Using The Nitrification Inhibitor 3, 5-dimethylpyrazole To Determine Net Nitrogen Mineralization And Denitrification Rates Simultaneously At Varying Water Contents

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
January 2017

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Abstract

This study investigated the use of a nitrification inhibitor (NI) as a means of simultaneously measuring the rates of net nitrogen (N) mineralization, as an accumulation of ammonium (NH_4^+), and denitrification, as a loss of nitrate (NO_3^-) throughout a 28-day incubation. The NI assay method, using 3, 5-dimethylpyrazole (DMP) as the NI, was evaluated using two levels of inhibition (0 and 200 mg DMP kg^{-1}) in two soil textures (fine vs. coarse) over a wide range of water-filled pore space (% WFPS) (35, 50, 85%). The lack of an increase in NO_3^- and an accumulation of NH_4^+ indicated that DMP was inhibiting nitrification, and clay content did not interfere with the efficacy of DMP. Microbial respiration (CO_2 production) did not significantly decrease with DMP application suggesting no adverse microbial effect. Water content (10 levels from 20 - 110% WFPS) influenced N mineralization and denitrification and differed between soil textures (fine vs. coarse) and carbon intensity (pig slurry, corn residues (High) vs. no manure, no residues (Low)). The N mineralization rate was greater in fine-textured soil above 70% WFPS; the denitrification rate was greater in coarse soil at 110% WFPS. The N mineralization was greater in low carbon intensity soil as apparent immobilization occurred above 85% WFPS in high carbon soil; the denitrification rate was greater in high carbon soil at above 80% WFPS. The NI method effectively assessed the influence of water content on the N mineralization and denitrification in soils differing in texture and carbon amendment.

List of Abbreviations Used

- NI (nitrification inhibitor)
- DMP (3, 5-dimethylpyrazole)
- WFPS (water-filled pore space)
- NH_4^+-N (ammonium nitrogen)
- NO_3^--N (nitrate nitrogen)
- CO_2 (carbon dioxide)
- TSN (total soluble nitrogen)
- DOC (dissolved organic carbon)
- DON (dissolved organic nitrogen)
- TEA (terminal electron acceptor)
- N_2O (nitrous oxide)

Acknowledgements

I would like to thank my supervisors Dr. David Burton, Department of Plant, Food and Environmental Sciences, Faculty of Agriculture, Dalhousie University and Dr. Bernie Zebarth, Agriculture and Agri-Food Canada for their guidance and support during my graduate studies. I would also like to thank my committee, Dr. Alex Georgallas and Dr. Gordon Price, Department of Engineering, Faculty of Agriculture, Dalhousie University.

Chapter 1.0 Introduction

1.1 Introduction

In agricultural crop production nitrogen (N), a macronutrient essential for plant growth, is typically limiting (Robertson and Groffman 2007). Producers add manure and inorganic fertilizers to the soil to meet crop N demand (Havlin et al. 2013). However, N losses of applied fertilizers can be substantial in humid climates due to excess precipitation in the spring and early fall (Zebarth et al. 2009). It is estimated that approximately 40% of soluble N going into the soils in Canadian agriculture systems is lost by leaching and denitrification (St. Luce et al. 2011; Janzen et al. 2003). Thus, from an economic and environmental perspective, it is necessary to improve N use efficiency in humid climates, such as Eastern Canada, by developing fertilizer recommendations that account for all the N sources available for plant uptake throughout the growing season (Sharifi et al. 2007; Zebarth et al. 2009; Dessureault-Rompré et al. 2011a). This is particularly true for soil organic N pools, which can supply 30 to 100% of the N required by the growing crop (Drury et al. 2003). However, the supply of N from these organic N pools is highly variable among fields and years and is therefore difficult to predict (Zebarth et al. 2012).

The biochemical processes that transform soil mineral N, the primary form of N taken up by plants, are controlled by the nature, distribution and activity of soil microorganisms (Robertson and Groffman 2007). The processes of N mineralization, nitrification and denitrification are major microbe facilitated processes governing soil mineral N availability originating from the soil organic N pool (St. Luce et al. 2011; Curtin and Campbell 2008). As these processes are facilitated by factors that influence microbial growth and activity, such as substrate availability, soil water

content and temperature (Zak et al. 1999), it is important to determine the extent to which microbial activity is affected by these factors. In humid climates with high annual precipitation it is important to examine how soil water content regulates the rate of net N mineralization and how this relates to nitrate (NO_3^-) accumulation and losses of during denitrification (Dessureault-Rompré et al. 2011b; Georgallas et al. 2012).

As the processes of net N mineralization and denitrification occur simultaneously in soils, and they have interdependent intermediates, it would be valuable for a laboratory assay to be able to assess these processes simultaneously in the same vessel. The quantity of ammonium (NH_4^+) accumulation in soil cannot be used as an indication of net N mineralization as it is rapidly converted to NO_3^- under aerobic conditions (Havlin et al. 2013). Many studies have reported net N mineralization as an accumulation of both NH_4^+ and NO_3^- (Stanford and Epstein 1974; Paul et al. 2003; Franzluebbers 1999). However, the denitrification of any NO_3^- that is produced, particularly at high water content, would cause N mineralization to be under estimated. The addition of a nitrification inhibitor (NI) to the assay system provides an opportunity to measure both processes simultaneously as a NI temporarily blocks the conversion of NH_4^+ to NO_3^- (Zerulla et al. 2001), allowing for concurrent measurements of the accumulation of NH_4^+ as a result of net N mineralization and the disappearance of NO_3^- as a result of denitrification (Khosa et al. 2012).

This study will use a NI to examine the effects of a wide range of soil water contents, representative of soil water available during the growing season, on the

rates of net N mineralization and denitrification. The NI technique will be applied to assess the effect of water content on net N mineralization and denitrification rates in contrasting soils, differing in soil texture and carbon status.

1.2 Literature Review

1.2.1 Factors Controlling Microbial Activity in Soil

Soil microbial activity is governed by two major environmental factors, water content and temperature (Voroney 2007). At low water content, water availability is an important constraint to microbial activity due to the limited accessibility of carbon (C) substrates for microbial growth and metabolic functions (St. Luce et al. 2011; Agehara and Warncke 2005; Voroney 2007). At high water content gaseous diffusion is limited by water-filled pore space (WFPS) and as a result microbial activity is limited by restricted oxygen (O_2) supply (Brady and Weil 2002c).

Soil structure influences the ability of a soil to hold water. Bulk density and total porosity together can be used to indicate pore space that could be air- or water-filled and, depending on the texture, how these pores are distributed in the soil (Brady and Weil 2002a).

The size, shape and connectivity of soil pores regulate soil aeration and water content (Voroney 2007). The water content of soil macropores alters the availability of O_2 in soils by limiting the amount of O_2 diffusion into smaller pore spaces where microbes perform their metabolic functions (Voroney 2007). When microbes utilize all of the dissolved O_2, the soil environment can change from aerobic to anaerobic (Voroney 2007). Aerobic microbial activity is dominant when the diffusion of O_2 into soil macropores is not limited (Brady and Weil 2002c). While anaerobic microbial

activity is dominant when O_2 is limited in soil macropores (Havlin et al. 2013). Aerobic and anaerobic activity can occur across a wide range of water contents, although it is generally accepted that below 60% WFPS aerobic activity dominates, and that above 80% WFPS anaerobic activity predominates (Linn and Doran 1984; Havlin et al. 2013).

The maximum rate of aerobic microbial activity, as measured by aerobic respiration, has been observed in the range of 50 - 70% WFPS (Linn and Doran 1984; Franzluebbers 1999; Havlin et al. 2013). Linn and Doran (1984) found positive linear relationships between WFPS and aerobic activity over the range of 30 - 60% WFPS and negative linear relationships between 60 - 70% WFPS.

Another critical factor is the ability of water to solubilize C substrates for microbial metabolic functions, and the temperature of the soil influences this process (Voroney 2007; Zak et al. 1999). As temperature increases in the soil there is an increase in the solubilization of substrates increasing biochemical reactions and the decomposition of organic substrates (Voroney 2007; Zak et al. 1999).

1.2.2 Microbe-Facilitated N Conversion Processes

There are three major processes mediated by microbes that control the availability of mineral N in the soil throughout the growing season. These processes are net N mineralization, nitrification, and denitrification.

1.2.2.1 Net N Mineralization

When heterotrophic soil fauna and microbes (bacteria and fungi) decompose C-based material with a narrow (<20:1) C:N ratio to acquire energy, N is released or mineralized (Robertson and Groffman 2007). Mineralization of N is a catabolic

process that results in carbon dioxide (CO_2) production. Biosynthesis associated with microbial growth on substrates with a wide (>30:1) C:N ratio results in inorganic N being converted to organic N, resulting in negative net mineralization or immobilization. Soil N accounts for 0.02 to 0.5% of the total soil mass and most of this N (95%) is found as organic N in the surface layer of the topsoil (Havlin et al. 2013). The fraction of organic N that is most susceptible to mineralization is the decomposable amino acids and organic bound NH_4^+ (Havlin et al. 2013).

While Gross N mineralization represents the total extent of N mineralization, net N mineralization is the balance between gross N mineralization and N immobilization (Robertson and Groffman 2007). Microbes immobilize N to support biosynthesis and growth (Robertson and Groffman 2007; Paul et al. 2003). Gross N mineralization and N immobilization occur simultaneously in soil and the extent of net N mineralization (or immobilization) and therefore the availability of mineral N depends on the C:N ratio of the C substrate (Robertson and Groffman 2007). In general, degradation of a C substrate with a C:N ratio > 30 results in net N immobilization, while net N mineralization occurs when the C:N ratio is < 20 (Robertson and Groffman 2007; St. Luce et al. 2011; Havlin et al. 2013).

1.2.2.2 Nitrification

Nitrification is the microbial-mediated oxidation of NH_4^+ to produce NO_3^-. The process is primarily catalyzed by chemoautotrophic bacteria in most soils, and the reaction typically occurs under aerobic conditions (Firestone and Davidson 1989; Groffman 1991). Nitrifying microbes (e.g., Nitrosomonas) first oxidize NH_4^+ to nitrite (NO_2^-) and subsequently a second group of microbes (e.g., Nitrobacter) convert NO_2^-

to NO_3^- (Havlin et al. 2013). The NH_4^+ introduced into the soil from fertilizers, and NH_4^+ produced from the mineralization of organic N are subject to nitrification (Slangen and Kerkhoff 1984; Groffman 1991; Havlin et al. 2013).

1.2.2.3 Denitrification

Denitrification is the anaerobic respiratory reduction of NO_3^- by heterotrophic microbes (Groffman 1991) resulting in a progressive set of reactions that convert NO_3^- to NO_2^- and then to gaseous N compounds such as nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) (Firestone and Davidson 1989). Denitrification is regulated by O_2 supply as influenced by the water content and the quality and quantity of C substrate (Georgallas et al. 2012; Baggs et al. 2000), and oxides of nitrogen for use as a terminal electron acceptors (TEA) under anaerobic conditions (Burton et al. 2012).

1.2.3 Factors Regulating the Rate of Net N Mineralization

From the seminal work by Stanford and Smith (1972), the mineralization of soil organic N follows a first-order kinetic model. The model includes a mineralization rate constant and a term quantifying the amount of potentially mineralizable soil N present in the soil (Dessureault-Rompré et al. 2010). Juma et al. (1984) observed that N mineralization in soil is not likely the result of a single homogeneous and discrete pool of organic N and that this process is often modeled as occurring as a result of one, two or three first order kinetically defined pools. The rate of net N mineralization is regulated by three major factors: (1) the quality and the quantity of potentially mineralizable organic N, (2) soil temperature, and (3) the

soil water content and its impact on soil aeration status (Robertson and Groffman 2007; St. Luce et al. 2011; Agehara and Warncke 2005; Zak et al. 1999).

