after application.

Figure 2-9: $\text{NO}_3^- \text{-N}$ quantities in the soil during the experimental season. Bars located above the graph denote early, middle, late and fall sampling periods.

This is likely a result of the large quantities of NO_3^- -N added directly in the case of AN compared with other treatments where changes in NO_3^- -N levels were a function of NO_3^- -N production through nitrification. As such, manure required relatively little time to nitrify NH_4^+ -N, while the organic N in alfalfa must undergo mineralization and then nitrification to attain substantial levels of NO_3^- -N. This explains the lag time in production between manure and alfalfa.

Nitrate also showed strong seasonal variations. There are three discernable increases in NO_3^- -N during the season. Unlike the initial pulse of NO_3^- -N, the two smaller pulses were greatest in magnitude and duration in the alfalfa treatments. During the second pulse (~ DOY 179) maximum NO_3^- -N levels were $8.3 \mu\text{g NO}_3^-$ -N g dry soil^{-1} in alfalfa plots which was more than twice as much as AN and manure plots which were both approximately $3 \mu\text{g NO}_3^-$ -N g dry soil^{-1} , while control plots contained $1.2 \mu\text{g NO}_3^-$ -N g dry soil^{-1} . The third and most prolonged increase in NO_3^- -N (~DOY 186-207) had even higher levels reaching $13 \mu\text{g NO}_3^-$ -N g dry soil^{-1} in alfalfa, followed closely by AN with $12.7 \mu\text{g NO}_3^-$ -N g dry soil^{-1} , manure with $8 \mu\text{g NO}_3^-$ -N g dry soil^{-1} and control with $6.9 \mu\text{g NO}_3^-$ -N g dry soil^{-1} . Similar to the first pulse there was a slight difference in timing with alfalfa increasing 3 days later than fertilizer and manure treatments, again, presumably attributable to time needed to mineralize and nitrify N. These dynamics suggest that despite little production of NH_4^+ -N, mineralization must have been occurring in order to supply N for nitrification and thus the production of NO_3^- -N.

Despite higher and more prolonged increases in NO_3^- -N levels in the alfalfa treatment during the second and third sampling periods, AN treatments resulted in the greatest increase in NO_3^- -N with a total of $185 \mu\text{g NO}_3^-$ -N g dry soil⁻¹, followed by alfalfa with $102 \mu\text{g NO}_3^-$ -N g dry soil⁻¹ and manure with $71 \mu\text{g NO}_3^-$ -N g dry soil⁻¹. However, average results show that alfalfa treatments were responsible for the release of larger quantities of NO_3^- -N in the middle, late and fall sampling periods with 4.5, 10.2 and 1.2 $\mu\text{g NO}_3^-$ -N g dry soil⁻¹ respectively, in comparison to AN which produced 2.0, 7.9 and 0.6 $\mu\text{g NO}_3^-$ -N g dry soil⁻¹ for the same three sampling periods (Table 2-7).

Table 2-7: Average NO_3^- -N production for different periods of time throughout the experimental season. Values in brackets indicate cumulative amount of NO_3^- -N produced.

Treatment	Average NO_3^- -N ($\mu\text{g NO}_3^-$ -N g dry soil ⁻¹)				
	Early Season (DOY 162-172)	Middle Season (DOY 176-186)	Late Season (DOY 190-207)	Fall (DOY 225-294)	Total
Control	3.88(45.3)a	0.90(10.4)a	4.21(39.3)a	0.75(5.1)a	9.71a
Alfalfa	7.08(34.7)a	4.45(21.8)b	10.21(40.0)b	1.19(3.5)a	22.93b
Manure	7.54(52.8)a	1.35(9.5)a	6.03(33.8)a	0.94(3.9)a	15.86ab
AN	28.22(76.5)b	2.01(5.5)ab	7.90(17.1)ab	0.59(1.0)a	38.72c

The differences seen here may indicate that while the initial amount of NO_3^- -N released was not as high as for AN treatments, the dynamics of when the release occurred may be useful when examining the release and availability of N for plant uptake. The Tukey's Kramer HSD test indicates that total production of NO_3^- -N in AN plots were significantly different from alfalfa, manure and control plots. Alfalfa and manure were not significantly different from one another but were both significantly different to control

plots. Results of the ANOVA indicate that amendment type had a significant effect on NO_3^- -N levels for the entire sampling period and for early, middle and late sampling periods (Table 2-4). Early in the sampling season, only AN produced significantly more NO_3^- than the control. As the sampling season progressed, alfalfa also became significant in producing higher quantities of NO_3^- and as a result produced significantly more NO_3^- in total. This indicates that some amendments, specifically alfalfa, require considerable periods of time to produce quantities of NO_3^- -N similar to AN. Temporal variations in NO_3^- -N levels treatments were often significantly and positively correlated to soil temperature and to a greater extent TDN (Appendix). Increases in soil temperature inherently increases microbial activity. Increased microbial activity increases the rate at which nitrification occurs and the relative quantities of NO_3^- -N in the soil. In the early period all treatments were also related strongly to WFPS (alfalfa $\rho = -0.45$, control $\rho = -0.43$ AN $\rho = -0.62$ and manure $\rho = -0.58$) (Appendix). Nitrification was governed by WFPS, increasing until WFPS reached a critical limit at approximately 60% at which point nitrification began to decrease. Water filled pore-space was statistically significant when compared to all treatments in the early sampling period. By the fall period it no longer affected alfalfa, but did affect all other treatments.

2.3.6. Total Dissolved Nitrogen Production

The dynamics of the TDN followed a pattern very similar to NO_3^- -N (Figure 2-10). The highest levels of TDN production occurred within the first 10 days. In all treatments the TDN levels were highest on DOY 163, only 3 days after the application of the treatments (Figure 2-10). Ammonium-nitrate had the highest levels with 208 $\mu\text{g N g}$

dry soil⁻¹ followed by manure with 108 µg N g dry soil⁻¹ and alfalfa with 96 µg N g dry soil⁻¹. Similar to NO₃⁻-N, TDN was subject to two more large fluctuations throughout the sampling period, both of which occurred at times corresponding to NO₃⁻-N peaks. Nitrate had a larger influence over the levels of TDN in the soil since, of the two N constituents, there is a substantially larger quantity of NO₃⁻-N than NH₄⁺-N. Results of the Spearman's rho correlation analysis confirm this. As shown in the Appendix, correlations between TDN and NO₃⁻-N are consistently higher than correlations between TDN and NH₄⁺-N. Furthermore, significant correlations occur more often between TDN and NO₃⁻-N than TDN and NH₄⁺-N. A summation of the average cumulative quantities of NO₃⁻-N and NH₄⁺-N for all sampling periods indicate that NO₃⁻-N ranged from 2.2 to 3.6 times greater accumulation in the soil than NH₄⁺-N. In both high fluctuations following the initial pulse, the alfalfa TDN levels were higher than all other variables, however, AN plots seem to respond faster than all other plots, with TDN levels increasing one sampling date earlier than other treatments. Levels of TDN rose towards the end of the season. DOY 294, the final sampling day, saw TDN higher than a number of sampling days early in the season indicating the possibility of a process of TDN release which functions slowly with time. ANOVA results show that TDN was not significantly related to the treatments when tested for the entire sampling period (Table 2-4). However treatment had a significant effect on TDN in all but the fall sampling period. The Spearman's Rho correlation showed few significant relationships throughout the sampling periods (Appendix). Early in the season TDN showed some significant relationships with VWC (AN ρ = -0.48 and manure ρ = -0.42) and WFPS (AN ρ = -0.48 and manure ρ = -0.41)

(Appendix). The middle sampling period had stronger correlations between TDN and soil temperature in most treatments while the late and fall sampling periods showed no significant correlations between TDN and any variable in all treatments (Appendix).

Figure 2-10: Total Dissolved Nitrogen (TDN) quantities during the experimental season. Bars located above the graph denote early, middle, late and fall sampling periods.

2.3.7. Dissolved Organic Carbon

Dissolved organic carbon was relatively low throughout the season with the exception of the initially high pulse seen in the early sampling period (Figure 2-11).

During the middle sampling period DOC experienced a secondary, smaller increase followed by a reduction before eventually returning to background levels. Although most variations were fairly gradual, there were short term differences between treatments within the early sampling period. Most notably, DOC levels in all treatments with the exception of alfalfa drop quickly on DOY163 before increasing on DOY 165 and eventually declining.

Figure 2-11: Dissolved Organic Carbon (DOC) quantities throughout the sampling season. Bars located above the graph denote early, middle, late and fall sampling periods.

DOC in Alfalfa plots declined more smoothly, showing a decrease on DOY 163. Treatment did not have a significant effect on DOC levels for any period nor for all days of the study period (Table 2-4). There were also very few significant correlations between DOC and other variables. Dissolved organic carbon was positively and significantly correlated to soil temperature in all treatments throughout the first sampling period (alfalfa $\rho = 0.81$, control $\rho = 0.42$ AN $\rho = 0.61$ and manure $\rho = 0.63$), however, these correlations did not continue in all treatments throughout the middle, late and fall sampling periods (Appendix). There were no other consistent relationships between DOC and environmental variables

2.4. Discussion

2.4.1. N Dynamics

2.4.1.1. Mineralization

Overall $\text{NH}_4^+\text{-N}$ was measured to be greatest in the AN plots however, 89% of this was in the first few days after application. Alfalfa and manure plots had lower concentrations of $\text{NH}_4^+\text{-N}$ overall but slightly higher concentrations of $\text{NH}_4^+\text{-N}$ in the later part of the experiment when plants would benefit most from higher quantities of inorganic N in the soil. Initial levels of $\text{NH}_4^+\text{-N}$ were high in all amendments, with AN plots having highest concentrations, followed by manure, alfalfa and controls. These high levels of $\text{NH}_4^+\text{-N}$ result from the fact that both AN and manure contain high levels of $\text{NH}_4^+\text{-N}$, while alfalfa contains little if any inorganic N. Within the first sampling period the quantities of $\text{NH}_4^+\text{-N}$ drop dramatically as $\text{NH}_4^+\text{-N}$ is either immobilized or

transformed. It is the dynamics that occur after this initial drop that indicate the effect that each amendment has on mineralization dynamics within the soil.

As Figure 2-8 shows, all amendments show an increase in $\text{NH}_4^+\text{-N}$ on DOY 179-182. Often the quality of the organic matter inputs, related to degradability resulting from low C:N ratio, is the key to mineralization (Huang et al., 2004; Robertson and Groffman, 2005). The results of the field sampling support these conclusions. The initial high quantities can be attributed to both AN and manure having high $\text{NH}_4^+\text{-N}$ contents prior to application. Alfalfa requires time to mineralize the high levels of organic N contained within it. Subsequent to the initial high levels of $\text{NH}_4^+\text{-N}$ there are three noticeable smaller increases in $\text{NH}_4^+\text{-N}$. In the first two increases in $\text{NH}_4^+\text{-N}$ on DOY 179-182 and 193-198, increases in $\text{NH}_4^+\text{-N}$ levels are dominant in the alfalfa plots (Figure 2-8). Alfalfa has been cited in the literature as possessing a C:N ratio of approximately 16:1 (Robertson and Groffman, 2005), which is similar to that of ours, 14.1. Presumably, this relatively low C:N ratio encouraged mineralization to occur rapidly and at high levels. The last increase on DOY 193-198 was greatest in manure plots which contains some grain straw used as bedding for dairy cattle. It is reasonable to infer that the relatively high C:N of grain straw (80:1) may have been responsible for the slow release of $\text{NH}_4^+\text{-N}$ from the manure amendments. Similar results have been cited in research in which N from manure took longer than synthetic fertilizer to produce available $\text{NH}_4^+\text{-N}$. (Mallory and Griffin, 2007). The authors conclude that the total substrate pool may be one of the greatest determining factors of how much N is

mineralized in the soil. This idea is in agreement with the findings from the analysis of TDN in which increased levels of $\text{NH}_4^+\text{-N}$ increased levels of TDN.