1.2.3.1 Quality and Quantity of Organic Nitrogen Substrate

The quality and quantity of organic N substrates determines the rate and pattern of N mineralization and immobilization (Robertson and Groffman 2007). As the composition or quality of organic N substrates varies from wide to narrow C:N ratios, so does the activity of the microbes responsible for degrading that substrate (Horwath 2007). Decomposition of a narrow C:N ratio (C:N < 20:1) substrate (e.g., rotted manure) results in more N mineralization as the microbial N demand for biosynthesis is being met resulting in excess N contained in the substrate being mineralized (Havlin et al. 2013). Substrate with a wide C:N ratio (C:N > 30:1) (e.g., grain straw) requires addition N to meet microbial N demand for biosynthesis, and soil mineral N is immobilized to support microbial growth (Havlin et al. 2013). Eventually, because of CO_2 production, the C:N ratio narrows (20 – 30:1) to the point where there is a transition from immobilization to mineralization. The duration of the transition from immobilization to mineralization depends on: (1) the quantity of substrate added; (2) the quantity of resistant components (e.g., lignins, waxes and fats) in the substrate; and (3) the level of substrate incorporation into the soil (Havlin et al. 2013).

The amount of soil organic matter (SOM) in the soil matrix is important to the overall supply of total C in the soil (Horwath 2007). Also, an increase in SOM can increase the size of the mineralizable N pool (Havlin et al. 2013). The light fraction of SOM and the particulate organic matter (POM) fraction regulate the turnover of C

and N, thus, a decline in the size of these fractions can alter the amount of N mineralized from this pool (Horwath 2007). In a long-term potato rotation study, Sharifi et al. (2008) found that cattle manure amended soils had greater organic C and N contents, due to significant increases in the POM fraction, and this increased the size of mineralizable N pool as it was 35% greater than the inorganic N only amendment.

1.2.3.2 Temperature

The rate of net N mineralization typically increases as temperature increases (Havlin et al. 2013; Zak et al. 1999; Agehara and Warncke 2005). Zak et al. (1999) noted that there was a significant effect of temperature on the amount of N mineralized, at low soil water contents, as the rates were greater at 25°C when compared to the treatments at 10°C and 5°C. Agehara and Warncke (2005) found that treatments at 20 - 25°C had significantly greater rates of net N mineralization when compared to the treatments at 10 - 15°C, inferring that the microbial population in the higher temperature was more adept at degrading the C substrate than the microbial community in the lower temperature. There was also a significant interaction between temperature and varying soil matric potential on the size of the mineralized N pool. The size of the mineralizable N pool declined as water potential varied from -0.01 to -0.30 MPa and the decrease was greatest at 25°C when compared to 5°C. It was concluded that at warmer soil temperatures the rates of microbial activity exceeded the diffusion of substrates to metabolically active cells. Whereas at lower temperatures the lower demand for substrate allowed diffusion to keep pace with microbial activity.

1.2.3.3 Water Content and its Effect on Aeration

Soil water content controls the growth and activity of microbes by i) influencing the water potential of microbial cells (Stark and Firestone 1995); ii) regulating the amount of gas and solute (substrate) diffusion into pore spaces (Zak et al. 1999; Rodrigo et al. 1997). Stark and Firestone (1995) indicated that water matric potential less than -0.6 MPa would limit microbial growth by way of cell dehydration. As a result the relationship between the rate of net N mineralization and water content is complex as there is a broad range of water contents that allow microbes to efficiently degrade C substrates (Georgallas et al. 2012; Pal and Broadbent 1975). Due to differences in soil texture, structure and the extent to which soil aggregation influences the size of pore spaces (Brady and Weil 2002a), the maximum rate of net N mineralization has been found to occur over a wide range (40 - 90%) of WFPS (Dessureault-Rompré et al. 2011b) and water potentials between -0.01 to -0.05 MPa (Myers et al. 1982; Miller and Johnson 1964). Franzluebbers (1999) found that the maximum rate of net N mineralization occurs between 36 - 43% WFPS in soils with intact aggregates, Guntinas et al. (2012) examined water contents of 25, 34 and 42% WFPS in a sandy loam and found that the mineralization rate was not significantly influenced by the water content, while Stanford and Epstein (1974) found that the maximum rate of net N mineralization occurs between 80 - 90% WFPS in finely ground homogenized soils.

The ability of the soil to hold water is determined by the distribution of pore sizes in the soil which is in turn a function of soil texture (Brady and Weil 2002a). Fine-textured soils (e.g., clay loams) have a greater total porosity and a greater

proportion of smaller pores when compared to coarse-textured soils (e.g., sandy loams) (Brady and Weil 2002a). With increasing water content above field capacity, the rates of aerobic microbial activity generally decline due to slow gas (O_2) diffusion (Dessureault-Rompré et al. 2011b; Georgallas et al. 2012). Drury et al. (2003) measured soil mineral N ($NH_4^+ + NO_3^-$) and found that at high water contents (e.g., 90 - 95% WFPS) significant net N mineralization occurred in a fine-textured clay loam, while the medium and coarse-textured soils had negligible or zero net N mineralization at these high water contents; although denitrification was not inhibited in the study and so denitrification was a likely source of lower soil mineral N in the coarse-textured soil. Agehara and Warncke (2005) observed that the rate of N mineralization at 50% water-holding capacity (WHC) was significantly lower than that observed at 70 and 90% WHC. With decreasing water content, microbes experience a decrease in intracellular water potential which limits growth and activity, reducing the rate of N mineralization in drier soils (Dessureault-Rompré et al. 2011b; Zak et al. 1999).

1.2.4 Factors Governing the Rate of Denitrification

The rate of denitrification is controlled by three main factors: (1) soil aeration and water content, (2) available soil NO_3^- and, (3) quality and quantity of C substrates (Groffman 1991; Havlin et al. 2013). Due to the high variability in these factors, denitrification is a highly variable process (Groffman 1991; Georgallas et al. 2012).

1.2.4.1 Water Content and its Influence on Aeration

Aerobic microbes preferentially utilize O_2 as a TEA, yet in its absence, denitrifiers can utilize NO_3^- as an alternate TEA (Burton et al. 2012). As the main condition for denitrification is a reduced supply of O_2 in the soil, numerous studies observe that denitrification occurs at a lower rates below 60% WFPS (Linn and Doran 1984; Burton et al. 2008; Gillam et al. 2008). Denitrification becomes a more important N conversion process when WFPS is at 70% or above (Linn and Doran 1984; Bateman and Baggs 2005; Burton et al. 2012).

The rate of denitrification over a wide range of water contents has been found to vary with soil texture (De Klein and Van Logtestijn 1996; Groffman and Tiedje 1991). Fine-textured soils (e.g., clay loams) have a higher total porosity and a greater number of smaller pores when compared to coarse-textured soils (e.g., sandy loams) (Brady and Weil 2002a). Denitrification has been observed to occur in anaerobic microsites even under low water contents (Groffman and Tiedje 1991). Denitrification was found to increase more sharply with increasing WFPS in a medium-textured loam than in a coarse-textured sand (De Klein and Logtestijn 1996) or a fine-textured clay loam (Groffman and Tiedje 1991). In a 30-day study, Aulakh et al. (1991a) observed that a sandy loam soil amended with hairy vetch had significantly greater cumulative denitrification losses (3.30 mg N kg^{-1}) than a silt loam-textured soil at 60% WFPS (1.64 mg N kg^{-1}) although at 90% WFPS the silt loam exhibited greater denitrification losses (64.08 mg N kg^{-1}) than the sandy loam-textured soil (51.14 mg N kg^{-1}).

1.2.4.2 Available Soil NO_3^-

In the absence of applied NO_3^- fertilizers, the availability of NO_3^- is dependent on nitrification (Robertson and Groffman 2007). The limitation of soil NO_3^- availability on the rate of denitrification is more pronounced in strictly anaerobic areas in non-fertilized soils (Firestone and Davidson 1989) as nitrification is inhibited and there is no exogenous supply of NO_3^- as is typical in aerobic areas (Havlin et al. 2013). Nitrate is a terminal electron acceptor used in the denitrification process as microbes can reduce NO_3^- under O_2 limited conditions (Havlin et al. 2013). Gillam et al. (2008) observed that an increase in the availability of NO_3^- did not affect cumulative denitrification, nor did the interaction with carbon addition, as NO_3^- was not required as a TEA indicated by low respiration rates and low carbon usage. While other studies found that an increase in NO_3^- availability did increase the denitrification rate (Strong and Fillery 2002; Jordan et al. 1998). Strong and Fillery (2002) found that in soils with low background NO_3^- (0 - 15 mg N kg^{-1} soil) the addition of NO_3^- increased the denitrification rate.

1.2.4.3 Quality and Quantity of Carbon Substrates

The availability of C substrate influences denitrification directly as a source of energy and electrons (deCatanzaro and Beauchamp 1985; Gillam et al. 2008; Burton et al. 2012), and indirectly, by consuming O_2 (Gillam et al. 2008; Beauchamp et al. 1989). The quality of the C substrate, as measured by the C N ratio, alters the O_2 consumption by microbes as narrow C:N ratio substrates increase the activity of aerobic microbes increasing O_2 consumption and creating anaerobic microsites

(Georgallas et al. 2012; Baggs et al. 2000). In a study by Gillam et al. (2008) the addition of a narrow C:N ratio substrate, red clover plant tissue, resulted in greater cumulative denitrification when compared to the addition of wide C:N ratio substrate. This result was postulated to be the effect of rapid respiration at the start of the incubation in the red clover treatment, which reduced O_2 availability in microsites where denitrification would be occurring. Similarly, deCatanzaro and Beauchamp (1989) found higher rates of denitrification in soils amended with narrow C:N ratio alfalfa, when compared to wide C:N ratio straw, which was attributed to the alfalfa decomposing more rapidly due to its narrow C:N ratio and low lignin content, providing more readily available soluble C substrate for the denitrifying microbes. Also, it was found that the non-amended soils had the lowest soluble C concentrations and lowest denitrification rate when compared to C amended treatments, as indicated by the considerably slower loss of NO_3^- during the incubation.

1.2.5 Factors Controlling the Efficacy of Nitrification Inhibitors

The oxidation of NH_4^+ to NO_3^- can occur rapidly in well-aerated soils (Havlin et al. 2013; Agehara and Warncke 2005). The challenge of measuring N mineralization by the accumulation of the product of mineralization (NH_4^+) in soil, is that nitrification is at the same time converting NH_4^+ to NO_3^- which may then be lost as a result of denitrification (Havlin et al. 2013), resulting in an underestimation of net N mineralization. A NI can be used to stop the nitrification process during the incubation period, allowing for the accumulation of NH_4^+ to reflect the magnitude of net N mineralization.

A NI can inhibit the growth and activity of nitrifying bacteria for 2 - 8 weeks to allow NH_4^+ to accumulate in soil (Havlin et al. 2013). A NI acts on nitrifying bacteria, such as Nitrosomonas (Slangen and Kerkhoff 1986; Zerulla et al. 2001), by deactivating the enzyme ammonium monooxygenase (McCarty et al. 1999; Di and Cameron 2011). The efficiency of a NI is dependent on the nature of the NI, which varies among inhibitors (Slangen and Kerkhoff 1984). The persistence of a NI and the effect of soil water content are of greatest importance in soil incubations which involve a range of high and low WFPS (Khosa et al. 2012) as the quantity of water-filled soil pores in a certain volume of soil will determine whether conditions are optimal for either nitrification or denitrification to occur (Menedez et al. 2009), and the persistence of a NI can vary over a range of water contents (Menendez et al. 2012).

Nitrapyrin (Nserve) and Dicyandiamide (DCD) are two inhibitors that are used extensively throughout the world (Zerulla et al. 2001) yet both display efficacy shortcomings, with regard to lower rates of persistence of the NI when compared to the pyrazole compound 3, 4-dimethylpyrazole (DMPP). Zerulla et al. (2001) compared the efficacy of DMPP to DCD and found that DMPP persisted in the soil for a longer duration than DCD. Khosa et al. (2012) found that 3, 5-dimethylpyrazole (DMP) was more persistent in the soil over a six-week incubation period than either Nitrapyrin or Phenylacetylene and highly effective at high WFPS as indicated by an accumulation of NH_4^+ at 90% WFPS.

Soil water content can affect the persistence of a NI in the soil (Menedez et al. 2012). Under aerobic conditions, nitrification is more apt to proceed oxidizing more

DMPP than in wetter soil. Menedez et al. (2012) observed DMPP in soil at water contents of 40, 60 and 80% WFPS over a 51-day incubation and found that persistence of DMPP was greater at 80% WFPS than at 40% WFPS.

There is a need to simultaneously investigate the influence of water content on the rates of net N mineralization and denitrification, as these processes reflect the microbial mediated soil N processes that contribute to soil mineral N supply during a growing season (Drury et al. 2003; Dessureault-Rompré et al. 2011b; Georgallas et al. 2012). Using DMP to inhibit nitrification will allow the simultaneous measurement of N mineralization (NH_4^+ accumulation) and denitrification (NO_3^- disappearance) at higher WFPS contents, something that has not previously been achieved (Dessureault-Rompré et al. 2011b; Georgallas et al. 2012; Paul et al. 2003).

1.3 Objectives

The overall objective was to investigate how soil water content, as measured by WFPS, influences the rates of net N mineralization and denitrification in soil.

To achieve the overall objective, the following specific objectives were examined:

1. Evaluate of the potential to use nitrification inhibitor DMP (NI method) for the simultaneous measurement of net N mineralization and denitrification rates in soil.
2. Assess the effect of a comprehensive range of water contents on simultaneous measurement of net N mineralization and denitrification rates using the NI method.
3. Examine how the effect of water content on net N mineralization and denitrification rates varies in soils that differ in texture and carbon availability using the NI method.

Chapter 2.0 Materials and Methods

2.1 Soils used in the Experiment

To examine the effect of differences of texture and carbon intensity on the measurements of N mineralization and denitrification using the NI method, soils from two long-term experiments were collected from different regions in Eastern Canada (Table 2.1) were collected from the surface 0 - 15 cm, air-dried and passed through a 2 mm sieve. Soil pH was measured in a 1:5 dilution of dry soil to $CaCl_2$ solution (Hendershot et al. 2008). Particle size distribution was assessed using the hydrometer method (Brewster 2001; Sheldrick and Wang 1993). A 1 g sample of soil was finely ground and organic C and total N were measured by the dry combustion method using an Elementar VarioMax Carbon and Nitrogen Analyzer (Skjemstad and Baldock 2008). Soil gravimetric water content of the air-dried soil was measured by loss of weight upon drying a 10 g sub-sample at 105°C for 48 hours.

2.1.1 Water-Filled Pore Space Determination

Water-filled pore space was calculated using the bulk densities that were established in the experimental vessels and assumed soil particle densities of 2.53 g cm^{-3} (clay loam) and 2.65 g cm^{-3} (sandy loam) (Carter and Ball 1993).

Table 2.1. Description of soils used in the experiment

Experiment & year collected	Soil name	Location	Amendment and management
1 (2011) and 2 (2014)	Brookston clay loam	Hon. Eugene F. Whelan Research Farm, Woodslee, ON (42.2° N, 82.7° W)	Crop *(corn)* residues returned, winter wheat-corn-soybean rotation
1 (2011)	Fox sandy loam	Martin Farm, Harrow, ON	Crop (*corn)* residues returned, corn-soybean
2 (2014)	Harrow sandy loam	HSG-A, Harrow, ON (42.0° N, 82.9° W)	Crop *(corn)* residues returned, winter wheat-corn-soybean
3 (2011)	Batiscan sandy loam	Laval University Experimental Farm, Saint-Augustine-de-Desmaures, QC (46° 44' N, 71° 31' W)	Crop *(corn)* residues exported, No applied N, winter wheat-corn-soybean with minimum tillage
3 (2011)	Batiscan sandy loam	Laval University Experimental Farm, Saint-Augustine-de-Desmaures, QC (46° 44' N, 71° 31' W)	Crop *(corn)* residues returned, pig slurry applied, winter wheat-corn-soybean with minimum tillage

Table 2.2. Characterization of soils used in the experiment

Experiment	Soil name and type	Clay	Silt	Sand	pH	Total C	Total N
		($g\ kg^{-1}$)				($g\ kg^{-1}$)	($g\ kg^{-1}$)
1	Brookston CL	23	34	43	6.0	1.53	0.169
1	Fox SL	12	17	71	5.0	1.19	0.121
2	Brookston CL	20	33	47	6.6	1.25	0.127
2	Harrow SL	11	35	54	6.6	1.29	0.127
3	Batiscan SL (H)	12	24	64	5.9	1.92	0.196
3	Batiscan SL (L)	13	22	65	6.0	1.64	0.157

2.2 Experimental Design

The three specific objectives outlined above were met through a series of three experiments.

2.2.1 Experiment 1

The objective was to verify that the proposed NI method, using DMP, allowed for the simultaneous measurement of net nitrogen mineralization as the accumulation of NH_4^+, and denitrification as the loss of NO_3^- over the desired incubation period within the same vessel. This was assessed by confirmation that (1) nitrification was inhibited in the treated soil as indicated by the lack of the appearance of NO_3^-; and (2) DMP did not have an adverse effect on soil microbial activity as measured by soil respiration.

The experiment used a factorial arrangement of treatments in a completely randomized design. Treatments included two levels of N inhibitor [with (DMP+) or without (DMP-) as NI], two soil types [clay loam (CL) or sandy loam (SL)] and three water contents (35%, 50% and 85% WFPS) with three sampling times (0, 14 and 28 days). Each treatment combination was replicated four times for a total of 144 experimental units.

Nitrification inhibition efficacy was determined, by modifying the NI DMP method developed by Khosa et al. (2012), in two different textured soils, a sandy loam and a clay loam from similar cropping rotations in Harrow, Ontario. Soils were chosen to vary in clay content, as this is the factor most likely to influence the nitrification inhibitor's adsorption to clay and its inhibitory effect (Barth et al. 2001).

Water contents were chosen to represent the full range of water contents at which net N mineralization, nitrification and denitrification are occurring (Linn and Doran 1984). The 35% WFPS was chosen to assess whether low water content limits the distribution of the NI, resulting in incomplete inhibition. The 50% WFPS was chosen as this is an optimal water content at which nitrification is occurring. The 85% WFPS was chosen as this high water content limits aeration in the pore spaces providing optimal conditions for the measurement of denitrification.

The incubation lengths were chosen based on the four-week length of nitrification inhibition using DMP found by Khosa et al. (2012).

2.2.2 Experiment 2

The objective was to use the NI method to measure the rates of net N mineralization and denitrification simultaneously in two soils with contrasting soil texture across a range of soil water contents.

The experiment used a factorial arrangement of treatments in a completely randomized design. Treatments consisted of two soil types (CL and SL) and ten water contents (20, 35, 50, 65, 75, 80, 85, 90, 95 and 110% WFPS) and with three sampling times (0, 14 and 28 days). Each treatment combination was replicated four times for a total of 240 experimental units.

Soils that varied in clay content were assessed to evaluate the rates net N mineralization and denitrification over a wide range of water contents. A sandy loam and a clay loam from Harrow, Ontario were chosen to represent a variation is clay content in a soil that otherwise had a similar pedogenic and agronomic history. Clay content was chosen as the focus as this physical soil property can influence microbe-

facilitated processes in soil due to the greater number of habitable and oxygenated micropore spaces in fine-textured soil. Soil respiration was also measured to determine the rate of microbial activity in the soil over the 28-day incubation.

The ten water contents were 20, 35, 50, 65, 75, 80, 85, 90, 95 and 110% WFPS. The 20 - 75% WFPS water contents were chosen as this is the range at which net N mineralization is expected to exceed denitrification. The 80 - 110% WFPS water contents were chosen as this is the range at which denitrification is expected to exceed net N mineralization, particularly under flooded conditions (e.g., 110% WFPS).

2.2.3 Experiment 3

The objective was to use the NI method to measure the rates of net N mineralization and denitrification simultaneously in two soils with contrasting carbon availability as in the study by Pelster et al. (2012), over the range of water contents used in the previous experiment.

The experiment used a factorial arrangement of treatments in a completely randomized design. Treatments included two soils [low carbon input intensity (L) or high carbon input intensity (H)], ten water contents (20, 35, 50, 65, 75, 80, 85, 90, 95 and 110% WFPS) with three sampling times (0, 14 and 28 days). Each treatment combination was replicated four times for a total of 240 experimental units.

Soils that vary in carbon availability were assessed to evaluate the impact of carbon supply on rates of net N mineralization and denitrification over a wide range of water contents. A sandy loam soil with a history of amendment, over a three year period, with pig slurry and crop residues returned was contrasted to the same soil

type to which no applied N and crop residues had been added. The soils were obtained from a research trial near Saint Augustine-de-Desmaures, Quebec (Pelster et al. 2012) in the corn year of the rotation. Soils were chosen to vary in carbon availability as this factor can influence microbe-facilitated processes in soil. Carbon substrate containing organic N has been found to regulate the amount of N mineralized and denitrified in soil. Soil respiration was also measured to determine the rate of microbial activity in the soil over the 28-day incubation.

The ten water contents were 20, 35, 50, 65, 75, 80, 85, 90, 95 and 110% WFPS. The 20 - 75% WFPS water contents were chosen, as this is the range at which net N mineralization is expected to exceed denitrification. Water contents of 80 - 110% WFPS were chosen as this is the range at which denitrification is expected to exceed net N mineralization, particularly under flooded conditions (e.g., 110% WFPS).

2.2.4 Nitrification Inhibition Assay Method

All three experiments were incubations conducted under laboratory conditions based on methods described by Khosa et al. (2012) which used short-term aerobic incubation methods as described by Curtin and Campbell (2008).

In experiment 1, for each combination of soil type and water content, two amendment treatments were applied. The first amendment was a solution designed to deliver 200 mg DMP kg^{-1} soil (DMP+) to allow for an evaluation of nitrification inhibition efficacy; the production of NO_3^- was used as evidence of a failure of the NI to inhibit nitrification. The second amendment was distilled water only (DMP-); this treatment served to demonstrate the magnitude of nitrifier activity in the absence of

the NI and as a measure of respiration in the absence of NI to allow evaluation of the effect of DMP on the soil microbial population.

Amendments as described above were added to the measured bulk soil of clay loam and sandy loam, and soil was wetted based on the bulk density for each soil type determined in a pre-trial experiment that evaluated the water and air-filled porosity of each soil, particularly above 85% WFPS, in a 50 mL volume within a 100 mL vessel (SCP Science, cat # 010-501-028). Bulk densities used were 1.15 g cm^{-3} for clay loam and 1.30 g cm^{-3} for sandy loam. Treatments of 35, 50 and 85% WFPS were brought to an initial WFPS of 5% less the target water content for the lower water contents and 40% less the target water content for the high water content and soil was pre-incubated in poly bags for 10 days to allow the DMP to take effect (Khosa et al. 2012), and to allow the flush of N mineralization that occurs after an air-dried soil undergoes rewetting (Stanford et al. 1974; Davidson 1991). Experimental soil was placed in an incubation chamber at a constant temperature of 25°C with humidity at 70%.

At the mid-point of the pre-incubation wetted and weighed soil was transferred but not packed into 100 mL polypropylene tubes (SCP Science, cat # 010-501-028). On day 0 of incubation, 20 mg NH_4^+-N kg^{-1} (as NH_4Cl) was applied by pipette (experiment 1 only) and soil was gently packed to a constant volume of 50 mL to reach the desired bulk density and then tubes were capped with Parafilm. Water content was monitored and maintained by the addition of water if loss was more than 1 g over the duration of the incubation.