In addition to quality related to ease of degradability, aeration is also an important aspect of mineralization since the mineralization process is carried out by aerobic heterotrophs. To determine the effects of aeration it is useful to evaluate the WFPS throughout the sampling season. Changes in WFPS throughout the season in relation to $\text{NH}_4^+\text{-N}$ production are similar. Increases in WFPS correspond directly to drops in the quantity of $\text{NH}_4^+\text{-N}$, while low WFPS correspond to increased levels of $\text{NH}_4^+\text{-N}$. Presumably, this is a result of increased levels of mineralization under aerobic conditions. The one exception to this occurs on DOY 255 when WFPS remained high while $\text{NH}_4^+\text{-N}$ in manure treatments increased noticeably. While WFPS may not account for the late season $\text{NH}_4^+\text{-N}$ dynamics, it is possible that soil temperature may have had an important effect on microbial activity. After examining soil temperatures we observed that increases in soil temperature coincide with increases in $\text{NH}_4^+\text{-N}$, and vice versa. Unlike WFPS, $\text{NH}_4^+\text{-N}$ in the manure treatment on DOY 255 coincides well with soil temperature. Soil temperature actually increased almost 7°C between DOY 250 and 255, indicating how influential the increase in soil temperature can be on microbial dynamics.

2.4.1.2. $\text{N}_2\text{O-N}$ Production

Two separate processes, nitrification and denitrification, are capable of producing $\text{N}_2\text{O-N}$. The design of the experiment excludes the possibility of determining for certain which process was responsible for the production of $\text{N}_2\text{O-N}$. Increasing this difficulty is

the fact that both processes can occur within the soil at the same time depending on soil and environmental conditions. However, a closer examination of the field conditions during sampling makes it possible to infer which of these two processes were most likely to contribute to the production of biogenic GHG. Presumably, nitrification was more likely to contribute to the production of $\text{N}_2\text{O-N}$ than denitrification due to drier aerobic conditions within the soil. To determine the processes at work, the controlling factors of both nitrification and denitrification were examined in depth. In the case of nitrification, $\text{NH}_4^+\text{-N}$ content, aeration and to a lesser extent pH are considered the most important parameters (Coyne and Frye, 2005). In the case of denitrification, $\text{NO}_3^-\text{-N}$ content and O_2 availability were examined as they are the two parameters which are essential to the denitrification process (Martens, 2005).

2.4.1.3. Water-Filled Pore Space

As the results of the field experiment show, rainfall induced large variations in the level of WFPS. Water-filled pore space and O_2 levels strongly govern the processes of nitrification and denitrification. Linn and Doran (1984) suggested that microbial respiration reaches a maximum at 60% WFPS during lab incubations. Although there is some disagreement on the exact WFPS at which nitrification begins to decline and denitrification increases, 60% WFPS is generally accepted as a threshold (Clayton et al. 1997; Robertson and Groffman, 2005). The results show that WFPS remained below 60% WFPS, gas flux measurements were taken at some time following rainfall events. In addition, it is quite likely that anaerobic micro-sites still existed within the soil at WFPS <60% and could have contributed to the production of $\text{N}_2\text{O-N}$ from the resultant

denitrification. Sporadic increases and decreases in $\text{CO}_2\text{-C}$ show that respiration was most likely inhibited at more than one point throughout the field study, indicating WFPS had most likely reached this limit and therefore decreased anaerobic activity. Batemen and Baggs (2005) also found that WFPS was strongly related to the mode of $\text{N}_2\text{O-N}$ production. Their results showed that $\text{N}_2\text{O-N}$ production was highest at 70% WFPS. At this point results of their acetylene inhibition indicated that denitrification was taking place. Further results between 20-60% WFPS showed that nitrification was the predominant mode of $\text{N}_2\text{O-N}$ production. At WFPS below 20% nitrification seemed to be inhibited, but increased between 20-35% WFPS. Our findings are in direct agreement with theirs. The lowest levels of $\text{N}_2\text{O-N}$ occurred during points at which WFPS dropped, with the exception of the results during the fall period at which point the levels of inorganic N had presumably returned to background levels and $\text{N}_2\text{O-N}$ decreased as a result of lacking suitable substrates (i.e. $\text{NO}_3^- \text{-N}$ and DOC). van Groenigan et al. (2005) observed instances in which WFPS reached 78%, which actually caused a delay in the production of $\text{N}_2\text{O-N}$ until slightly aerobic conditions returned. This may explain why $\text{N}_2\text{O-N}$ production remained high at 52% WFPS compared to the generally accepted 60% WFPS. The majority of papers reviewed on the subject have shown strong relationships between WFPS and $\text{N}_2\text{O-N}$ production, all of which follow similar patterns cited in the previous examples (Clayton 1997; Smith et al. 1998; De Neve and Hofman 2000; Dobbie and Smith 2001; Ruser et al. 2006).

2.4.1.4. Soil Temperature

Temperature greatly affects biological processes within the soil. Maag and Vinther (1996) showed that as temperature increased so did N_2O -N production. Our results are similar in that high fluxes on N_2O -N are related to higher soil temperature. While the soil temperature remained near 20°C throughout the summer, large short term temperature fluxes in the early and mid sampling periods corresponded directly to the high production of N_2O -N. Similarly, Kaiser et al. (1998) found that time was attributed to being the most important seasonal variable. Our results span a much shorter, temperature dropped, so did the production of N_2O -N.

2.4.1.5. Influence of soil pH

While soil pH plays an important role in almost all soil processes including nitrification and denitrification, there seems to be little evidence that any N_2O -N production was inhibited or increased as a result of the specific field conditions during this field experiment. The pH in the study area was approximately 6.6 which is well within the acceptable range under which both nitrification and denitrification occur. Coyne and Frye (2005) point out that nitrification was inhibited at pH levels below 5.5 or above 8. These results were also found by Cheng et al. (2004) in which nitrification in various agricultural soils were inhibited at pH levels below 5. Their study further states that low pH levels are responsible for incomplete conversion of NH_4^+ -N to NO_3^- -N. Conversely, denitrification requires pH ranging from 6 to 8 (Martens, 2005). There is evidence that states that N_2O -N production from denitrification in soils at differing levels of aeration and pH reach a maximum at 6.5, while minimums occurred at pH levels of 6

and 8 (Stevens et al. 1998) At any rate, the field conditions of soil pH in this study do not seem conducive to limiting or enhancing either process.

2.4.1.6. Influence of Chemical Characteristics

In most cases, the results of literature regarding the subject indicate that the addition of N fertilizer amendments increase the levels of N_2O -N production (Lessard et al. 1995; de Klein et al. 1996; Rochette et al. 2004; Gillam et al. 2007; Rochette et al. 2007). However, there exists some disagreement regarding which type of amendment contributes most to the increase in N_2O -N production. Some of these papers state that synthetic fertilizers like AN produce greater quantities of N_2O -N (Gregorich et al, 2005). The highest cumulative levels of N_2O -N produced during the field experiment were seen in the alfalfa plots, followed by manure, AN and control plots. The highest fluxes of N_2O -N occurred in the manure plots with $151 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$, followed by alfalfa with $143 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$, AN with $73 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$ and control containing $37 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$. Quantities of N_2O -N returned to their lowest levels by the end of the experiment at 0.7 to $2.4 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$.

The highest N_2O -N fluxes occurred on DOY 168 with the exception of AN plots which occurred on DOY 172. An examination of environmental and response variables indicate that the greatest production of N_2O -N early in the season can be associated strongly with denitrification (Figure 2-5). An examination of the environmental and response variables show that during the first 3 sampling days WFPS was low, approximately 30%. On DOY 163 quantities of NH_4^+ -N were at their highest point for

the entire season with the exception of the control plot. Certainly some mineralization was occurring in order to transform organic N to inorganic N in alfalfa plots. Manure and AN plots would have contained high $\text{NH}_4^+\text{-N}$ levels at the time of addition, however levels increased further indicating mineralization occurred here as well. One day later on DOY 165, sharp decreases in $\text{NH}_4^+\text{-N}$ began to occur in all amendments. At this point, $\text{NO}_3^-\text{-N}$ increased to its highest concentrations in manure and fertilizer plots, while one sampling date later on DOY 168, alfalfa and control plots reach a maximum concentration. This can be attributed to immediate nitrification of available $\text{NH}_4^+\text{-N}$ in the soil. The high $\text{N}_2\text{O-N}$ fluxes on DOY 165-168 supports this. However, denitrification would not likely have become an important process until day 167 when there was almost 28 mm of rainfall. This rainfall increased WFPS to 52% (DOY 168). The remaining $\text{N}_2\text{O-N}$ production later in the experimental period can be largely attributed to nitrification, although not exclusively. Results show that when $\text{N}_2\text{O-N}$ fluxes increased, $\text{NH}_4^+\text{-N}$ levels decreased while levels of $\text{NO}_3^-\text{-N}$ increased. Further noticeable increases in $\text{N}_2\text{O-N}$ fluxes occurred on DOY 179. High WFPS and declines in the quantity of $\text{NO}_3^-\text{-N}$ followed by declines in the quantity of $\text{NH}_4^+\text{-N}$ indicate that this mixture of environmental and chemical characteristics in the soil made it possible that nitrification and denitrification both contributed to $\text{N}_2\text{O-N}$ production. At this point rainfall events had been consistent but small resulting in WFPS below 40%, well below the threshold of 60%. This is not to say that denitrification was not occurring on anaerobic micro-sites. Sharp decreases in $\text{NO}_3^-\text{-N}$ indicate that denitrification was likely occurring and contributing to $\text{N}_2\text{O-N}$ production, however at rates far lower than what can be attributed

to nitrification. Leaching could also have contributed to losses of NO_3^- -N from the soil. These results are in sharp contrast to the dynamics seen during DOY 186-198. At this time N_2O -N fluxes decreased as WFPS underwent a steady decline, and NO_3^- -N increased substantially. During this period it is likely that nitrification was the main process leading to these relatively small N_2O -N fluxes.

The field experiment findings are in agreement with those of Ellert and Janzen (2008) in which they monitored N_2O -N production in soils influenced by alfalfa, manure and fertilizer. Their results show that alfalfa was also responsible for the highest production of N_2O -N over a three year study period, followed by manure and fertilizer. These findings stress the importance of understanding long term N dynamics related to organic matter containing large quantities of N as opposed to concentrating strictly on short term additions of inorganic N, even in studies where alfalfa was examined as crop in rotation rather than an amendment. Their results also indicate that legumes that previously fixed atmospheric N contribute significantly to the production of N_2O -N as they decay. Ranges of N_2O -N production in their field trials were comparable to ours. Levels in their manure and fertilizer study were at a maximum in spring with approximately $280 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$ and $200 \mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$ respectively for selected cropping systems. Larsson et al. (1998) in a study examining the addition of grass and alfalfa mulches to the soil surface found that alfalfa addition to the soil showed the largest release of N_2O -N. The dynamics seen in that experiment were the same as ours, showing the highest production of N_2O -N within 20 days of application. As in our study, they attribute the production of N_2O -N to a combination of nitrification and

denitrification. The authors state that by increasing the amount of available N in addition to high levels of degradable C microbial populations may increase. As the organic matter is broken down, respiration by microbes increased, thereby decreasing the O₂ levels in the soil and causing anaerobic micro sites which further contribute to denitrification. While the peak production of N₂O-N was more than twice as high in their study, approximately 410 µg N₂O-N m⁻² hr⁻¹ compared to 143 µg N₂O-N m⁻² hr⁻¹ in ours, their mulches were applied directly to the surface, as compared to ours which were incorporated into the soil which may have accounted for some differences in the actual amount of N₂O-N produced (Flessa and Beese (1995)), found that incorporation of sugar beet residues, also high in N, resulted in high N₂O-N fluxes approximately 1 week after incorporation into the soil. Their maximum values were close to ours, with highest levels occurring at 140 µg N₂O-N m⁻² hr⁻¹. Although a large portion of the current research deals with pig slurry, the addition of animal manure and the subsequent production of N₂O-N has been noted throughout the literature. Rochette et al. (2004) studied N₂O-N emission following additions of pig slurry, and observed that short episodes of N₂O-N production were influenced by the addition of such an amendment. The authors cite the addition of large amounts of NH₄⁺-N and organic C as a driving factor in N₂O-N production under conditions conducive to denitrification. Consistent with our results, Rochette et al. (2007) using dairy cattle manure showed that dairy cattle manure is responsible for higher N₂O-N fluxes than synthetic fertilizers as a result of greater organic C inputs. The addition of C was a major contributing factor since it relieved any microbial constraint that may have limited N₂O-N production through denitrification (Rochette et al. 2007).