Experiments 2 and 3 followed the same procedure described above with two exceptions. First, all soil received a solution designed to deliver 200 mg DMP kg^{-1} and second, on day 0 the only amendment applied was a solution designed to deliver 100 mg NO_3^--N kg^{-1} (as KNO_3). In experiments 2 and 3 the production of NH_4^+ was used as a measure of net N mineralization and the consumption of NO_3^- as a measure of denitrification.

2.3 Soil Respiration

Soil respiration was assessed at three time periods: at two hours after the soil was packed to bulk density (t=0), and at two (t=14 days) and four (t=28 days) weeks to determine the microbial activity in the soil based on the quantity of carbon dioxide (CO_2) generated in the headspace gas over a 30-minute sampling interval. Prior to destructive sampling of soils, the Parafilm was removed one hour prior to CO_2 sampling to allow gas to equilibrate and the headspace volume (109 mL) was flushed with compressed air for 5 seconds prior to capping tube with a cap fitted with a rubber septum. Compressed air (30 mL) was injected into the tube to maintain positive pressure inside the tube for subsequent gas sampling. Three headspace gas (10 mL) samples were taken using a syringe and stored in 6 mL evacuated exetainer at 10 minutes intervals after capping of the tube. Headspace gas samples (0.5 mL) were analyzed for CO_2 and N_2O concentrations using the Varian Star 3800 Gas Chromatograph with an attached thermal conductivity detector and electron capture detector respectively (Varian, Mississauga, ON) as described by Burton et al. (2008).

2.4 Soil Mineral N and Dissolved Organic Carbon Analysis

Extraction of mineral N, by destructive sampling, was done at three time points: at two hours after the soil was packed to bulk density (t=0), and at two (t=14 days) and four (t=28 days) weeks following the method described by Maynard et al. (2008). Soil mineral N and dissolved organic C (DOC) concentrations were measured by adding 75 mL of 0.5 M K_2SO_4 to the incubation vessel which was then capped and shaken for one hour on a lateral shaker then soil slurries were left to settle out upright for one hour and then filtered through a Whatman no. 40 paper. Extracts were frozen at -20°C until colorimetric analysis of NH_4^+-N, NO_3^--N, total soluble N (TSN) and DOC concentrations using a Technicon AutoAnalyzer II system, following the protocols: Technicon Industrial Method #98-70W for NH_4^+-N; Technicon Industrial Method #100-70W for NO_3^--N; AutoAnalyzer Application Method #G-086-93 A for TSN and Technicon Industrial Method #455-76W/A for DOC, respectively (Technicon Industrial Systems 1978a; Technicon Industrial Systems 1978b; Bran+Luebbe 1993; Technicon Industrial Systems 1978c). The mineral N data was used to confirm nitrification inhibition during the incubation (experiment 1) and as measure of net N mineralization and denitrification (experiment 2 and 3) as an increase over time in the concentration of NH_4^+ and a decrease over time in the concentration of NO_3^-, respectively. The TSN data was evaluated to describe the size of the labile organic N pool as it includes the inorganic and organic N fractions in the samples (Experiment 3 at 80 - 110% WFPS only). Dissolved organic N (DON) concentrations were calculated by subtracting inorganic N (NH_4^+ + NO_3^-) from TSN. The DOC was used as a measure of the available organic C as a substrate source for

soil microbes (Experiment 3 at 80 - 110% WFPS only). Soil mineral N and DOC concentrations were calculated at each time-period and rate calculations were determined by slope of the linear regression (Linest function in Microsoft Excel) of NH_4^+-N or NO_3^--N concentrations over time (0, 14 and 28 days).

2.5 Statistical Analyses

The experimental data was analyzed using the statistical software JMP by SAS (SAS, Cary, NC, USA). The experiment used a factorial arrangement of treatments in a completely randomized design. Data was tested for normality and transformations were not necessary. Analysis of variance (ANOVA) was performed on the rates of net N mineralization, denitrification and respiration, total soluble nitrogen and dissolved organic carbon concentrations. Means comparison (standard least squares) was done following ANOVA using Tukey's HSD test and Student's t test. Regression techniques were utilized to examine the relationships between water content and the rates of net N mineralization and denitrification.

Chapter 3.0 Results and Discussion

3.1 Experiment 1

The objective of experiment 1 was to demonstrate the effectiveness of the nitrification inhibitor DMP, substantiating its suitability for use as a method to simultaneously measure net N mineralization and denitrification rates in two soils and over a range of water contents. This approach (the NI method) was chosen due to the demonstrated persistence of nitrifier inhibition in the soil over a 6-week period (Khosa 2012). Here we considered (1) three water contents to assess the influence of aeration status on inhibitor effectiveness and (2) two textures to assess the impact of potential sorption of DMP to clay surfaces limiting the effectiveness of the DMP. The experimental approach was to measure the appearance, or lack of, NO_3^- in an NH_4^+ amended soil as evidence of the effectiveness of the inhibition of nitrification in the presence of DMP in two contrasting soil types and over three water contents. Measurements of CO_2 production were conducted to determine whether DMP had an impact on microbial respiration.

3.1.1 Change in NO_3^--N Concentration

The addition of DMP effectively blocked the production of NO_3^--N. The increase in NO_3^--N concentration observed in the absence of DMP addition (1.03 mg N kg^{-1} d^{-1}) was not apparent when DMP was added (-0.009 mg N kg^{-1} d^{-1}) (Table 3.1). In the absence of DMP, water content did influence the change in the NO_3^- concentration (Table 3.1) as there was greater change at 35% (1.62 mg N kg^{-1} d^{-1}) and 50% (1.65 mg N kg^{-1} d^{-1}) WFPS than at 85% WFPS (-0.20 mg N kg^{-1} d^{-1}). The impact of DMP addition on the change in NO_3^--N concentration did not differ

between soil types yet it did differ between water contents (Table 3.1).

Water content did have a significant effect on the change in the NO_3^--N concentration in the presence of DMP and this differed between soil types (Table 3.1). The accumulation of NO_3^- at 35% (0.007 mg N kg^{-1} d^{-1}) and 50% (0.006 mg N kg^{-1} d^{-1}) WFPS suggested that nitrification exceeded denitrification and the loss of NO_3^- at 85% WFPS (-0.04 mg N kg^{-1} d^{-1}) suggested that nitrification was less than denitrification (Table 3.1). The change in the NO_3^--N concentration was greater at 35% and 50% WFPS in the CL-textured soil than at 85% WFPS in the CL-textured soil and at all three water contents in the SL-textured soil (Table 3.1).

Table 3.1. Change in soil NO_3^--N concentration (slope) in CL- and SL-textured soil during a 28-day incubation in experiment 1. Analysis of variance (ANOVA) for treatments of soil texture (ST), water content (WC), and DMP addition (DMP +/-).

Treatment	Change in NO_3^--N Concentration (mg N kg^{-1} d^{-1})		
DMP +/-			
DMP+	-0.009	b	
DMP-	1.03	a	
DMP+			
ST	**WC (% WFPS)**		
CL	-0.027		
SL	0.009		
	35	0.007	a
	50	0.006	a
	85	-0.04	b
CL	35	0.016	a
	50	0.033	a
	85	-0.08	b
SL	35	-0.003	a
	50	-0.02	ab
	85	-0.03	a
ANOVA		**Prob > F**	
DMP Addition (+/-)		<0.0001*	
DMP Addition x WC		<0.0001*	
ST x DMP Addition		0.0753	
DMP+			
ST		0.9653	
WC		0.0154*	
ST x WC		0.0037*	

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different.*

3.1.2 Change in NH_4^+-N Concentration

Because the conversion of NH_4^+ to NO_3^- is blocked in the presence of DMP, the accumulation of NH_4^+ is interpreted as a quantitative measure of N mineralization. In DMP+ treatments, N mineralization (the accumulation of NH_4^+-N) was greater in CL-textured (1.23 mg N kg^{-1} d^{-1}) soils than in SL-textured (0.913 mg N kg^{-1} d^{-1}) soils (Table 3.2).

Table 3.2. Change in soil NH_4^+-N concentration (slope) in CL- and SL-textured soil in DMP+ treatments between day 0 and day 28 in experiment 1. Analysis of variance (ANOVA) for treatments of soil texture (ST) and water content (WC).

Treatment	Change in NH_4^+-N Concentration (mg N kg^{-1} d^{-1})
ST	
CL	1.23 a
SL	0.913 b
ANOVA	**Prob > F**
ST	0.0001*
WC	0.3798
ST x WC	0.0875

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different.*

3.1.3 Soil Respiration

The respiration of the soil microbial community, measured as CO_2 production, was undertaken to determine whether DMP addition had any impact on microbial activity. The addition of DMP increased respiration (18.3 μg CO_2-C kg^{-1} min^{-1}) relative to soils not receiving DMP (14.8 μg CO_2-C kg^{-1} min^{-1}) and this effect was similar in the two soils (Table 3.3).

Water content had a significant effect on respiration in the presence of DMP following pre-incubation (day 0 for N addition), as rates decreased with increasing water content and decreased more rapidly with increasing water content, for the SL-textured soil compared with the CL-textured soil (Table 3.3).

Table 3.3. Respiration rate (slope) in CL- and SL-textured soil at three water contents (% WFPS) on day 0 in experiment 1. Analysis of variance (ANOVA) for treatments of DMP addition (DMP+/-), soil texture (ST) and water content (WC).

Treatment	Microbial Respiration Rate ($\mu g\ CO_2\text{-}C\ kg^{-1}\ min^{-1}$)	
DMP (+/-)		
DMP+	18.3 a	
DMP-	14.8 b	
DMP+		
ST	**WC (% WFPS)**	
CL	21.0 a	
SL	15.4 b	
	35	23.6 a
	50	18.7 b
	85	12.3 c
CL	35	25.8 a
	50	20.8 b
	85	16.5 c
SL	35	21.4 b
	50	16.7 c
	85	7.9 d
ANOVA	**Prob > F**	
DMP Addition (+/-)	<0.0001*	
ST (DMP +/-)	<0.0001*	
WC (DMP +/-)	<0.0001*	
DMP Addition x ST	0.0520	
DMP Addition x WC	0.7635	
DMP+		
ST	<0.0001*	
WC	<0.0001*	
ST x WC	0.0347*	

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different.*

3.1.4 Discussion

The addition of DMP was effective in inhibiting nitrification in soils differing in texture and water content. This observation is consistent with the findings of previous short-term experiments using DMPP (Barth et al. 2008; Barth et al. 2001). In the current study, soil texture did not influence the effectiveness of DMP which is

different from previous work by Barth et al. (2001) in which adsorption of a N pyrazole compound (DMPP) to clay was found to reduce the efficiency of nitrification inhibition resulting in nitrification inhibition in sandy-textured soils being more effective than in loamy-textured soil. As well, Barth et al. (2008) found that inhibition of nitrification was more effective in a sandy loam-textured soil than in a loam-textured soil.

Water content did not impact the inhibitory effect of DMP in both soils. These results are consistent with a study by Chen et al. (2010) in which efficacy was also determined by the accumulation of NO_3^- at 40 and 60% WFPS. In the current study, with increasing water content NO_3^- did not accumulate indicating that DMP was effective in inhibiting nitrification.

With the addition of DMP, the accumulation of NH_4^+-N along with the lack of NO_3^- production indicated that microbes were not nitrifying the added NH_4^+. The change in NH_4^+ was greater in the CL-textured soil than in the SL-textured soil. The adsorption and diffusion of NH_4^+ in soil below field capacity would be expected to decrease in a coarse textured soil (Barth et al. 2008; Stark and Firestone 1995).