Differences in N₂O-N production between manure and synthetic N plots are largely attributable to increases in denitrification. The results of their study showed levels of N₂O-N considerably higher than ours, almost 1500 µg N₂O-N m⁻² hr⁻¹ compared to our manure with 151 µg N₂O-N m⁻² hr⁻¹. The N₂O-N dynamics in our study were similar in that manure was consistently higher than fertilizer. Like our study, the fluxes occurred almost immediately after the application of manure. Lessard et al. (1996) found similar results in their application of manure to corn crops. Not only were their levels comparable to those recorded throughout our sampling period, but the dynamics were also similar. Emission of N₂O-N early in the sampling period even under areas planted with corn likely results from little plant uptake of N during the initial growth stages. In their study, like ours, fluxes in the manure treatments occurred almost immediately. Amendments were mixed into the soil in their experiment, as were ours, which as they point out, may cause fluxes to occur more quickly than if the amendment were simply applied to the surface. Stevens and Laughlin (2001) also found that the addition of cattle slurry greatly increased fluxes of N₂O-N. Although their fluxes occurred over shorter periods of time, the effects of slurry application were nonetheless similar.

In our study, the addition of synthetic fertilizers caused an increase in the quantity of N₂O-N produced throughout the sampling period. These results were similar to those of Venterea et al. (2005) in their study of tillage and fertilizer interaction. Regardless of tillage method, the application of fertilizers resulted in an increase in N₂O-N production within 1-3 weeks following application. A study by van Groenigen et al. (2004) also found that fertilizer produced less N₂O-N than slurry in an examination of amendment in

relation to soil type. Clearly, the addition of C to the soil is a major factor that contributes to higher levels of N_2O -N production.

The proportion of N_2O -N losses relative to the total N measured in our experiment was very small and similar to those values in the literature (Whalen et al. 2000; Helgason et al. 2005; Mkhabela et al., 2007; Ellert and Janzen, 2008). Mkhabela et al. recorded cumulative losses of 0.07, 1.5 and 0.07% of the total N applied from slurry in three short term field experiments. Throughout our experimental season 0.29% of the total N applied in the slurry was lost to denitrification, while 0.40 and 0.23% were lost in alfalfa and AN plots respectively. Whalen et al (2000) indicated losses of 0.05 to 1.0% over an 11 day sampling period subsequent to the application of swine slurry. Ellert and Janzen (2008) measured cumulative losses of approximately 0.95 and 1.32% in manure and non-manure amended treatments applied over alfalfa and corn systems. Helgason et al. (2005) indicate similar cumulative losses of 1.18% which are in general agreement with the current international findings which indicate N_2O losses average approximately 1.25% of the total N applied as fertilizer. While our findings were lower than the generally accepted 1.25%, our experiment lasted only 133 days. If application had taken place in early spring when denitrification occurs more rapidly as a result of saturated conditions, N_2O -N production may have been closer to the values found in other research. Furthermore, the data indicates that in comparison to the total volume of N applied, alfalfa produced 1.4 times more N_2O -N than manure and 1.7 times more N_2O -N than fertilizer.

2.4.2. Carbon Dynamics

2.4.2.1. Mineralization

In aerobic soil conditions $\text{CO}_2\text{-C}$ production occurs from heterotrophic respiration. In soils, $\text{CO}_2\text{-C}$ is produced by two means, aerobic decomposition of C and anaerobic decomposition. In aerobic decomposition heterotrophic organisms produce CO_2 as well as consume O_2 for energy purposes (Cambardella, 2005). As a result, $\text{CO}_2\text{-C}$ is returned to the atmosphere. The rate at which this breakdown occurs is strongly related to the quality of the amendment. In this case quality refers to the decomposability or susceptibility of OM to decomposition under standard conditions (Cambardella, 2005). Fungi, which are the principal decomposers of fresh organic matter, are able to produce enzymes capable of breaking down all OM; however specialized fungi are required to break down lignin which has a high C:N ratio. This again stresses the importance of the C:N ratio in relation to the speed at which organic matter transformations occur. The addition of any new substrate to the soil however causes an increase in microbial activity that in turn increases $\text{CO}_2\text{-C}$ production as a result of increased respiration. Increased microbial activity soon ceases as C sources become limiting, at which point the microbial population dies off, releasing C and other nutrient such as N into the soil. Anaerobic decomposition is much slower and not usually a major process in agricultural soils where conditions are not usually saturated for long periods of time, however, saturation could result in the production of methane. At any rate, it is important to note that some $\text{CO}_2\text{-C}$ production will occur as microbes use $\text{NO}_3^-\text{-N}$ as a terminal electron acceptor rather than O_2 . In this case, the production of $\text{N}_2\text{O-N}$ is increased.

2.4.2.2. Water-Filled Pore Space

According to Camberdella (2005), the optimum production of CO₂-C occurs when large soil macro-pores are well drained while capillary pores remain saturated as a result of matric forces. The results of our study show that WFPS is an important environmental variable controlling the production of CO₂-C. Inspection of the dynamics of CO₂-C production in relation to WFPS shows a strong inverse relationship between the two. Carbon dioxide production reached a maximum in the first three sampling days, which also had the lowest WFPS recorded throughout the season. As WFPS peaked on DOY 168-172, CO₂-C production reached its lowest level. These dynamics continue throughout the duration of the sampling season. Furthermore, the results of a regression analysis of log transformed CO₂-C emissions compared to WFPS showed a significant correlation ($P < 0.05$). Based on the variation in CO₂-C fluxes we can therefore deduce that at no less than three points during the sampling season the soil reached anaerobic conditions. Similar results were recorded by Hernandez-Ramirez et al. (2009) in which they observed that accumulated rainfall was significantly associated with the production of CO₂-C. Their results indicated that rainfall often increased microbial activity responsible for CO₂-C production. The authors state that accumulated rainfall was the most important short term driver of CO₂-C production. Beare et al. (2009) found in their study on compaction that peak CO₂ production occurred at 45% WFPS, while overall production of CO₂ was reduced in compacted soils in comparison to non-compacted soils.. Although they recorded quantities of CO₂-C between 3 and 7 $\mu\text{g CO}_2\text{-C g dry soil}^{-1} \text{ d}^{-1}$ in compacted and non-compacted soils respectively, initial levels of CO₂-C

production prior to re-wetting were similar to ours. Furthermore, the authors point out that $0.45 \text{ cm}^3 \text{ cm}^{-3}$ was the optimum WFPS for production of $\text{CO}_2\text{-C}$. Our results are in agreement with these findings given that the large $\text{CO}_2\text{-C}$ production was experienced on DOY 182, 193 and 198 when WFPS was 30-45%. Drury et al. (1998) found the N_2O production and production of CO_2 was strongly correlated, which stresses the importance of changes in moisture on $\text{CO}_2\text{-C}$ production. Likewise, Franzluebbers et al. (1995), Akinremi et al (1998) and Ruser et al. (2006) also found results indicating the importance of WFPS in relation $\text{CO}_2\text{-C}$ production. Conversely, Paul et al (1999) found that $\text{CO}_2\text{-C}$ flux was not significantly correlated with soil moisture.

2.4.2.3. Temperature

Both the $\text{CO}_2\text{-C}$ dynamics and Spearman's Rho correlation show that temperature did have a significant effect on the amount of $\text{CO}_2\text{-C}$ produced throughout the duration of the sampling period ($P < 0.05$). All increases in $\text{CO}_2\text{-C}$ levels corresponded to increases in soil temperature, while drops corresponded to decreases in soil temperature. This is not surprising given that, as stated earlier in the discussion of $\text{N}_2\text{O-N}$, microbial processes responsible for GHG production are largely governed by soil temperature. The dependence of $\text{CO}_2\text{-C}$ production is often cited in relation to the Arrhenius model which predicts increases in $\text{CO}_2\text{-C}$ based on temperature increases (Chapman and Thurlow, 1996). Increased emission of $\text{CO}_2\text{-C}$ occurs as microbial activity increases which is strongly governed by temperature. Feirer et al. (2003) studied the response of microbial populations and $\text{CO}_2\text{-C}$ production in surface and subsurface layers. They were able to conclude that with N and C amendments respiration rates increase exponentially between

10°C and 35°C. Furthermore, their results indicate that surface and subsurface soil between 0-15 cm possessed a Q_{10} value between 2.8 and 3.0. Similarly, studies of intact soil cores collected from forested sites showed the same exponential increase in respiration with $\text{CO}_2\text{-C}$ at WFPS between 20 and 50% (Fang and Moncrieff, 2000).

2.4.2.4. Influence of Chemical Properties

$\text{CO}_2\text{-C}$ emission was highest in soil amended with alfalfa followed by that amended with manure, fertilizer and control. Therefore, amendments containing the highest levels of C produced the highest levels of $\text{CO}_2\text{-C}$. This occurred as a result of increased microbial respiration during C mineralization.

Chantigny et al. (2002) found that the application of alfalfa resulted in higher levels of $\text{CO}_2\text{-C}$ production followed by manure and lowest in control treatments. Carbon dioxide fluxes in their study increased 3-29 days after application and declined after 56-84 days in sandy loam soil, dynamics which are similar to ours. Carbon degradability and N availability were cited as one possible reason for these results. Their findings and ours suggest that high quantities of lignin in cattle manure were most likely responsible for lower levels of $\text{CO}_2\text{-C}$ production than alfalfa since lignin is not as easily decomposed as OM with lower C:N ratios like alfalfa. Likewise, Ellert and Janzen (2006) found that the termination of alfalfa crops led to higher $\text{CO}_2\text{-C}$ fluxes than addition of manure or fertilizer. Although our results showed considerably higher levels of $\text{CO}_2\text{-C}$ production immediately after application the dynamics were nonetheless similar.

Rochette and Gregorich (1998) also showed that there was a significant difference in CO₂-C between manure treatments and control plots, but no significant difference between fertilizer and control plots. Although their results showed higher production of CO₂-C, approximately 48 g CO₂-C m² d⁻¹ compared to our CO₂-C production of only 13 g CO₂-C m² d⁻¹ in the slurry treatments, their stockpiled manure contained more C. Regardless of this the CO₂-C dynamics and production in relation to other treatments including fertilizer and control were similar. Fertilizer fluxes were only 6-7 g CO₂-C m² d⁻¹ lower than ours. In a long-term study on the application of pig slurry CO₂-C fluxes were once again substantially higher than ours, almost 10 times higher in their heavily fertilized treatments, however, at more moderate application, 60 Mg ha⁻¹ rates of CO₂-C production were only twice that recorded in our field measurements (Rochette et al. 2000). Furthermore, the differences between pig slurry and cattle slurry may have contributed greatly to these differences. Specifically, pig slurry contains high levels of carbonates which have the ability to increase CO₂-C production (Chantigny et al. 2002).