After 10 days of pre-incubation with DMP, the addition of DMP did not prove toxic to the microbial population as the soil respiration rate did not decrease in this treatment. Similarly, a study by Kong et al. (2016) indicated that non-target microbes and their functions were not adversely affected by DMPP. The increases in respiration caused by the addition of DMP in the current study may be the result of microbes accessing new carbon substrate as a result of the DMP essentially killing other microbes the soil (Maienza et al. 2014). Maienza et al. (2014) observed an

increase in respiration in DMPP applied soil when compared to the control and the manure + DMPP treatment.

3.2 Experiment 2

In the second experiment, the net N mineralization rate and denitrification rate in two contrasting soil textures and over 10 water contents were determined by measuring the accumulation of NH_4^+-N and loss of added NO_3^--N along with measurements of N_2O production and microbial respiration (CO_2 production). The comparison of two soil textures (fine- and coarse-textured) was undertaken to assess the amount of N mineralized and denitrified and the influence of aeration on N mineralization, denitrification and respiration by considering 10 water contents in soils with different pore size distributions. Water-filled pore space values in the CL-textured soil had a range of 17 - 107% WFPS and the SL-textured soil had a range of 21 - 111% WFPS.

3.2.1 Net Nitrogen Mineralization Rate

Soil texture did not have a significant effect on the net N mineralization rate (Table 3.4). Water content had a significant effect on the net N mineralization rate and there was a significant interaction between water content and soil texture (Table 3.4). The greatest N mineralization rates were found at intermediate water contents (51 - 86% WFPS) in SL-textured soil. The lowest rates of N mineralization in SL-textured soil occurred at the lowest (21 & 36% WFPS) and highest (91 - 111% WFPS) water contents (Table 3.4). In the CL-textured soil, there were no significant differences in the net N mineralization rate at water contents greater than 17% WFPS (Table 3.4).

Table 3.4. Net N mineralization rate (slope) in CL- and SL-textured soil over a wide range of water contents during a 28-day incubation in experiment 2. Analysis of variance (ANOVA) for treatments of soil texture (ST) and water content (WC).

Treatment	Net N Mineralization Rate ($mg\ N\ kg^{-1}\ d^{-1}$)	
ST	**WC (% WFPS)**	
CL	17	0.300 cdef
	32	0.466 abcdef
	47	0.412 abcdef
	62	0.506 abcde
	72	0.455 abcdef
	77	0.580 ab
	82	0.506 abcde
	87	0.479 abcde
	92	0.502 abcde
	107	0.547 abc
SL	21	0.347 bcdef
	36	0.275 ef
	51	0.557 abc
	66	0.689 a
	76	0.609 ab
	81	0.543 abcd
	86	0.501 abcde
	91	0.359 bcdef
	96	0.261 def
	111	0.208 f
ANOVA	**Prob > F**	
ST	0.0856	
WC	<0.0001*	
ST x WC	<0.0001*	

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Tukey's HSD test and * significantly different.*

3.2.2 Denitrification Rate

Soil texture, water content and the interaction between the two ($p < 0.0001$) influenced the denitrification rate (Table 3.5). The rate increased dramatically at 90% WFPS in both the SL-textured soil at (2.73 mg N kg^{-1} d^{-1}) and CL-textured soil (1.17 mg N kg^{-1} d^{-1}) with SL-textured soil having the higher denitrification rate (Table 3.5). There was no significant difference in denitrification rates below 90% WFPS and above 95% WFPS in both soil textures (Fig. 3.1). The numerically greatest denitrification rates were found at 110% WFPS.

Further, soil texture and water content influenced the concentration of N_2O over a 30-minute sampling period on day 0, 14 and 28 as indicated by a significant interaction (Table A.1.). Emissions were numerically largest in the CL-textured soil at 65% WFPS on day 0 (0.006 mg N kg^{-1}) (Fig. A.1).

Table 3.5. Denitrification rate (slope) in CL- and SL-textured soil over a range of water contents during a 28-day incubation in experiment 2. Analysis of variance (ANOVA) for treatments of soil texture (ST) and water content (WC).

Treatment	Denitrification Rate (mg N kg^{-1} d^{-1})
ST	
CL	1.02 b
SL	1.25 a
ANOVA	**Prob > F**
ST	0.0162*
WC	<0.0001*
ST x WC	<0.0001*

- ** significantly different*

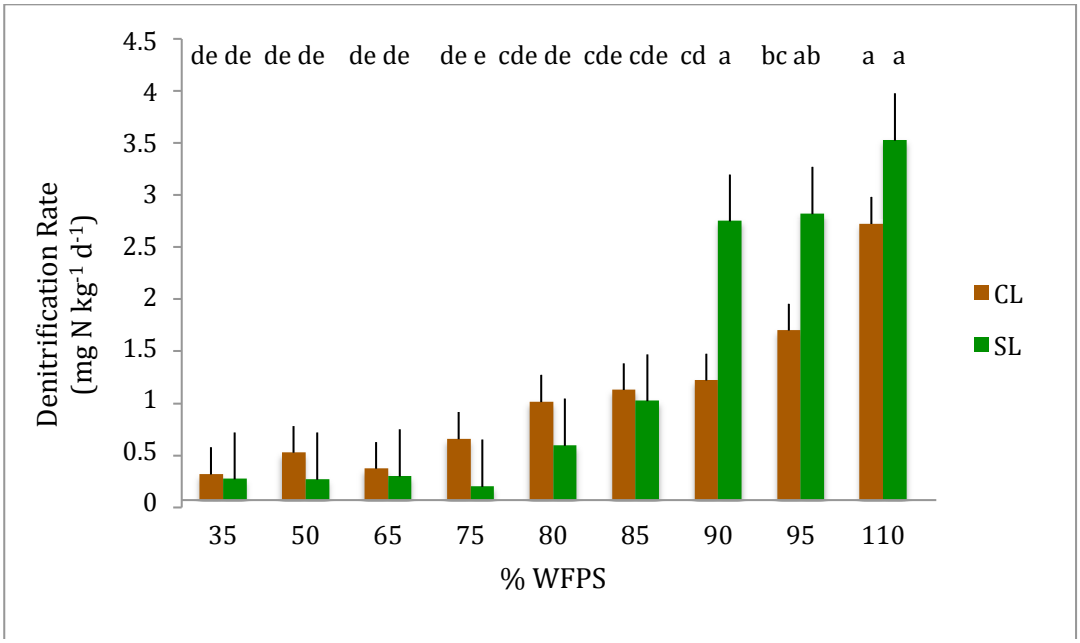

Figure 3.1. Denitrification rate (slope) in CL- and SL-textured soil at nine water contents (± 5% WFPS) during a 28-day incubation in experiment 2 (least squares means). Treatments with same letter not significantly different ($p > 0.05$) based on Tukey's HSD test.

3.2.3 Soil Respiration

Measurements taken 10 days after DMP addition on day 0 indicated that neither soil texture nor water content had a significant effect on the rate of respiration (Table A.2; Fig. A.2; Fig. A.3). On day 14, soil texture had a significant effect on the rate as the SL-textured soil exhibited a greater respiration rate than the CL-textured soil (Table A.2; Fig. A.2). On day 28, water content had a significant effect on the respiration rate as rates were lower at 20% and 35% WFPS (4.5 and 4.6 μg CO_2-C kg^{-1} min^{-1} respectively) than at 80% WFPS (9.3 μg CO_2-C kg^{-1} min^{-1}) where the greatest rate was observed (Fig. A.3).

3.2.4 Discussion

A simple, cost-effective method to concurrently measure N mineralization and denitrification in soils would be invaluable in studies of the influence of environmental variables on N cycling. The current study attempted to determine whether concurrent measurement could be achieved using NI method. The most common approaches have been to use the measurement of the difference in the sum of NH_4^+-N + NO_3^--N or *mineral N* between sample dates as an estimate of net N mineralization (Reichman et al. 1966; Stanford and Smith 1972; Campbell et al. 1974; Myers et al. 1982). The measurement of NH_4^+ alone is not effective when investigating the production of NH_4^+ in an aerated soil as it can be nitrified quickly. As well, Sierra (1997) and Dinesh and Dubey (1998) calculated net N mineralization rates based on the accumulation of NO_2^--N + NO_3^--N. This approach is problematic in soils with high water content as an unknown amount of nitrate can be lost via denitrification.

With respect to measurement of the denitrification, previous work relied on the measurement of gaseous N products and incorporated the use of acetylene gas to block the conversion of N_2O to N_2 (Aulakh et al. 1984, 2000; Gillam et al. 2008). Most agree this method is only a short-term method as longer term incubations can lead to an underestimation of total denitrification as nitrification is also blocked limiting NO_3^- availability in the soil (Gillam et al. 2008; Groffman et al. 2006). Further, a more complex and costly method, such as tracer studies using stable isotope ^{15}N has been used to identify and quantify the gaseous products of denitrification (Bateman and Baggs 2005). In the current study, DMP was highly

effective at blocking the production of NO_3^- allowing for the timely measurements of the decline in added NO_3^- as a gauge of denitrification in a short-term incubation.

In surveying the literature there are few studies that have measured net N mineralization and denitrification simultaneously over a wide range of water contents particularly above field capacity (Franzluebbers 1999; Aulakh et al. 2000a; Drury et al. 2003), in part due to the impact of denitrification on the accumulation of nitrate at high water content removing an unknown amount of the mineralized N.

The net N mineralization rate was influenced by water content and this differed between soil textures. Both soil types in this experiment contained similar total C [1.25 (CL) and 1.29 (SL) g kg^{-1}] and the same total N (0.127 g kg^{-1}) and were collected in the spring (after the corn year) from a winter wheat-corn-soybean rotation. We therefore anticipated the composition of the C substrate would be similar and that this factor alone was not driving the mineralization process; rather, the accessibility and solubility of that substrate for microbes determined the level of mineralization occurring. Some research suggests that sandy soils exhibit a higher rate of mineralization, when compared to silt or clay, as there is greater aeration and less protection of labile organic matter (St. Luce et al. 2010; Griffin 2008; Sahrawat 2008) and the clay content could be a central factor in determining the availability of labile organic N (Curtin and Wen 1999; Ros et al. 2011; Hassink 1997).

The N mineralization rate in the coarse-textured soil declined at low and high water contents relative to intermediate water contents, while the mineralization rate in fine-textured soil was relatively similar at high water contents as at low and intermediate water contents. In drier soil, the rate of diffusion of solubilized

substrate (Skopp et al. 1990), a lowered intracellular water potential (Stark and Firestone 1995), as well as reduced microbe mobility (Agehara and Warncke 2005; Killham et al. 1993) can result in much lower rates of mineralization. In the current study rates in the fine-textured soil rose above 87% WFPS, while rates in the coarse-textured soil declined possibly due to differences in pore structure due to flooded conditions as the fine-textured soil had more smaller, habitable pore spaces than the coarse-textured soil. As well, an increase in accessibility of labile organic N in smaller pore spaces as a result of an increase in water content leads to an increase in N mineralization (Brady and Weil 2002a). This observation is similar to work by Drury et al. (2003) over a three-month incubation, which found that net N mineralization, as measured by as accumulation of soil mineral N, was occurring at 95% WFPS in a Brookston clay loam soil, whereas the accumulation of soil mineral N declined in the SL-textured soils over the same time period due to probable denitrifying activity.

The denitrification rate was influenced by soil texture and water content as indicated by the significant two-way interaction. The denitrification rate rose linearly above 70% WFPS in both soil textures although it was a sharper increase in the coarse-textured soil. Groffman and Tiedje (1991) observed that with increasing % WFPS a loam-textured soil exhibited a sharper increase in the rate of denitrification when compared to a clay loam-textured soil, noting that coarse-textured soils are more directly influenced by the amount of water present than fine-textured soils.

The denitrification rate was much greater at 110% WFPS in both soil textures

than all other water contents. In a similar study, Aulakh et al. (2000a) observed that rates at 90% and 120% WFPS in a sandy loam were four to six times greater than at 60% WFPS. The greater rates of denitrification at the higher % WFPS reflected the increased anaerobic conditions in the micropores caused by water blocking gas exchange within the macropores.

The loss of N_2O was greater in the fine-textured soil and greatest at 75, 80 and 110% WFPS on day 14. Bateman and Baggs (2005) measured $^{14+15}N\text{-}N_2O$ over a 24-day incubation of a silt loam soil and found (1) the total $^{14+15}N\text{-}N_2O$ emissions at 70% WFPS in a fertilized treatment were six times greater than the total emissions at 60% WFPS and 16 times greater than at 20% WFPS over the entire incubation period.

Soil respiration rates were not significantly influenced by soil texture or water content on day 0. Overall, microbial activity was relatively constant over the full range of water contents with the exception of 95% WFPS in the SL-textured soil (7.3 μg CO_2-C kg^{-1} min^{-1}) which demonstrated a significant interaction between water content and soil texture. Previous work has found that respiration rates (CO_2 production) are controlled by drainage and air-filled porosity (Groffman and Tiedje 1991) and % WFPS (Linn and Doran 1984; Bateman and Baggs 2005). Groffman and Tiedje (1991) found that a well-drained clay loam exhibited higher CO_2 emissions at higher water contents than a poorly-drained clay loam. Over an 18-day incubation, Linn and Doran (1984) found that % WFPS influenced CO_2 production in a silty clay loam as the rates were lower between 20 and 35% WFPS than between 50 and 97% WFPS, although the maximum rate occurred at 60% WFPS in their study. In a 24-day

incubation study, Bateman and Baggs (2005) observed that the water content also influenced respiration rates in a silt loam; the flux in CO_2-C emissions was significantly higher at 70% WFPS, on day 1, than at 20 - 60% WFPS, yet by day 24 the reverse was true. In the current study, respiration activity declined between day 0 and 28 in all water contents across both textures presumably due to substrate limitation.

3.3 Experiment 3

The net N mineralization rate (NH_4^+ accumulation) and denitrification rate (NO_3^- disappearance) were measured in a sandy loam with two contrasting carbon amendment intensities over 10 water contents. The difference in carbon amendment intensity was anticipated to create differences in oxygen demand within each soil and was expected to influence soil microbial processes at various water contents. Measurements of CO_2 production, dissolved organic C, total soluble N and N_2O emissions were undertaken to provide supplementary information on soil microbial processes occurring and help to explain the observed trends in N mineralization and denitrification. The comparison of two soil differing in the intensity of carbon amendment (high carbon amendment - pig slurry, crop residues returned v. low carbon amendment - no N fertilizer, crop residues exported) was undertaken to determine whether the intensity of carbon amendment, and the resulting impact on oxygen demand, influenced N mineralization, denitrification and respiration rates as a function of water content.

3.3.1 Net N Mineralization Rate

The interaction between carbon amendment and water content had a significant effect on the N mineralization rate (Table 3.6). The influence of water content differed between the two carbon amendments (Table 3.6). The NH_4^+-N concentrations in these treatments were decreasing (immobilization) in the low carbon intensity soil at 35% WFPS and between 85 and 110% WFPS in the high carbon intensity soil (Fig. 3.2). Between 20 and 75% WFPS in the low carbon intensity soil, rates were not significantly different with the exception of 35% WFPS. In the high carbon intensity soil N mineralization rates were variable and the only significant difference was found between the higher water contents of 85 and 110% WFPS and water contents below 80% WFPS (Table 3.6). The numerically greatest rates were found at 95% WFPS in low carbon intensity soil (0.264 mg N kg^{-1} d^{-1}) followed by 20% WFPS in the high carbon intensity soil (0.231 mg N kg^{-1} d^{-1}) (Table 3.6).

Table 3.6. Net N mineralization rate (slope) in low (L) carbon intensity soil and high (H) carbon intensity soil over a wide range of water contents during a 28-day incubation in experiment 3. Analysis of variance (ANOVA) for treatments of carbon amendment (CA) and water content (WC).

Treatment		N Mineralization Rate ($mg\ N\ kg^{-1}\ d^{-1}$)	
CA		**WC (% WFPS)**	
L		0.105	a
H		-0.027	b
L	20	0.045	abcde
	35	-0.105	ef
	50	0.082	abcde
	65	0.037	abcde
	75	0.074	abcde
	80	0.127	abcd
	85	0.138	abc
	90	0.209	abc
	95	0.264	a
	110	0.178	abc
H			
	20	0.231	ab
	35	0.076	abcde
	50	0.019	abcde
	65	0.001	cde
	75	0.029	bcde
	80	0.093	abcde
	85	-0.245	f
	90	-0.095	def
	95	-0.120	def
	110	-0.261	f
ANOVA		**Prob > F**	
CA		<0.0001*	
WC		0.0006*	
CA x WC		<0.0001*	

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different*

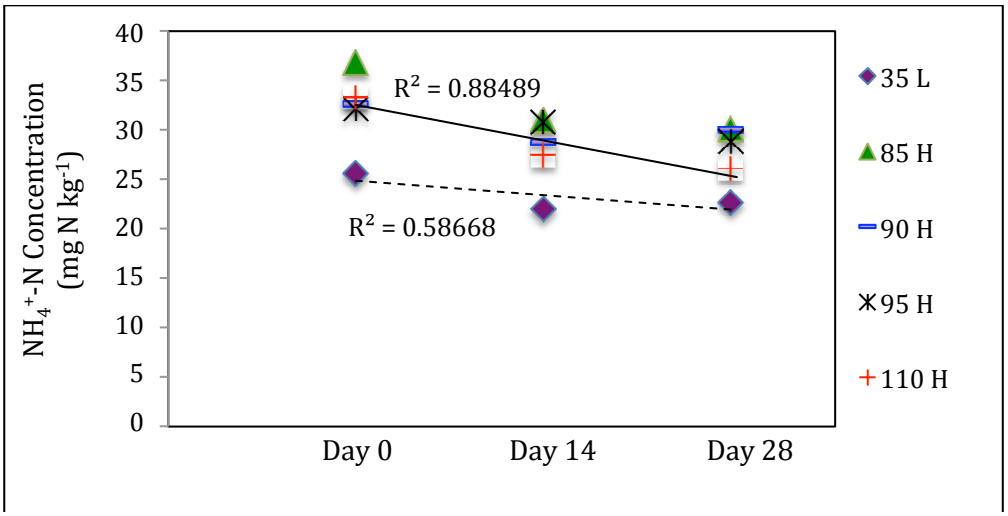

Figure 3.2. Concentration of NH_4^+-N in low carbon intensity (L) and high carbon intensity (H) soil at 35 to 110% WFPS on day 0, 14 and 28 in experiment 3. Linear regressions for treatments L 35% WFPS = dashed line, H 110% WFPS = solid line.

3.3.2 Dissolved Organic Carbon and Total Soluble and Dissolved Organic Nitrogen

Dissolved organic carbon (DOC) and total soluble nitrogen (TSN) were measured on treatments above 80% WFPS and used to calculate dissolved organic nitrogen (DON) for days 0, 14 and 28.

Water content and carbon amendment exhibited a significant interaction ($p = 0.0115$) (Table A.4) which influenced the DOC concentration on day 0; it was greater in the high carbon intensity soil between 80 and 90% WFPS (Fig. 3.3). Over the incubation period DOC was highly influenced by the carbon amendment and was greater in the high carbon intensity soil on day 14 (127 mg C kg^{-1}) and day 28 (130 mg C kg^{-1}) than the low carbon intensity soil on day 14 (115 mg C kg^{-1}) and day 28 (124 mg C kg^{-1}) (Table A.4; Fig. 3.4). As well, on days 14 and 28 water content had a significant effect on DOC as the greatest concentrations were found in at 110%

WFPS - 126 mg C kg^{-1} on day 14 ($p = 0.0059$) and 135 mg C kg^{-1} on day 28 ($p < 0.0001$) (Fig. A.4).

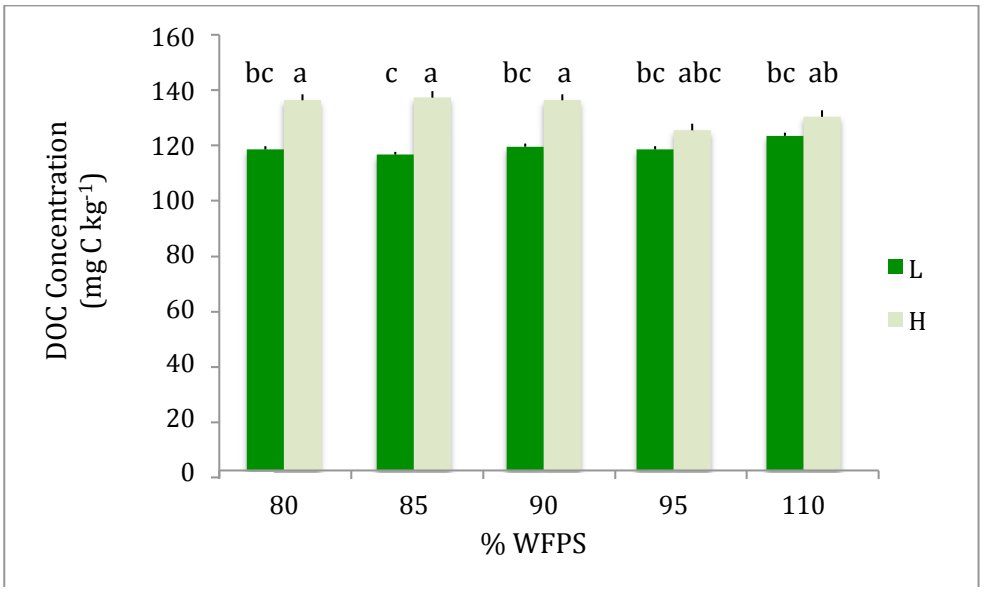

Figure 3.3. Dissolved organic carbon (DOC) concentration in low (L) carbon intensity and high (H) carbon intensity soil at 80 - 110% WFPS on day 0 (least squares means) in experiment 3. Treatments with same letter not significantly different ($p > 0.05$) based on Tukey's HSD test.

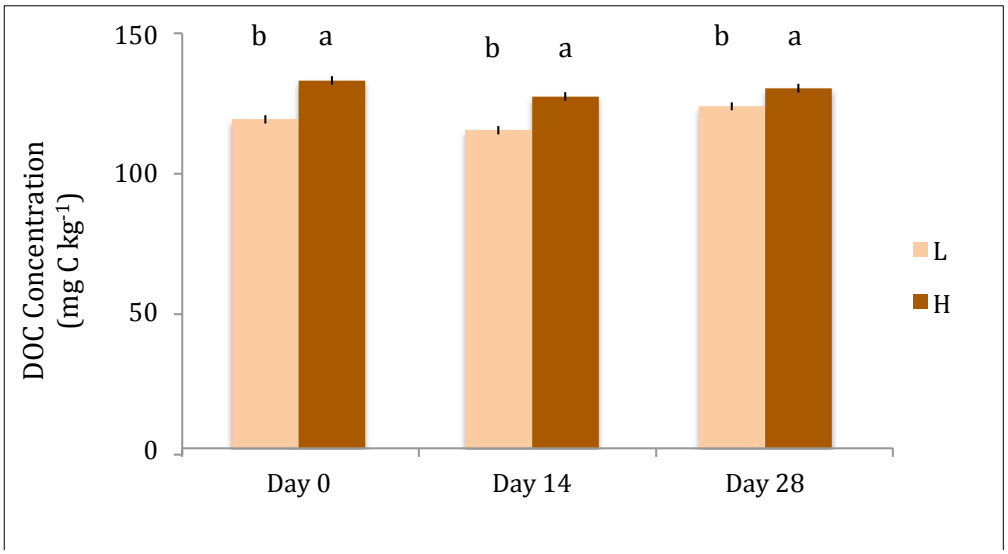

Figure 3.4. Dissolved organic C (DOC) concentration in low (L) carbon intensity and high (H) carbon intensity soil (least squares means) at 80 - 110% WFPS during a 28-day incubation in experiment 3. Treatments with same letter not significantly different ($p > 0.05$) based on Student's t test.