In general, the results indicate that little CO₂-C production resulted from the application of synthetic fertilizer or in control plots. A lack of C substrates in both cases is most likely responsible for a much lower initial production of CO₂-C than that seen in alfalfa and manure. Seasonal dynamics beyond this point could be attributed solely to temporal variations in temperature and rainfall.

2.5.Conclusion

We have examined the transformation of N as well as the emissions of both N_2O -N and CO_2 -C in soil which has received different fertilizer amendments. The greatest amount of N_2O -N was emitted from the manure treatments early in the experimental period, followed by alfalfa, fertilizer and control plots. However, alfalfa treatment was responsible for the greatest cumulative emission during the 133 day experimental period. Manure, fertilizer and control plots followed alfalfa in quantity of N_2O -N production respectively. The initial increase in N_2O -N was attributed to both nitrification and denitrification. As a legume alfalfa naturally contains high amounts of organic N that is easily mineralized and subsequently nitrified. Furthermore, soil conditions, most notably WFPS, influenced both nitrification and denitrification which contributed to N_2O -N production. We conclude that under field conditions the incorporation of legumes such as alfalfa to the soil produces greater quantities of N_2O -N than manure and synthetic fertilizers. Although these are small losses of N from the soil, this is a potent GHG and a leak from the system that should be minimized

Fluctuations in CO_2 -C were found to vary in response to additions of easily degradable C substrates and environmental conditions. Both the highest single recorded measurements of CO_2 -C production throughout the experimental period as well as the highest cumulative CO_2 -C production were measured in alfalfa plots. Low C:N ratios in alfalfa increased microbial activity and respiration. Manure produced less CO_2 -C as a result of slightly lower C content when compared to alfalfa, however, the nature of the organic matter found in the manure, mainly straw, has a high C:N ratio. Generally

speaking, straw is not easily broken down. Increased microbial activity is required in order to break down high lignin containing straw bedding. Low CO₂-C fluxes were observed in both fertilizer treatments and control treatments because no additional C substrates were observed.

Our results show that the production of GHG is strongly influenced by both soil transformation processes and environmental variables. High production of N₂O-N was strongly dependent on nitrification and denitrification which both depend heavily on the addition of C substrates and environmental conditions. High mineralization rates liberated substantial amounts of NH₄⁺-N, which was subsequently nitrified to NO₃⁻-N and enhanced N₂O-N production while also increasing the denitrification process under certain circumstances. We conclude that in evaluating the relative benefit of fertilizer amendments to the soil it is necessary to consider all soil N losses and transformations.

The findings of this study indicate that while the addition of synthetic fertilizers deliver large quantities of inorganic N to the soil quickly, these additions may be transformed and consequently lost quickly. Although cumulative N losses were higher in alfalfa treatments than any other amendment, the actual mass of N lost was low (<1%) and its high relative quantity of organic N compared to all other amendments meant that it had the ability to sustain prolonged production of inorganic N throughout the growing season. The usefulness of alfalfa as a soil N fertilizer is important and may at times be overlooked. When considering the timing of N release for crops such as corn which require large amounts of N in early July, our research has shown that other amendments

may not be as effective in delivering N at this time as alfalfa. Furthermore, when assessing the emissions of GHG associated with each amendment, although alfalfa results in greater CO₂ and N₂O production in the field, it does not have the off-site GHG emissions of manure and AN. Manure may release considerable GHGs during storage while manufacturing AN is an energy intensive operation. It is realistic to assume both manure and AN produce more GHG when these factors are accounted for, further increasing the appeal of alfalfa as N fertilizer.

3. Production of N₂O and CO₂ from compacted and non-compacted soils amended with ammonium nitrate, alfalfa and manure

3.1.Introduction

Soil compaction, especially in humid locales such as Eastern Ontario, is one of the most problematic aspects of modern mechanized agriculture. These problems arise in part due to high water content present in soils which causes a reduction in the load support capacity of soils (Hamza and Anderson., 2005). In addition, the fine texture of soils in these areas often compounds already serious environmental factors (Lapen et al., 2001). As compaction occurs, soil structure is greatly affected, causing a reduction in aggregate size and soil porosity which can effect soil processes such as carbon (C) and nitrogen (N) mineralization, nitrification, and denitrification (Bakken, 1987; Hassink, 1992; Jensen et al., 1996; Lipiec and Stepniewski, 1995; De Neve and Hoffman, 2000; Sitaula, 2000; van Groenigan, 2005; Beare et al., 2009). One reason why the loss of soil structure leads to changes in these processes is related to the destruction of soil pore space which serves as both substrate protection and as a habitat for the bacteria, fungi micro- and mesofauna that are responsible for the transformations and eventual loss of N and C from the agricultural system. Jansen et al. (1996) state that mineralization rates decreased in non-cropped, compacted soils. In addition, CO₂-C fluxes decreased upon compaction. Studies by De Neve and Hofman (2000) indicate that compaction in amended soil did not affect rates of N mineralization but did cause a depression in C

mineralization. Further studies by Breland and Hansen (1996) determined that an increase in bulk density from 1.0 g cm^{-3} to 1.4 g cm^{-3} reduced N mineralization by up to 18%.

Nitrous oxide production as a result of nitrification and denitrification has been found to be influenced by compaction as in N and C mineralization. Bakken et al. (1987) indicate that losses of $\text{N}_2\text{O-N}$ through denitrification in compacted soils were substantially higher than losses in non-compacted soils. The literature is generally in agreement that increases in soil compaction increase losses of N through denitrification (Whalley et al. 1995; Lipiec and Stepniewski, 1995; Ball et al., 1999; Sitaula et al., 2000).

Nitrogen is essential for successful plant nutrition and growth since N serves as the most important macronutrient in plant development and is required for the manufacturing of proteins, amino acids and enzymes within various plants and microorganisms including bacteria and fungi (Brady and Weil, 2002). The importance of N in agroecosystems has led to the increased usage of synthetic fertilizers as well as organic fertilizers such as legumes and manure (Chantigny et al. 2002). The different characteristics of these amendments have the ability to greatly increase greenhouse gas (GHG) production from soils especially in humid environments like Eastern Ontario. Production of N_2O may be caused through both nitrification and denitrification depending on field conditions (Ruser et al. 2006; Coyne and Frye, 2005; Beauchamp 1997). Increased additions of organic matter also lead to the production of CO_2 through C mineralization (Beare et al. 2009). Although the production of GHG is of increasing

importance due to climate change, the loss of soil nutrients and decreased productivity that result are more critical concerns to farmers in the short term. As such, it is important to understand the dynamics that take place within the soil and the processes that govern N and C loss to better understand the importance of proper fertilization timing and techniques.

The focus of this incubation study is to assess the rate at which soils release N_2O and CO_2 under conditions which have been altered both chemically and physically. Specifically, the focus will be on the addition of N amendments to the soil including inorganic fertilizer (ammonium-nitrate, AN), manure and alfalfa and the alteration of soil physical structure through compaction.

3.2. Materials and Methods

3.2.1. Trial Site and Soil

A 34 day laboratory incubation experiment was undertaken to determine the consequences of different amendments and soil bulk density on the rate of N_2O -N lost as a result of nitrification and denitrification. In addition CO_2 -C emissions were examined as a by-product of soil microbial activity related to soil N transformations. The incubation experiment was conducted on a Melanic Brunisol (Canadian Soil Classification System) with a pH of 6.6 and a loam texture obtained from the Eastern Cereal and Oilseed Research Center, Ottawa, ON, Canada ($45^{\circ} 22' N$, $75^{\circ} 43' W$). Soil was collected April 2009 from a previously undisturbed plot that had been used as a control site for a long-term tillage experiment. Prior to application of amendments the soil was air-dried,

crushed and sieved to $< 2\text{mm}$ in an effort to attain full homogenization of the soil. Large plant litter and rocks were removed. The experiment consisted of a 2 factor design where the first factor was an amendment treatment (4 levels): Control, alfalfa at 332.75 g m^{-2} (3.6 g N kg^{-1}), ammonium-nitrate fertilizer (AN) at 11.2 g m^{-2} (6.8 g N kg^{-1}), and manure at 3 L m^{-2} (30 g N kg^{-1}), and the second factor was a compaction treatment (2 levels): non-compacted soil at 0.010 g mm^{-3} (NC) and compacted soil at 0.015 g mm^{-3} (CS). Each treatment consisted of 5 replicates. Application rates for manure slurry were based on normal annual application rates or a small local dairy operation. Rates were approximately equivalent to 30000 L ha^{-1} , whereas AN was based on recommended application rate for maize at $100\text{ lbs N acre}^{-1}$ (112 kg N ha^{-1}). However, a miscalculation resulted in the over application of AN. Application of alfalfa was based on the average amount of alfalfa produced per square meter on a typical dairy farm in the area.

3.2.2. Sample preparation

Prior to application of the amendments, 7 kg of air dried soil was divided into four clean trays, each tray containing 1750 g . Upon addition of each individual amendment, 315 ml of water was added to bring all soil to approximately 18% gravimetric water content (GWC) with the exception of the manure treatment which only required 265 ml of water based on the high volumetric water content (VWC) of the slurry prior to application. After addition of water, GWC was approximately 2% higher than the average GWC (16%) found in previous growing seasons in the research plots where the soil was collected. This was done intentionally to account for any loss of moisture while soils were exposed to laboratory air. Upon application of each amendment, soil was divided

into individual weighing dishes, each containing the equivalent amount of soil necessary to reach the desired BD. Soil was then placed into 135 cm³ brass cores which had been covered on the bottom by fibreglass mesh. The placement of the mesh was intended to keep the soil intact in the cores while allowing aeration from both the top and the bottom of the core. While the soils for the NC treatment fit easily into the brass cores, a large hammer was required to compress soils for the CS treatments. Soil cores were then placed into 1.5 L Mason jars fitted with rubber gas sampling septa. Each jar was purged with lab air for 30 seconds to ensure that CO₂ levels were not above ambient concentrations prior to being sealed. The jars were then sealed and placed in boxes for the duration of the incubation except when removed for sampling purposes. The incubation took place for 34 days at room temperature. The physical characteristics are listed in Table 3-1. Jars were weighed at the following hours after setup: 45, 69, 94, 215, 314, and 503 and 800. At these times each jar was corrected for moisture. An eye dropper was used to add small increments of water to the soil until the jar and soil weight had returned to their initial weights.

Table 3-1: Relationship between bulk density, total porosity, gravimetric water content, volumetric water content and water filled pore space in non-compacted and compacted soils

	Bulk Density (g cm ⁻³)	Total Porosity (cm ³ cm ⁻³)	Gravimetric water content (g g dry soil ⁻¹)	Volumetric water content (Θ) cm ³ cm ⁻³	WFPS (cm ³ cm ⁻³)
Non- compacted	1.0	0.62	23.3	23	37
Compacted	1.5	0.43	15.6	23	54

3.2.3. Gas sampling and analyses

At each sampling interval, a 30-cm³ syringe fitted with a 2 way Luer-lock was used to sample headspace gas. Samples were collected with the syringe, injected into, and stored in pre-evacuated (0.0005 kPa) 12-ml vials (Exetainers, Labco Inc., UK) containing approximately 0.3g magnesium perchlorate as a drying agent (Beare et al. 2009). Nitrous Oxide was analyzed using a gas chromatograph (Varian 3800, Varian Inc, Walnut Creek, CA) equipped with an electron capture detector (ECD) and a headspace autoinjector (Combi Pal, CTC Analytics, Zurich, Switzerland). The ECD was operated at 390 °C using an Ar/CH₄ carrier gas at 70 mL min⁻¹. Gas separation was obtained using a Haysep N 80/100 pre-column (0.32 cm diam. x 50 cm length) and Haysep D 80/100 mesh analytical columns (0.32 cm diam. x 200 cm length) in column oven maintained at 65 °C (Gregorich et al. 2007). Carbon dioxide was measured using a flame ionization detector (FID). The FID was operated at 250 °C using a He carrier gas at 15 mL min⁻¹ (Beare et al. 2009). The concentration of N₂O (μL N₂O-N L⁻¹ min⁻¹) was estimated from chromatogram area based on a quadratic equation. Following each sampling period, mason jars were evacuated with a standard lab gas to replenish the oxygen content of the incubation jars.

To determine the mass of N₂O and CO₂ emitted from the soils on g g⁻¹ dry soil throughout the sampling period the following equation was applied:

$$\text{Emission of } N_2O = \frac{(N * P) \left(\frac{R}{T}\right) \left(\frac{V}{W}\right) M}{t}$$

Where N is the N₂O-N concentration at time of sampling; T = In situ air temperature (K)(K=273+C°); V/W = Chamber headspace volume (L)/ (W) of the soil cores (g); M = molar conversion for N₂O (28.02 g mol⁻¹); t = time in hours since last sampling. Values approximately double the average recorded concentration were deemed outliers and were removed from analysis. These values were attributed to sampling error and equipment malfunction. Values outside the detection range of the instrumentation for N₂O-N (approximately 30 ppmv) were also removed. Both N₂O and CO₂ gas fluxes are expressed on a dry weight basis (ex. µg N₂O-N g⁻¹ dry soil hour⁻¹) since amendments were applied on this basis.

Volumetric water content (θ) and water filled pore space (WFPS) were determined as follows: $\theta = v \times \beta / \rho_w$ where ρ_w is the density of water (g m⁻³), v is the gravimetric water content (g g⁻¹) and β is the soil bulk density (g mm⁻³) ; WFPS= (% θ)/(1-(β/ρ_s)).

3.2.4. Statistical Analysis

The result of the compaction and amendment treatments were analyzed using a two-way Analysis of Variance (ANOVA). Net N₂O-N and CO₂-C production were tested in comparison to other soil variables including WFPS, porosity, VWC and GWC. In all cases gas flux data was either log or -log transformed to normalize variance. Subsequent to testing, transformed variables were back calculated (log¹⁰ transformed) to attain proper values. Tukey's HSD (Honestly Significant Difference) test was used to compare the means of the transformed data. Tukey's HSD test compares all possible means to

determine which if any are significantly different from one another while reducing the likelihood of making a type I error. Statistical analysis was done using JMP7 statistical software (SAS Institute Inc.).

3.3. Results

3.3.1. Nitrous Oxide Emissions

Figure 3-1 and Figure 3-2 show N₂O-N emissions in non-compacted and compacted soils throughout the experiment. Both non-compacted and compacted soils exhibited similar patterns in N₂O-N production for all treatments excluding the AN amendment in which higher than usual fluxes resulted from over application of AN. Nitrous oxide production was rapid within the first 100 hrs in alfalfa and manure treatments and then declined as the experiment progressed. Alfalfa amended soil showed the highest initial production of N₂O-N (0.0023 $\mu\text{g kg}^{-1} \text{ hr}^{-1}$ in non-compacted, 0.033 $\mu\text{g kg}^{-1} \text{ dry soil hour}^{-1}$ in compacted) and remained consistently higher than both manure and control treatments throughout the experiment. N₂O-N production from soils treated with manure also exceeded that of control samples (0.0010 $\mu\text{g kg}^{-1} \text{ dry soil hour}^{-1}$ in non-compacted soil, and 0.028 $\mu\text{g kg}^{-1} \text{ dry soil hour}^{-1}$ in compacted). Regardless of amendment type, compacted treatments experienced considerably higher production of N₂O-N than non-compacted treatments.

The results for the AN treatment were problematic due to calculation errors made during fertilizer application, in which rates far exceeded normal field conditions (approximately 6 times more N was applied than regular field application). As a result,

N_2O -N emissions varied substantially in their pattern of production in both NC and CS soils amended with AN. Gaps in the AN treatment data in Figure 3-2 result from concentrations of N_2O -N above the detection level of the GC instrument.

Figure 3-1: N_2O -N emissions over time in non-compacted soils. *Note that AN results are expressed on a secondary y-axis for clarity

Figure 3-2: N₂O-N emissions over time in compacted soils. *Note that AN is expressed on a secondary y-axis for clarity

For all amendment treatments, cumulative production of N₂O-N was higher in CS than NC soils (Figure 3-3). This pattern was also found for the AN amended soils in spite of over application.

Figure 3-3: Cumulative N_2O-N production through time for all amendment and compaction treatments

Nitrous oxide emission data was separated into early (0-143 hours), middle (143-314 hours) and total (0 – 820 hours) periods based on flux patterns. Data subsequent to hour 314 was not analyzed as a separate period as fluxes returned to background levels. Ammonium nitrate treatments were not included in the analysis as a result of over application. Results from the 2-factor ANOVA indicated that amendment and

compaction treatments were significant as was the interaction of these two factors for all 3 periods (Table 3-2).

Table 3-2: 2-Factor Analysis of Variance results examining the effects of amendment (A) and compaction (C) (NC = no compaction, CS = compaction) treatments on N₂O-N emissions. In effects test Prob>F below 0.05 indicates statistical significance. For Tukey's Kramer HSD different letters indicate significant differences between treatment combinations.

	Early			Middle			Total		
	DF	F Ratio	Prob>F	D	F Ratio	Prob>F	DF	F Ratio	Prob>F
Amendment (A)	2	9.07	0.0012	2	10.74	0.0005	2	7.30	0.0033
Compaction (C)	1	28.28	<0.0001	1	16.28	0.0005	1	26.04	<0.0001
A × C	2	7.06	0.0039	2	7.63	0.0027	2	5.44	0.0112
Tukey's Kramer	Early			Middle			Total		
µg kg dry soil ⁻¹									
Alfalfa (CS)	1.70 a			0.07 a			1.82 a		
Manure(CS)	1.00 ab			0.25 b			1.51 ab		
Control(CS)	0.17 bc			0.03 b			0.40 bc		
Alfalfa(NC)	0.15 bc			0.04 b			0.24 bc		
Manure(NC)	0.09 bc			0.04 b			0.17 c		
Control(NC)	0.05 c			0.02 b			0.09 c		

Results from the Tukey's Kramer HSD test (Table 3-2) indicate that the interaction of the compaction and amendment treatments led to significantly more total cumulative N₂O-N emissions in compacted alfalfa and in compacted manure amended soils than in their non-compacted counterparts. However, compaction did not significantly affect N₂O-N emissions in the control soils (Table 3-2). This result was entirely due to differences in N₂O-N emissions during the early period (0-143 hours) for the manure treatment while N₂O-N emissions continued to remain significantly greater in compacted than in non-compacted plots during the middle period. Overall alfalfa amended soils produced 4.7

and 2.6 times more $\text{N}_2\text{O-N}$ than control soils with compacted and non-compacted treatments, respectively, while manure amended soils produced 3.9 and 1.9 times more $\text{N}_2\text{O-N}$ than control soils with compacted and non-compacted treatments, respectively. However, of these comparisons, the only significant difference was between the compacted alfalfa amended soils and compacted control soils.

3.3.2. Carbon Dioxide Emissions

Unlike $\text{N}_2\text{O-N}$ production, over fertilization with AN was of little consequence in production of $\text{CO}_2\text{-C}$ (Figure 3-4 and Figure 3-5). Carbon dioxide production had similar initially high fluxes followed by a declining pattern in both compacted and non-compacted soils. In both cases alfalfa amended soils consistently showed higher emissions of $\text{CO}_2\text{-C}$ than all other treatments for at least the first 300 hours. Within the first 24 hrs of sampling $\text{CO}_2\text{-C}$ production was ~2 times higher in non-compacted and compacted control treatments respectively than compacted and non-compacted manure amended soils. Manure was only slightly higher than control and AN amended soils for both non-compacted and compacted treatments.

Figure 3-4: CO₂-C production through time during the incubation experiment in non-compacted plots

Figure 3-5: CO₂-C production through time during the incubation experiment in compacted plots

In both compacted and non-compacted soil, cumulative CO₂-C fluxes were highest in alfalfa amended soils, followed by manure, AN and controls (Figure 3-6). Lower emission of CO₂-C in the AN plots were likely caused by reduced microbial activity resulting from an increase in pH due to over application of AN.

Figure 3-6: Cumulative $\text{CO}_2\text{-C}$ production through time for all amendment and compaction treatments

As with the $\text{N}_2\text{O-N}$ emission data, $\text{CO}_2\text{-C}$ emissions were divided into early, middle and total periods. To be consistent with this previous analysis, the AN treatment was not included in the analysis due to over application of this amendment. Results of the 2-factor ANOVA indicated that amendment, compaction treatment and the interaction

of these two factors were significant for each period (Table 3-3). The Tukey's Kramer HSD test indicated that during each period, the non-compacted alfalfa amended soils had significantly higher CO₂-C emissions than any other treatment combination (Table 3-3). Compaction did not affect the CO₂-C emissions from manure amended soils but the manure treatment led to more CO₂-C production than control soils. Finally, compaction also had no influence on the CO₂-C emissions from control soils. CO₂-C production decreased as the C:N ratio of the amendment type increased and soil compaction increased. Total cumulative values show that nearly 4 times the amount of CO₂-C is produced in alfalfa amended soils vs. control soils while manure amended soils only produced approximately 1.5 times the amount of CO₂-C vs. control soils.

Table 3-3: 2-Factor Analysis of Variance results examining the effects of amendment (A) and compaction (C) (NC = no compaction, CS = compaction) treatments on CO₂-C emissions. In effects test Prob>F below 0.05 indicates statistical significance. For Tukey's Kramer HSD different letters indicate significant differences between treatment combinations.

	Early				Middle				Total	
	D	F Ratio	Prob>F	DF	F Ratio	Prob>F	DF	F Ratio	Prob>F	
Amendment(A)	2	2562.00	<0.0001	2	1158.63	<0.0001	2	2442.98	<0.0001	
Compaction(C)	1	14.04	0.0010	1	12.67	0.0016	1	18.06	0.0003	
A × C	2	7.16	0.0036	2	9.07	0.0012	2	8.62	0.0015	
Tukey's Kramer	Early				Middle				Total	
µg kg dry soil ⁻¹										
Alfalfa (CS)	807.02 b				237.87 b				1298.07 b	
Manure(CS)	228.47 c				147.69 c				591.98 c	
Control(CS)	148.58 d				81.69 d				360.70 d	
Alfalfa(NC)	886.04 a				265.51 a				1439.70 a	
Manure(NC)	231.86 c				148.75 c				611.97 c	
Control(NC)	155.92 d				83.63 d				379.98 d	

3.4.Discussion

Changes in the soil environment have the ability to alter the way in which soil microorganisms function. In the case of N_2O -N production, the most important two processes at work are nitrification and denitrification. Due to the nature of the experiment, it is not possible to distinguish which process were responsible for the majority of N_2O -N production; however it should be noted that both take place simultaneously in the soil, and thus, both may contribute to N_2O -N production. It is unlikely however that both processes are equally responsible for equivalent proportions of N_2O -N production. It is more likely that nitrification was the key factor governing the production of N_2O -N. Nitrification is an aerobic process in which reduced forms of N are oxidized and thus form NO_2^- and NO_3^- (Hillel et al. 2005). The process is reliant on the following important environmental factors: NH_4^+ availability, aeration, and pH (Hillel et al. 2005). Based on the parameters imposed on the study, we can conclude that there is little chance that limitations to nitrification existed.

3.4.1. Influence of Compaction

The results of the experiment show that there was a significant response to compaction when examining the production of N_2O -N. N_2O -N production from the compacted treatments ranged from 4 times higher in control plots to 28 times higher in manure plots than non-compacted plots, while on average the increase in N_2O -N emission was approximately 14 times greater in compacted plots than in non-compacted plots

when amendments were compared at the initial stages of the incubation. Our flux values are similar to other studies examining the effects of compaction (Ruser et al. 2006; Beare et al. 2009). Other studies show similar relationships between N_2O -N production and compaction although flux magnitudes vary (van Groenigen et al., 2005; Flessa et al., 2002; Sitaula et al., 2000).