On day 0 the interaction between carbon amendment and water content influenced the concentrations of total soluble N ($p = 0.0232$) (Table 3.7). The only significant difference found was between 90% and 110 % WFPS in the low carbon intensity soil (Table 3.7).

On day 0, dissolved organic N (DON) concentrations were influenced by the carbon amendment ($p < 0.0001$) and water content ($p = 0.0009$) (Table A.5) and were greater in the high carbon intensity soil (25.1 mg N kg^{-1}) (Fig. 3.5) and above 85% WFPS (Table A.6). On day 14 and 28, DON concentrations were influenced by the interaction between carbon amendment and water content (Table A.5; Table A.6) and DON was greater in the high carbon intensity soil (79 mg N kg^{-1} and 102 mg N kg^{-1}) (Table A.6). Over 28 days, the DOC/DON ratio was wider in the low carbon intensity soil (15) than the high carbon intensity soil (6) (Table 3.7).

Table 3.7. Total soluble nitrogen (TSN) concentration in low (L) carbon intensity and high (H) carbon intensity soil on day 0 and mean DOC/DON ratio during a 28-day incubation in experiment 3. Analysis of variance (ANOVA) for treatments of carbon amendment (CA) and water content (WC) on day 0.

Treatment		TSN Concentration (mg N kg^{-1})	DOC/DON
CA	**WC (% WFPS)**		
L		161 b	15
H		173 a	6
L	80	161 bc	
	85	161 bc	
	90	158 c	
	95	160 bc	
	110	164 b	
H	80	171 a	
	85	174 a	
	90	174 a	
	95	172 a	
	110	174 a	
ANOVA	**Prob > F**		
CA	<0.0001*		
WC	0.2242		
CA x WC	0.0232*		

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different*

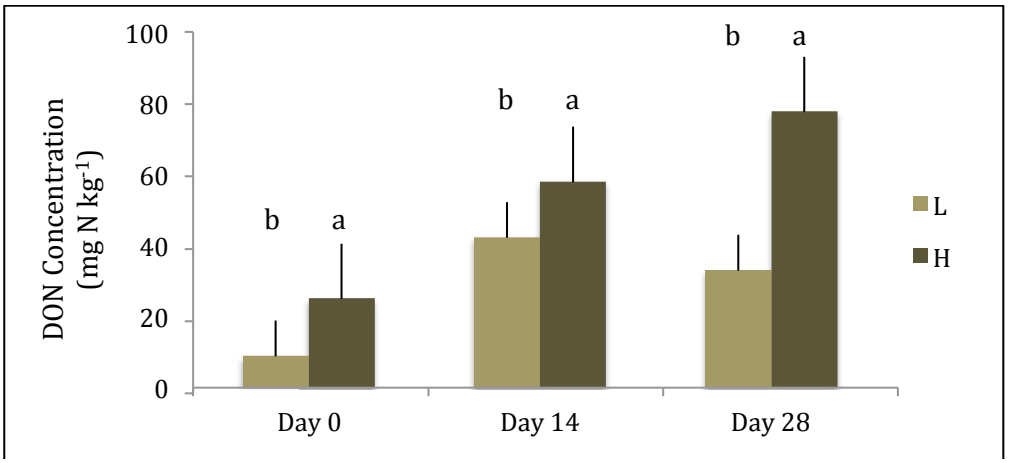

Figure 3.5. Dissolved organic N concentration (DON) in low (L) carbon intensity and high (H) carbon intensity soil on day 0, 14 and 28 (least squares means) in experiment 3. Treatments with same letter not significantly different (p > 0.05) based on Student's t test.

3.3.3 Denitrification Rate

The interaction between carbon amendment and water content ($p < 0.0001$) had a significant effect on the denitrification rate (Table 3.8). The significant interaction between water content and carbon amendment was evident between the low and high carbon intensity soils above 80% WFPS (Fig. 3.6). The numerically greatest denitrification rates were found at 110% WFPS for both the high carbon intensity soil (3.90 mg N kg^{-1} d^{-1}) and in the low carbon intensity soil at (1.55 mg N kg^{-1} d^{-1}) (Fig. 3.6). The lowest rates were found at 50% WFPS in the high carbon intensity soil (0.170 mg N kg^{-1} d^{-1}) and in the low carbon intensity soil (0.190 mg N kg^{-1} d^{-1}) (Fig. 3.6).

Measurements of cumulative N_2O emissions (as a source of denitrification loss) were taken over a 30-minute sampling period on day 0, 14 and 28. The interaction between carbon amendment and water content occurred on day 0, 14 and 28. (Table A.7). On day 0, the N_2O concentrations were higher in the high carbon intensity soil at 35, 75 and 80% WFPS than those in the low carbon intensity soil (Fig. 3.7).

Table 3.8. Denitrification rate (slope) in low (L) carbon intensity and high (H) carbon intensity soil over a wide range of water contents during a 28-day incubation in experiment 3. Analysis of variance (ANOVA) for treatments of carbon amendment (CA) and water content (WC).

Treatment	Denitrification Rate ($mg\ N\ kg^{-1}\ d^{-1}$)	
CA		
L	0.724 b	
H	1.41 a	
ANOVA		**Prob > F**
CA		<0.0001*
WC		<0.0001*
CA x WC		<0.0001*

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different.*

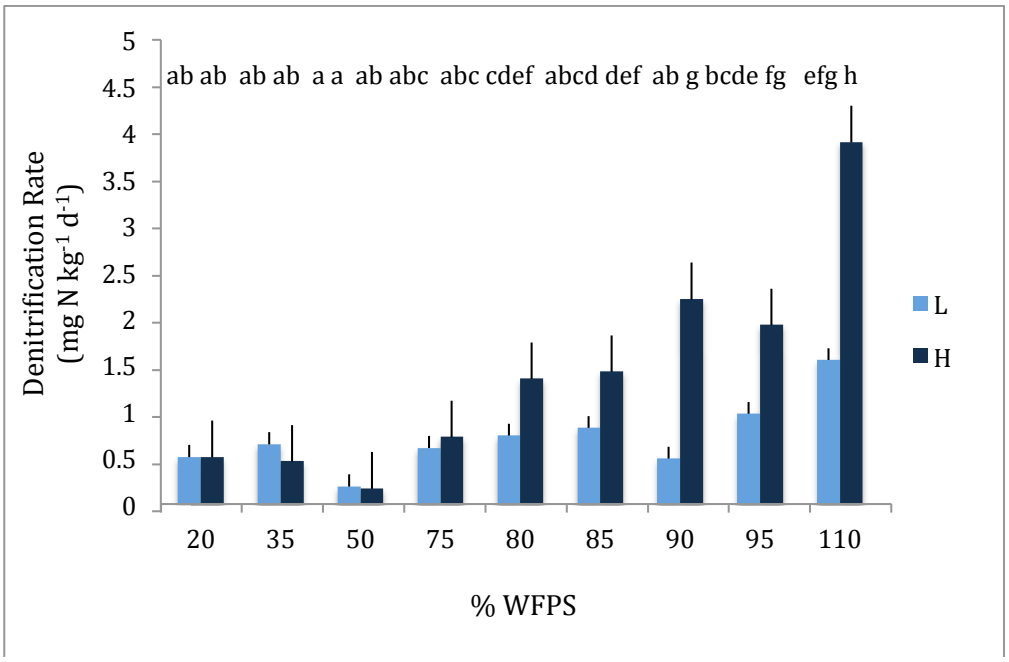

Figure 3.6. Denitrification rate (slope) in low (L) carbon intensity and high (H) carbon intensity soil over a wide range of water contents during a 28-day incubation (least squares means) in experiment 3. Treatments with same letter not significantly different (p > 0.05) based on Tukey's HSD test.

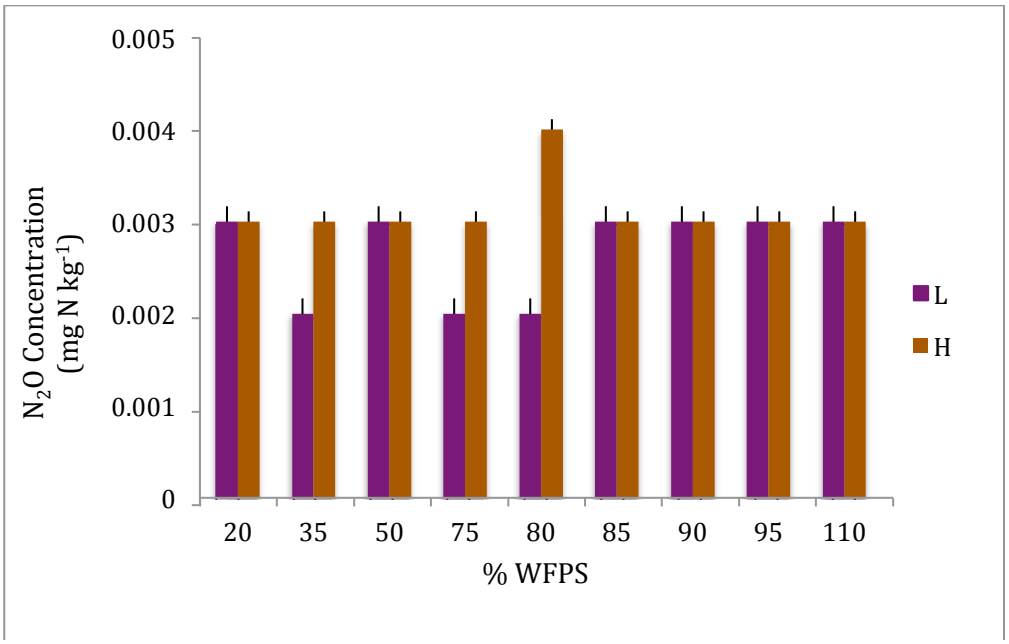

Figure 3.7. Concentration of nitrous oxide (N_2O) in low (L) carbon intensity and high (H) carbon intensity soil over a wide range of water contents on day 0 during a 28-day incubation (least squares means) in experiment 3.

3.3.4 Soil Respiration

Carbon amendment and water content had a significant effect on the respiration rate on day 0 (Table A.8; Fig. 3.8). The rate was greater in the high carbon intensity soil (23.9 µg CO_2-C kg^{-1} min^{-1}) (Table A.8). The lowest rate was observed at 110% WFPS (14.7 µg CO_2-C kg^{-1} min^{-1}) and the greatest rate was observed at 80% WFPS (27.0 µg CO_2-C kg^{-1} min^{-1}) (Fig. 3.8). On day 14, carbon amendment had a significant effect on the respiration rate, as it was greater in the high carbon intensity soil (11.9 µg CO_2-C kg^{-1} min^{-1}) (Table A.8). On day 28, there was no significance effect (Table A.8) therefore means were not reported here.