As compaction occurs, the changes in BD lead to important changes in other soil physical properties. As BD increases, there is a general decrease in porosity, and an increase in WFPS as a result of physical destruction of macropores within the soil. The results of our compaction study suggest that the production of N_2O -N was strongly influenced by the WFPS. The compacted cores had a higher average emission of N_2O -N than the non-compacted cores. At a WFPS of 54 %, the average emission of all 4 amendment plots initially was $8.94 \mu\text{g N}_2\text{O-N g}^{-1} \text{ dry soil hr}^{-1}$, while the non-compacted soil cores with a WFPS of 37% averaged $0.57 \mu\text{g N}_2\text{O-N g}^{-1} \text{ dry soil hr}^{-1}$ at the beginning of the study. Without further information there is no way in which we can determine unequivocally which soil processes were causing the production of N_2O -N. However, we can draw conclusions based on aeration status and comparison to other research.

Ruser et al. (2006) undertook field experiments using similar treatments, including compaction and amendment addition. While their study resulted in a wide range of N_2O flux values, often far above ours, similar patterns were seen. Compacted soils consistently had fluxes greater than those in non-compacted soils. Furthermore, based on ^{15}N labelling results, the authors concluded that soil moisture had a significant

consequence on nitrification and denitrification levels with denitrification the predominant N_2O production process at levels $\geq 70\%$ WFPS. As a result, they concluded that nitrification was the cause of N_2O production at WFPS below this level. Bateman and Baggs (2005) used acetylene inhibition techniques in their examination of the effects of WFPS on N_2O production. They found that fertilized soils at 70% WFPS produced significantly more N_2O than soils at 20% WFPS. They were also able to determine that autotrophic nitrification was responsible for the majority of the N_2O production at WFPS between 20%-60%. They also show the sharpest increase in N_2O production between 50-60% WFPS. Moreover, results of ^{15}N labelling studies in the same experiment confirmed the results of the acetylene inhibition, showing that autotrophic nitrification is the primary source of N_2O production between 35% - 65% WFPS, which is the same range of WFPS in the compacted and non-compacted soils in our incubation study indicating similar processes were likely occurring. In a study examining N_2O production in soils receiving urine, dung and compaction, van Groenigan et al. (2005) were able to draw similar conclusions. Results from their study showed that compaction was significant in N_2O production and that the most significant effects on N_2O production could be linked to changes in WFPS. Furthermore, high levels of compaction increase the duration of high WFPS, further increasing the likelihood of increased N_2O emission by denitrification. Sitaula et al. (2000) found that an increase in bulk density decreased the overall diffusivity of the soil. As the diffusivity of soil decreases, the likelihood of imposing anaerobic conditions on the soil increases. Since denitrification is an anaerobic process, an increase in bulk density would decrease diffusivity while increasing WFPS and thus increasing the

chances that denitrification could occur in saturated micropores. Sitaula et al. (2000) suggest that denitrification may start at levels > 45 % WFPS. This is the lowest WFPS at which denitrification has been suggested to occur, however, the authors do not state that levels of denitrification would be higher than nitrification at this point, only that both may occur.

In general, most studies state that WFPS is one of the most limiting factors of N₂O production, and that WFPS above 60% is widely regarded as the point at which denitrification begins to take place (Clayton et al. 1997; Robertson and Groffman, 2005). However, in this study, it would be likely that most of the N₂O production occurred largely as a result of nitrification. The WFPS of both CS and NC treated soils were below 60% and as such, probably not denitrifying on a large scale. However, it is likely that denitrification was occurring to some extent throughout the experiment. When the soils were compacted into cores, micro sites at which denitrification could occur were probably formed. Denitrification would take place in the micro-sites as long as suitable conditions and a constant supply of NO₃⁻ were available.

3.4.2. Influence of amendment type

The availability of NH₄⁺ in the soil is the single most crucial factor in determining the amount of N₂O produced (Robertson and Groffman, 2005). Soil microbes require a constant supply of N, preferably in the form of NH₄⁺ to survive. The availability of NH₄⁺ and therefore amendments containing NH₄⁺ are important in controlling nitrification and denitrification (Beauchamp, 1997).

Throughout the incubation experiment, the levels of NH_4^+ and NO_3^- were not measured in the soil; rather, all conclusions were derived based solely on the nature of the amendment at the time it was added to the soil.

Excluding the results from the erroneous AN treatment, cores treated with alfalfa had the highest initial production of N_2O and the highest cumulative production. High N_2O fluxes would be expected of alfalfa since it is a legume and naturally fixes di-nitrogen (N_2) gas from the atmosphere. With the addition of alfalfa, microbial communities will act quickly to mineralize C and N for growth purposes. This process has the ability to cause a net N mineralization and thus an increase in the levels of available inorganic N (Robertson and Groffman. 2005). A number of studies have found similar results where soils amended with alfalfa have larger N_2O emissions than soils amended with other fertilizers. For example, Chantigny et al. (2002) found that N_2O production was greater in plots fertilized with alfalfa than with cattle manure. Their results stated that legumes produce more N_2O through denitrification than manures. Carpenter-Boggs et al. (2000) showed that plots fertilized with alfalfa in the spring had higher levels of mineralization than other cover crops such as soybean and corn. They further state that due to N fixation, alfalfa has a relatively small C/N ratio and this ratio is important in determining preferential net N mineralization rather than net N immobilization. They concluded that rotations with legumes increase N mineralization. Larsson et al (1998) found N_2O production was highest in field soils amended with N-rich mulches such as alfalfa, and that this was seen primarily within the first month after addition. Larsson et al (1998) state that the addition of easily mineralized substrates will

increase N_2O fluxes not only due to an increase in nitrification resulting from available NH_4^+ , but also denitrification from more available NO_3^- as a result of increased nitrification. Furthermore, an increase in NO_3^- from nitrification has the ability to decrease the O_2 in the soil as aerobic microbes consume it. Changes in O_2 levels increase the presence of anaerobic micro sites and further enhance denitrification. Beauchamp et al. (2004) also state that legumes decompose faster than non-leguminous plants, and in turn, release inorganic N into the soil quickly. As far as mechanisms responsible for N_2O production, they support the view that denitrification is dependent on high soil water contents.

After alfalfa, N_2O production was greatest in manure amended soils. High levels of NH_4^+ in the soil amended with manure are conducive to high levels of nitrification. Like alfalfa, manure generally undergoes mineralization prior to undergoing processes like denitrification. Upon application of manure, organic matter therein is quickly mineralized and nitrified, thus producing N_2O . Oenema et al. (1997) state that after application, manure will be mineralized quickly, citing that urea, amino acids, peptides and bacterial cells will be mineralized within the first few days and lead to an increase in NH_4^+ and ammonia (NH_3). Other constituents however will remain more resistant and take months to mineralize. Our results show that these patterns have persisted throughout the duration of the experiment, particularly in the non-compacted soils. Results from non-compacted soils indicate that while alfalfa emissions were initially higher and cumulatively higher than manure, there was a brief period between hour 100 and 200 that manure N_2O production surpassed that of alfalfa. It is possible that this period of high

N₂O-N production could be attributable to nitrification of large quantities of NH₄⁺ released during mineralization of easily degradable components found in manure. Increased levels of NO₃⁻ that result from nitrification could also lead to an increase in denitrification as denitrifiers use NO₃⁻ as electron donors. The dynamics seem to indicate that high levels of N₂O emitted at the beginning of the experiment result from a mix of denitrification and nitrification. Findings by de Klein et al. (1996) in their acetylene inhibition found that initial rates of NO₃⁻ are low; however levels increase as nitrification takes place. This lends support to the evidence found in this study when N₂O-N emitted from manure amended soil briefly surpassed that of alfalfa amended soils. These findings support the idea that the occurrence of denitrification may have resulted because of decreased O₂ levels and increased NO₃⁻ levels, but were most likely not the most important process in the production of N₂O-N.

As mentioned previously, the soils amended with AN had much higher levels of N₂O production on a cumulative scale as a result of over application based on calculation errors. While emission rates relative to other treatments may not be comparable, we can still draw conclusions based on patterns seen here. In general, it appears that there is a higher likelihood that denitrification played a more substantial role in N₂O production than in either alfalfa or manure. As Lampe et al. (2006) point out, this is most likely as a result of a large NO₃⁻ pool available at the beginning of the experiment. This is in sharp contrast to alfalfa which must undergo mineralization then nitrification before accumulating a substantial pool of NO₃⁻, and manure which must undergo nitrification to accumulate NO₃⁻ in the soil. However, rates in our study would still most likely be lower

than those of Lampe et al. (2006) since WFPS was lower in the current study. There were large differences in production of N_2O between the early and later stages of the experiment in the AN amended soils (Figure 3-1 and Figure 3-2). The compacted soils had considerably higher initial production of N_2O , indicating that there may be a quick initial period of nitrification of NH_4^+ or denitrification or of available NO_3^- not seen in the non-compacted soil. A sharp decline in N_2O -N production after 200 hours in the CS and 300 in the NC soils then occurred. This earlier decline in N_2O -N production in the CS could have occurred as a result of accelerated losses of NO_3^- in addition to NH_4^+ resulting from denitrification prior to 200 hours. The secondary spike in N_2O production at 263 hrs in the CS treatment could have resulted from increased levels of NO_3^- liberated through nitrification. In terms of the relative N_2O production among amendment types, Jarecki et al. (2008) found that AN amended soils had higher levels of cumulative N_2O production in sandy loam soil than soils amended with manure. Unfortunately this conclusion cannot be speculated on given the application errors of AN in the current study.

3.4.3. Influence of pH

Although pH was not measured, it is unlikely that there were any major changes throughout the incubation in the alfalfa, manure and control soil treatments. However, as a result of over application of AN, changes may have arisen in pH in the AN treatments. For both NC and CS treatments, N_2O -N production increased dramatically in AN treated soils towards the end of the experiment. By this point N_2O -N production in alfalfa, manure and control treatments had all returned to background levels. Over application of

AN may have temporarily increased the pH in the soil as a result of hydrolysis as NH_4^+ was converted to NH_3 . This increase in pH could have temporarily reduced the activity of enzymes. The decrease in the activity of this enzyme would have reduced the conversion of NH_3 to NO_3^- . Once pH levels returned to normal after volatilization reduced the soil pH, these enzymatic processes resumed, allowing high quantities of NH_4^+ to be quickly nitrified and lost as $\text{N}_2\text{O-N}$. Furthermore, the absence of enzymatic activity could have led to elevated levels of nitrite (NO_2) in the soil which is toxic to many organisms and would have contributed to the decreases in $\text{N}_2\text{O-N}$ production 400-500 hours into the incubation. To our knowledge pH remained at or near background levels which were approximately 6.6 (Gregorich et al., 2006) in the alfalfa, manure and control treatments.

3.4.4. CO_2 Production

Like $\text{N}_2\text{O-N}$ production, $\text{CO}_2\text{-C}$ production was greatest in both NC and CS treatments and all amendment treatments including control at the beginning of the incubation and subsequently declined throughout the experiment. In all cases $\text{CO}_2\text{-C}$ production was highest in the alfalfa, and followed by manure amended soils with control and AN amended soils having similar $\text{CO}_2\text{-C}$ emissions. However, unlike N_2O , $\text{CO}_2\text{-C}$ production was higher in non-compacted soils than in compacted soils. The $\text{CO}_2\text{-C}$ fluxes in compacted soils were 13%, 14%, 34% and 15% higher in non-compacted alfalfa, control, fertilizer and manure amended soils respectively. There was also little connection between N application and $\text{CO}_2\text{-C}$.

Ruser et al. (2006) measured slightly higher but non-significant levels of CO₂ flux from non-compacted soil ridges compared to compacted interrow soil. Their rates were higher than ours ranging from 8-35 mg CO₂-C m⁻² h⁻¹, in contrast to ours, which were 0.048 to 4.8 mg CO₂-C m⁻² h⁻¹ in fertilized and alfalfa plots respectively. Jensen et al. (1996) found that total porosity may have an effect on C mineralization and in turn the production of CO₂. Jensen et al. (1996) concluded that it was not a reduction in pore size inhibiting microbial access to pore space that reduced CO₂ production, but the anaerobic conditions imposed on soil pores that inhibited C mineralization as it is an aerobic process. Conversely, De Neve et al. (2000) found that there were no clear differences in C mineralization between soils at different compaction rates. Findings indicate that differences in C mineralization may occur at bulk densities above 1.6 g cm⁻³. Beare et al. (2009) also found significant relationships between compaction and CO₂ production, suggesting that CO₂ production peaked at 45% WFPS and began to decline with further increases in WFPS. Our NC soils at 37% WFPS were likely beginning to reach a possible maximum in CO₂ production, while the CS with a higher WFPS most likely had CO₂-C production limited to some extent by reduced aeration.

3.4.5. Influence of amendments

The relative rates of CO₂-C production were directly related to the quality of the material added to the soil. Alfalfa produced approximately 2 times more CO₂-C than manure which showed the second highest cumulative fluxes. Alfalfa contains higher levels of C available for microbial consumption. In the process, CO₂ is produced as a bi-product of microbial respiration. Depending on the amendment and the relative quantity

of organic compounds, C consumption will take place on a smaller or greater scale. In terms of time in which breakdown occurs, the order is usually as follows: sugars, starches, simple proteins>crude proteins>hemi cellulose>cellulose> fats and waxes> lignins and phenolic compounds (Brady and Weil, 2002). Although no actual sampling of the DOC was done, samples taken from field experiments throughout the summer show that the levels of DOC were highest in alfalfa (Figure 2-11). Total DOC remained in this order based on amendment type throughout the entire sampling period of 132 days, though the quantity greatly decreased. As with the incubation experiment, levels of CO₂-C did drop dramatically as levels of DOC dropped within the first 10 days. By the end of the experiment, CO₂-C production was similar across all treatments as was the case in the field study (Figure 3-4 and Figure 3-5). Ellert and Janzen (2008) found that the highest levels of CO₂ emissions in fall coincided with the termination of alfalfa crops. They further found that manure also increased CO₂ emissions while AN did not, a result similar to ours. The AN treatment most likely had little effect on CO₂-C production because there was no organic matter added to the soil. Nevertheless, CO₂ and N₂O production are often coupled. The reasoning is that during C consumption, the production of CO₂ may decrease O₂ levels and thus increase anaerobic sites in the soil leading to denitrification (Ellert and Janzen, 2008).

Rochette et al. (2000) state that low CO₂ fluxes result from low temperatures, low root respiration and low oxidation by heterotrophs. Soil temperatures in our incubation were sufficiently high to avoid this problem and as there was no plant heterotrophic activity to have been the driving cause of CO₂ production. Other research showed similar

results indicating that manure (in this case swine slurry) showed significantly higher levels of CO₂ production than soils treated with AN, which in turn showed no significant difference from control plots (Jarecki et al. 2007)

3.5. Conclusion

Compaction of soil resulted in increased levels of N₂O-N and was partially attributed to the decrease in aeration within the soil as a result of decreased porosity and increased WFPS. This result supports the idea that compaction increases N losses in agricultural soils. Carbon dioxide production decreased as a result of increased compaction indicating that compaction seriously altered the activity of soil microbes.

The type of amendment had significant consequences on the release of N₂O-N and CO₂-C from the soil. Alfalfa amended soil resulted in the highest initial release of N₂O-N and CO₂-C, as well as the highest cumulative quantities over the experimental period. With all amendments, the initial production of N₂O-N and CO₂-C occurred quickly upon application to the soil and returned to background levels within ~350 hours of application. Under moisture levels and bulk density analogous to ideal field conditions, losses were attributed to microbial breakdown of N, mostly through nitrification, although we cannot determine for certain that low levels of denitrification did not occur. Our results indicate that the nature of organic and inorganic amendments as well as the state of the physical condition of the soil greatly alters the production of biogenic gasses which result in substantial losses of soil N

Lab incubation techniques are advantageous in studying soil N processes under ideal conditions. In this case, ideal conditions refer to high soil and air temperatures as well as stable WFPS. By maintaining steady levels of both, the fluctuations of other variables on the production of $\text{N}_2\text{O-N}$ can be studied without the confounding effects of fluctuating environmental variables influencing gas fluxes. However, lab incubations are unable to accurately reflect natural patterns with which such losses occur. The pattern of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ production in the lab incubation differed greatly from patterns in field incubation. Lab incubation results present a single high production of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ followed by a gradual decrease as compared to the field experiment which produced multiple large productions of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ as soil moisture and temperature varied. Lab incubations therefore represent potential losses as opposed to field experiments which reflect actual losses.

4.0 Summary & Conclusion

The purpose of this study was to identify how fertilizer amendments, environmental variables and soil physical properties interact to influence soil N transformations and gaseous losses of C and N.

While the incubation experiment allowed us to determine the relative rates at which $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ are transformed and lost from soil under controlled temperature and moisture conditions, the field study more accurately demonstrated how variable *in situ* soil N dynamics can be in relation to environmental variables.

The main findings of the studies are as follows.

- 1) The quality of the amendment applied and the ease with which microbes break down substrates had a significant effect on the amount of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ produced. Losses of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ are indicative of not only important greenhouse gas emissions but of soil transformation processes, including mineralization and nitrification. Given the similarity in total N applied with each amendment, amendments with lower C:N and organic N sources resulted in higher fluxes of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ indicating more rapid soil N transformation and higher levels of microbial respiration. Ammonium nitrate treated soils produced the highest cumulative quantities of $\text{N}_2\text{O-N}$; however, this was attributed to over application of the amendment. Otherwise both $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ cumulative production was greatest in soils amended with alfalfa, followed by manure and control. Carbon dioxide production was not significantly increased by

the over application of AN. In particular, the presence of large amounts of labile C resulted in faster N transformations in alfalfa amended soils than either manure or AN amended soils or control soils. Ammonium nitrate was responsible for adding the greatest quantities of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ to the soil. However, alfalfa also added significant quantities of mineral N to the soil. These results suggest that alfalfa may release inorganic N at rates and overall quantities conducive to crop growth.

- 2) The field and lab experiments highlight how temporal patterns of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ production can vary in response to environmental variables such as temperature and WFPS. Control of environmental variables including temperature and rainfall within a closed environment such as the lab incubation, resulted in a single large production of both $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ early in the experiment, while field experiments experienced great variation in timing and quantity of $\text{N}_2\text{O-N}$ and $\text{CO}_2\text{-C}$ production. The lab incubation experiment allowed monitoring of gas production at regular intervals without fear of missing important fluxes induced by rainfall and temperature variation. A major uncertainty in the assessment of total cumulative N losses in the field experiment is the interpolation of gas flux activity given the sporadic nature of these emissions in an uncontrolled environment. However, the field experiment was advantageous in that soils could be sampled in order to determine relative quantities of inorganic N, something that lab incubation did not allow. Unfortunately the benefits of this sampling

procedure were partially offset by unaccounted leaching losses of N as a result of large rainfall events.

- 3) The alteration of physical soil characteristics has an effect on the transformation and losses of soil N. Production of $\text{N}_2\text{O-N}$ was higher in compacted soils regardless of the amendment type. Bulk density only affected the production of $\text{CO}_2\text{-C}$ in alfalfa amended soils and it decreased with compaction. Increasing soil bulk density inhibits the activity of soil microorganisms by limiting access to organic matter as well as limiting O_2 levels, therefore, slowing N transformation processes. The issue is similar for $\text{N}_2\text{O-N}$ losses which increases with increasing bulk density as soil pore space is decreased and anaerobic micro-sites become more plentiful within the soil

Both incubation and field experiments possessed advantages and disadvantages where field experiments represent a more realistic view of N and C cycling in agroecosystems, while lab incubation studies are more suited for the study of specific processes. Although every effort was made to mimic the procedure used in production agriculture, subtle differences remain. Incorporation of amendments were much more thorough here than in practice. While our incorporation of amendments stressed homogeneity, this is often not the case in agricultural practices. Localized hotspots in the soil, including clumps of vegetation and points of manure injection into the soil may result in increased N transformations and GHG production. As a result, there are issues regarding experimental rates and quantities of GHG and mineral N produced that inherently differ between field conditions and experimental conditions. Despite these differences, the

findings of this study have indicated that N loss in agricultural settings has the potential to be a serious problem.

As mentioned earlier, manure can be difficult to deal with logistically. Farmers often apply manure long before planting and consequently lose substantial amounts of manure N before it is utilized for plant growth. Instead farmers apply AN in the spring to supplement soil N lost over winter. Typically, farmers give little credit to alfalfa when calculating N budgets for the upcoming growing season. One can argue that based on the results of the field experiment it is apparent that proper management of both manure and alfalfa can lead to the release of substantial quantities of N ~40 days after application. In some cases this could coincide with periods in which N is in highest demand in the soil.

In studying these components of the agroecosystem, we hoped to gain a better insight into how fertilizer application, be it from alfalfa, manure or AN, could be better managed to minimize losses and maximize available soil N. Based on the findings of both the field experiment and the lab experiment attention may be drawn to possible sources of mismanagement in agriculture which in turn leads to losses of N and contributes to a source of inefficiency in agriculture which can prove to be not only financially damaging to farmers but also environmentally damaging.

In general, farmers are often not aware that the rate of N transformation is very rapid. Often transformations are so rapid that it may be difficult for farmers to synchronize application methods and timing for maximum plant uptake. The results in this study emphasize the importance of environmental variables such as rainfall and

temperature on influencing rates of N emissions and transformations. Application of fertilizers, especially fertilizers containing high quantities of NO_3^- , need to be shifted away from periods where both of these environmental variables are not ideal for N retention in the soil. Manure especially is often applied in the fall when large quantities of NO_3^- are susceptible to leaching losses, as well as subject to higher than usual periods of denitrification. Our results indicate clearly that increasing WFPS has a serious effect on N losses from N_2O -N production. Furthermore, higher levels of compaction in soils late in the season increase the chances of N loss. In an effort to minimize clumping of soil and control weeds, alfalfa is often plowed down in the fall. Like manure this exposes N to transformations and losses which are unnecessary and wasteful. Our results indicate that alfalfa releases substantial quantities of inorganic N into the soil. In addition, the large amount of labile C contained within the alfalfa means that N is released quickly. A delay in the incorporation of alfalfa into the soil may decrease the losses of N from alfalfa through leaching.

It is important that further research on proposed changes to fertilizer application be studied. Production agriculture will only be willing to implement changes to fertilization practices that prove to be beneficial in the long-term and on a large scale. The use of N tracer studies in particular could prove useful in determining with more accuracy the rate at which transformations occur. Furthermore, studies incorporating plant growth are essential to determine how plant growth alters not only N transformations, but also losses. From a more holistic point of view, the use of organic fertilizers should be compared to synthetic fertilizers not only in their ability to produce

large quantities of inorganic N, but also taking into account other facets of fertilizer production as they relate to GHG production. Synthetic fertilizers are energy intensive to manufacture and contribute to other sources of GHG production besides losses in the field. Similarly, manure releases other forms of GHG during storage. These emissions should be taken into account when considering what type of fertilizer is most beneficial at the broad scale.

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5.0 Appendix

Table 5-1: Spearman's rho correlation coefficients for environmental and response variables associated with the alfalfa treatment for DOY 162-172. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	Soil Temp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.77	-0.86	-0.82	-0.43	-0.07	-0.77	-0.32	-0.48
WFPS		1	-0.75	-0.82	0.63	-.31	-0.69	-0.45	-0.56
Soil Temp			1	0.81	0.59	-0.10	0.81	0.34	0.37
CO ₂ -C				1	-0.43	-0.07	0.70	0.02	0.25
N ₂ O-N					1	0.14	-0.42	-0.05	0.34
Total N						1	0.10	0.61	0.54
DOC							1	0.34	0.43
NO ₃ ⁻ -N								1	0.63
NH ₄ ⁺ -N									1

Table 5-2: Spearman's rho correlation coefficients for environmental and response variables associated with the control treatment for DOY 162-172. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	Soil Temp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.82	-0.75	-0.57	0.67	-0.19	-0.09	-0.37	-0.40
WFPS		1	-0.82	-0.67	0.70	-0.15	-0.30	-0.43	-0.23
Soil Temp			1	0.78	-0.63	-0.17	0.42	0.59	0.04
CO ₂ -C				1	-0.55	-0.15	0.33	0.39	0.03
N ₂ O-N					1	-0.15	-0.24	-0.47	0.33
Total N						1	-0.22	0.46	-0.28
DOC							1	0.23	-0.23
NO ₃ ⁻ -N								1	0.17
NH ₄ ⁺ -N									1

Spearman's Rho variable by variable correlation for Control treatment between DOY 162-172

Table 5-3: Spearman's rho correlation coefficients for environmental and response variables associated with the AN treatment for DOY 162-172. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.88	-0.77	-0.77	0.44	-0.48	-0.27	-0.57	-0.62
WFPS		1	-0.82	-0.75	0.55	-0.48	-0.41	-0.62	-0.59
SoilTemp			1	0.81	-0.56	0.33	0.61	0.63	0.70
CO ₂ -C				1	-0.24	0.20	0.39	0.44	0.65
N ₂ O-N					1	-0.66	-0.14	0.72	-0.58
Total N						1	-0.13	0.82	0.62
DOC							1	0.16	0.62
NO ₃ ⁻ -N								1	0.81
NH ₄ ⁺ -N									1

Table 5-4: Spearman's rho correlation coefficients for environmental and response variables associated with the manure treatment for DOY 162-172. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.70	-0.62	-0.50	0.47	-0.42	-0.26	-0.59	-0.69
WFPS		1	-0.72	-0.53	0.60	-0.41	-0.45	-0.58	-0.60
SoilTemp			1	0.72	-0.51	0.14	0.63	0.52	0.74
CO ₂ -C				1	-0.25	-0.07	0.66	-0.26	0.63
N ₂ O-N					1	-0.19	-0.24	-0.20	-0.40
Total N						1	0.07	0.76	0.47
DOC							1	0.28	0.60
NO ₃ ⁻ -N								1	0.60
NH ₄ ⁺ -N									1

Table 5-5: Spearman's rho correlation coefficients for environmental and response variables associated with the alfalfa treatment for DOY 172-182. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.86	-0.52	0.19	-0.02	-0.29	-0.14	-0.31	-0.08
WFPS		1	-0.53	0.13	-0.12	-0.22	-0.13	-0.17	0.00
SoilTemp			1	0.00	-0.16	0.44	0.33	0.40	-0.04
CO ₂ -C				1	0.50	0.16	0.24	0.08	0.09
N ₂ O-N					1	0.15	0.03	0.00	-0.05
Total N						1	0.41	-0.92	-0.03
DOC							1	0.35	0.01
NO ₃ ⁻ -N								1	0.01
NH ₄ ⁺ -N									1

Table 5-6: Spearman's Rho rho correlation coefficients for environmental and response variables associated with the control treatment for DOY 172-182. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	.85	-0.58	-0.24	-0.10	-0.58	-0.36	-0.57	0.15
WFPS		1	-0.63	-0.23	-0.03	-0.51	-0.27	-0.37	0.17
SoilTemp			1	0.29	0.10	0.58	0.44	0.24	-0.11
CO ₂ -C				1	0.29	0.08	0.28	-0.49	-0.03
N ₂ O-N					1	0.33	0.22	-0.03	0.18
Total N						1	0.34	-0.35	-0.21
DOC							1	-0.14	-0.17
NO ₃ ⁻ -N								1	-0.18
NH ₄ ⁺ -N									1

Spearman's Rho variable by variable correlation for control treatment between DOY 172-182

Table 5-7: Spearman's rho correlation coefficients for environmental and response variables associated with the AN treatment for DOY 172-182. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.69	-0.48	0.03	0.13	-0.35	-0.08	-0.38	-0.09
WFPS		1	-0.52	-0.02	0.12	-0.17	-0.17	0.21	-0.15
SoilTemp			1	0.21	0.06	0.14	0.43	0.18	0.29
CO ₂ -C				1	0.30	-0.32	0.21	-0.45	0.10
N ₂ O-N					1	0.38	0.07	0.29	0.15
Total N						1	-0.27	-0.90	-0.13
DOC							1	-0.17	-0.11
NO ₃ ⁻ -N								1	0.12
NH ₄ ⁺ -N									1

Table 5-8: Spearman's rho correlation coefficients for environmental and response variables associated with the manure treatment for DOY 172-182. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.74	-0.04	-0.17	0.19	0.28	0.03	0.21	0.15
WFPS		1	-0.24	-0.24	-0.12	0.05	0.02	0.05	-0.10
SoilTemp			1	0.12	0.12	0.39	0.08	0.26	-0.16
CO ₂ -C				1	0.46	0.28	0.26	0.01	0.30
N ₂ O-N					1	0.44	0.29	0.34	0.45
Total N						1	0.37	0.83	-0.05
DOC							1	-0.30	0.17
NO ₃ ⁻ -N								1	0.17
NH ₄ ⁺ -N									1

Table 5-9: Spearman's rho correlation coefficients for environmental and response variables associated with the alfalfa treatment for DOY 190-207. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.60	-0.17	-0.06	0.18	-0.25	0.34	-0.40	-0.02
WFPS		1	-0.52	-0.08	-0.09	-0.27	0.13	-0.47	-0.36
SoilTemp			1	0.26	0.38	0.00	0.19	0.21	0.31
CO ₂ -C				1	0.50	0.00	0.11	-0.09	0.17
N ₂ O-N					1	0.07	0.11	-0.35	0.15
Total N						1	0.22	0.83	0.31
DOC							1	-0.12	0.17
NO ₃ ⁻ -N								1	0.38
NH ₄ ⁺ -N									1

Table 5-10: Spearman's rho correlation coefficients for environmental and response variables associated with the control treatment for DOY 190-207. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.49	-0.30	-0.56	-0.44	-0.67	0.19	-0.64	0.44
WFPS		1	-0.55	-0.51	0.42	-0.31	0.14	-0.22	0.03
SoilTemp			1	0.15	0.75	-0.01	-0.28	-0.12	0.11
CO ₂ -C				1	-0.34	0.48	0.04	0.42	0.08
N ₂ O-N					1	0.08	-0.29	0.41	-0.15
Total N						1	-0.05	-0.61	0.42
DOC							1	0.07	0.03
NO ₃ ⁻ -N								1	-0.38
NH ₄ ⁺ -N									1

Table 5-11: Spearman's rho correlation coefficients for environmental and response variables associated with the AN treatment for DOY 190-207. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.73	-0.53	-0.62	0.26	0.02	0.21	-0.24	-0.16
WFPS		1	-0.63	-0.52	0.19	-0.01	0.30	-0.28	-0.18
SoilTemp			1	0.47	0.43	-0.28	-0.13	0.17	0.22
CO ₂ -C				1	0.19	0.16	-0.46	0.12	0.25
N ₂ O-N					1	0.21	0.44	0.18	0.38
Total N						1	-0.05	0.73	0.05
DOC							1	-0.44	-0.05
NO ₃ ⁻ -N								1	0.20
NH ₄ ⁺ -N									1

Table 5-12: Spearman's rho correlation coefficients for environmental and response variables associated with the manure treatment for DOY 190-207. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.61	-0.42	-0.26	-0.09	-0.09	0.22	-0.28	0.02
WFPS		1	-0.72	-0.53	0.29	-0.05	-0.13	-0.06	0.02
SoilTemp			1	-0.32	0.51	-0.25	-0.20	0.06	-0.27
CO ₂ -C				1	0.26	0.28	-0.15	0.12	0.12
N ₂ O-N					1	0.11	-0.32	-0.11	0.26
Total N						1	-0.13	0.45	0.01
DOC							1	-0.27	-0.53
NO ₃ ⁻ -N								1	0.12
NH ₄ ⁺ -N									1

Table 5-13: Spearman's rho correlation coefficients for environmental and response variables associated with the alfalfa treatment for DOY 225-294. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.94	0.83	0.67	0.05	0.64	-0.45	0.26	0.36
WFPS		1	0.90	0.77	0.20	0.77	-0.24	0.31	0.45
SoilTemp			1	0.81	0.12	0.84	-0.39	0.42	0.51
CO ₂ -C				1	0.16	-0.63	0.16	-0.01	0.34
N ₂ O-N					1	0.28	-0.46	0.59	0.43
Total N						1	0.69	-0.24	-0.42
DOC							1	-0.04	-0.07
NO ₃ ⁻ -N								1	-0.04
NH ₄ ⁺ -N									1

Table 5-14: Spearman's rho correlation coefficients for environmental and response variables associated with the control treatment for DOY 225-294. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.77	0.45	0.38	-0.35	-0.75	0.21	0.63	0.45
WFPS		1	0.60	0.53	0.02	-0.69	0.04	0.55	0.50
SoilTemp			1	0.94	0.25	-0.87	-0.39	-0.77	0.62
CO ₂ -C				1	-0.16	-0.62	0.30	-0.62	0.65
N ₂ O-N					1	-0.27	-0.33	-0.33	0.06
Total N						1	0.22	0.95	0.70
DOC							1	0.22	0.05
NO ₃ ⁻ -N								1	0.49
NH ₄ ⁺ -N									1

Table 5-15: Spearman's rho correlation coefficients for environmental and response variables associated with the AN treatment for DOY 225-294. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.77	0.45	0.38	0.35	-0.75	0.21	-0.63	0.45
WFPS		1	0.60	0.53	-0.02	-0.69	0.04	-0.55	0.50
SoilTemp			1	0.94	0.25	-0.87	-0.39	-0.77	0.62
CO ₂ -C				1	0.16	-0.62	-0.49	-0.62	0.65
N ₂ O-N					1	-0.27	-0.30	-0.31	0.06
Total N						1	-0.33	0.95	-0.70
DOC							1	-0.22	-0.05
NO ₃ ⁻ -N								1	-0.49
NH ₄ ⁺ -N									1

Table 5-16: Spearman's rho correlation coefficients for environmental and response variables associated with the manure treatment for DOY 225-294. Significant correlations are shown in bold ($p < 0.05$).

	VWC	WFPS	SoilTemp	CO ₂ -C	N ₂ O-N	Total N	DOC	NO ₃ ⁻ -N	NH ₄ ⁺ -N
VWC	1	0.91	0.54	0.40	0.09	0.05	-0.11	-0.37	0.66
WFPS		1	0.45	0.31	0.01	-0.27	-0.08	-0.39	0.57
SoilTemp			1	0.83	-0.27	-0.17	-0.68	-0.52	0.41
CO ₂ -C				1	-0.02	0.33	-0.57	-0.14	0.25
N ₂ O-N					1	0.33	0.67	0.37	0.49
Total N						1	0.43	0.82	-0.11
DOC							1	0.49	0.20
NO ₃ ⁻ -N								1	-0.11
NH ₄ ⁺ -N									1