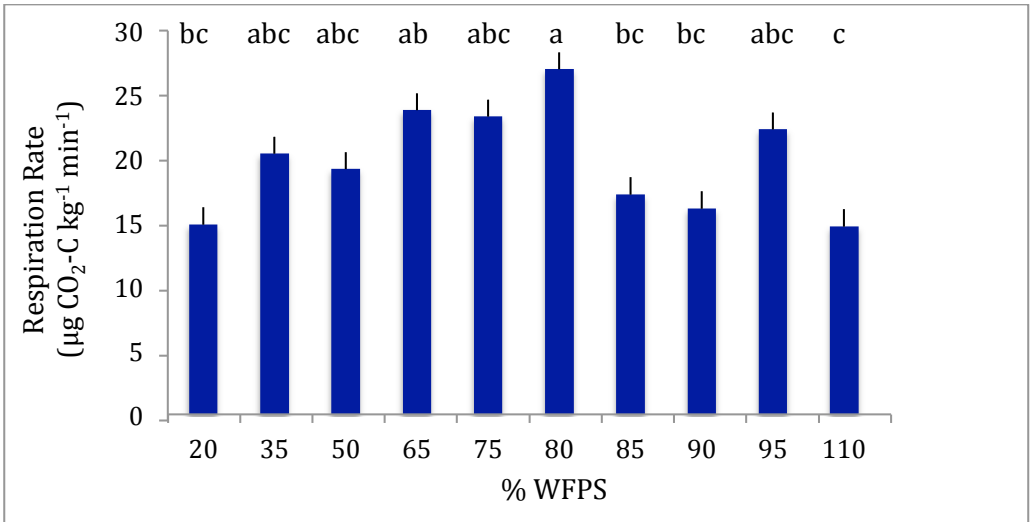

Figure 3.8. Respiration rate (slope) in low carbon intensity and high carbon intensity soil over a wide range of water contents on day 0 (least squares means) in experiment 3.

3.3.5 Discussion

In using the NI method, the rates of mineralization and denitrification as affected by carbon intensity (oxygen demand) and water content (% WFPS) were assessed. The oxidation of carbon-rich organic matter by heterotrophic microbes in soil requires O_2 as an electron acceptor in well-drained aerobic soil, and if the O_2 supply is reduced by high levels of microbial activity the soil can become anaerobic (Voroney 2007). While previous work has linked the source of carbon (e.g., crop residues and manures) to differing N mineralization and denitrification rates (Thomas et al. 2015; St. Luce et al. 2016; Sharifi et al. 2008; McKenney et al. 1993; deCatanzaro and Beauchamp 1989), few studies have assessed the interaction between oxygen demand as a result of differing carbon intensities and water content (Drury et al. 2003; de Neve and Hofman 2002; Aulakh et al. 2000b; Guo et al. 2010).

The mineralization rate differed between carbon intensities and the influence of water content differed between the low carbon intensity and high carbon intensity soil. The low carbon intensity soil exhibited a positive N mineralization rate of 0.105 mg N kg^{-1} d^{-1} while the high carbon intensity soil exhibited a negative N mineralization rate (N immobilization) of -0.027 mg N kg^{-1} d^{-1}, as concentrations of NH_4^+-N declined over time in the high carbon intensity soil. In the low carbon intensity soil at 35% WFPS and between 85 and 110% WFPS in the high carbon intensity soil, mineralization was not occurring, rather net N immobilization was occurring. Over a one month incubation, Drury et al. (2003) observed a sandy loam (bulk density of 1.30 Mg m^{-3}) amended with and without red clover and between 20 and 95% WFPS, and found that (1) the mineralization rate was not influenced by the addition of red clover residue and (2) mineral N decreased over the incubation to values lower than initial values at 90 and 95% WFPS. It was proposed that microbes were immobilizing N at higher water contents. While the soils used in this experiment were not N-limited due to the addition of 100 mg NO_3^- kg^{-1}, the sharp increase in the denitrification rate between 85 and 110% WFPS in the high carbon intensity soil indicated that microbes were more actively using all of the available C substrate under anaerobic conditions. Further, the DOC concentrations between 80 and 110% WFPS indicated that microbes in both treatments had available substrate, although the high carbon intensity soil (133 mg C kg^{-1}) had significantly greater DOC than the low carbon intensity soil (119 mg C kg^{-1}) on day 0 and throughout the incubation. The high carbon intensity soil's DOC/DON ratio of 6 over 28 days was narrower than the ratio in the low intensity soil which had a DOC/DON ratio of 15

indicating microbes in the high intensity soil could gain energy and increase in population size quicker. Respiration rates in the high carbon intensity soil also indicated that substrate was exhausted more rapidly which led to a decrease in O_2 supply limiting microbial catabolic activity. Nitrogen mineralization occurs when the C:N ratio of C substrate is < 20:1 and N immobilization is more likely to occur when the C:N ratio is > 30:1 (Robertson and Groffman 2007; St. Luce et al. 2011; Havlin et al. 2013). St. Luce et al. (2016) observed in a sandy loam-textured and a clay-textured soil, over 28 days and at 60% WFPS, with added particulate organic matter (POM) collected from corn-soybean-corn-forage-forage and the addition of corn POM only and found low mineralization rates and N immobilization occurring. It was suggested that in the short-term, the high ligno-cellulosic concentration found in these residues protected N-rich material from degradation.

In the present study, the composition of the total C and N content of the soil was similar in both treatments - total C [1.64 (L) and 1.92 (H) g kg^{-1}] and total N [0.157 (L) and 0.196 (H) g kg^{-1}]. Both soils were collected from the corn year of a corn-winter wheat-soybean rotation, although the accessibility to the substrate determined the level of mineralization occurring as indicated by the interaction between carbon amendment and water content. Net N mineralization rates were not significantly greater in the low carbon intensity soil (with the exception of 35% WFPS) yet the rates above 80% WFPS were two to seven times higher than rates below 75% WFPS. De Neve and Hofman (2002) observed, over 98 days, that the mineralization rate, in a loamy sand-textured soil amended with and without fresh carrot leaf residue and between 17 and 60% WFPS, was (1) significantly higher in

the non-amended soil (0.72 mg N kg^{-1} wk^{-1}) as compared to the amended soil (0.12 mg N kg^{-1} wk^{-1}) and (2) mineralization rates were significantly higher at 60% WFPS than at lower water contents in the non-amended soil. Further, Thomas et al. (2015) observed the mineralizable N pools over a 48-week incubation, at 55% WFPS, in the same sandy loam-textured soil used in the present study and found the mineralization rate to be significantly higher in the control or low carbon intensity soil (0.019 mg N kg^{-1} wk^{-1}) than the high carbon intensity soil (0.013 mg N kg^{-1} wk^{-1}) soil.

The denitrification rate was influenced by carbon amendment and water content and the interaction of these two factors was significant. The high carbon intensity soil exhibited a greater denitrification rate of 1.41 mg N kg^{-1} d^{-1} than the low carbon intensity soil at 0.724 mg N kg^{-1} d^{-1}. In the present study, the greatest denitrification rates were found at 110% WFPS for both the high carbon intensity (3.90 mg N kg^{-1} d^{-1}) and the low carbon intensity soil (1.55 mg N kg^{-1} d^{-1}). Aulakh et al. (2000b) observed a sandy loam with and without poultry manure over 16 days at 60, 90 and 120% WFPS and found that the denitrification rate, measured as cumulative N loss, was greater in the poultry-manure amended soil at 60 (7.2 mg N kg^{-1}), 90 (51.3 mg N kg^{-1}) and 120% WFPS (43.2 mg N kg^{-1}) than in the non-amended soil at 60 (4.5 mg N kg^{-1}), 90 (22.7 mg N kg^{-1}) and 120% WFPS (24.0 mg N kg^{-1}.

At the highest water content, the high carbon intensity soil was respiring more (17.1 µg CO_2-C kg^{-1} min^{-1}) than the low carbon intensity soil (12.2 µg CO_2-C kg^{-1} min^{-1}). This was attributed to the greater DOC concentrations in this soil as compared to the low carbon intensity soil and is consistent with the greater

denitrification rates observed at 110% WFPS as microbes in denitrification sites were unable to access O_2 and instead used NO_3^- as a TEA (Burton et al. 2008). Aulakh et al. (2000) also measured CO_2-C emissions and found greater rates in manure-amended soil at 60, 90 and 120% WFPS over the non-amended.

The lowest denitrification rates were found at 50% WFPS in both the high carbon intensity (0.170 mg N kg^{-1} d^{-1}) and low carbon intensity soil (0.190 mg N kg^{-1} d^{-1}). In a similar incubation study by Guo et al. (2010), the change in inorganic N (mainly NO_3^-) concentrations and total N_2O emissions was observed in a clay loam-textured soil sampled from fields in three rotations over 50 days at 30, 45, 60, 75 and 90% WFPS, and found that (1) all rotation treatments exhibited decreases in the inorganic N concentrations between 60% and 90% WFPS; (2) the monoculture corn had higher DOC and initial inorganic N than the other treatments relating to the significantly higher changes in inorganic N in this treatment and the corn-soybean rotation; (3) total N_2O emissions were influenced by water content to a greater degree than carbon amendment. In the present study, the high carbon intensity soil had a greater concentration of DOC and so higher rates were expected.

The highest and lowest N_2O emissions were observed in the low carbon intensity soil at 50% WFPS (6.1 μg N kg^{-1}) and at 75% WFPS (2.8 μg N kg^{-1}) and the high carbon intensity soil at 95% WFPS (5.5 μg N kg^{-1}) and at 50% WFPS (2.9 μg N kg^{-1}) respectively. Drury et al. (2003) found N_2O emissions were highest at 65% and 85% WFPS in a sandy loam over 12 weeks, and the amendment type was influential as emissions were greater in the red clover amended soil as compared to the non-amended soil. Lastly, in a study by deCatanzaro and Beauchamp (1989), the non-

amended soils had the lowest soluble C concentrations and lowest denitrification rates when compared to C amended treatments (alfalfa and straw), which was indicated by a gradual loss of NO_3^- over the incubation.

Results of this experiment indicated that the use of the NI method allowed the quantification both the N mineralization rate and the denitrification rate as NH_4^+ accumulation and NO_3^- disappearance respectively in soil containing C substrates with mainly narrow C:N ratios. Although N immobilization was a significant factor in the higher water contents in the high carbon intensity soil, which would require further study.

Chapter 4.0 Conclusion

4.1 Experiment 1

The ability of DMP to inhibit nitrification in arable soil that varied in clay and water content was confirmed. This assessment based on the measurements that indicated a lack of an increase in soil NO_3^-, as would be expected if nitrification occurred, and the soil respiration rate over a 28-day incubation period in each vessel. The influence of clay content was assessed, yet adsorption of DMP to fine-textured clay loam soil was not found to interfere with the inhibitory effect of DMP as both soil types equally inhibited nitrification. As nitrification is typically occurring at lower water contents the lack of NO_3^- accumulation at 35 and 50% WFPS was evidence that DMP was inhibiting nitrification. As well, soil microbial activity as indicated by the soil respiration rate did not significantly decrease with the application of DMP suggesting that DMP had no adverse effect on microbial activity.

4.2 Experiment 2

Concurrent measurements of the rate of N mineralization and denitrification, via the NI method, in arable soil that varied in texture and water content were used in the evaluation to find out how soil texture influences the kinetics of these processes. The rate of mineralization was greatest in the intermediate water contents in both soil textures. The rate was consistently greater in the CL-textured soil between 72 and 107% WFPS, indicating greater N mineralizing activity in smaller pore spaces and greater accessibility of solubilized substrate when compared to the larger pore spaces found in the SL-textured soil at the same water contents.

The rate of denitrification was much lower in both soil textures at the lower water contents, and progressively grew greater toward the highest water content in both soils textures due to the increased anaerobic conditions created by the addition of water blocking the oxygen gas exchange within the pores.

The NI method was able to simultaneously measure net N mineralization and denitrification rates in these two soils of contrasting texture across the full range of water contents considered.

4.3 Experiment 3

The NI method was further validated, as rates of N mineralization and denitrification were determined in soil that varied in carbon amendment and water content. Carbon amendment and water content had a significant effect on the mineralization rate as the low carbon intensity soil exhibited a greater rate. The high carbon intensity soil contained more DOC and exhibited a slightly narrower DOC/DON ratio, yet it did not experience a greater rate of mineralization specifically above 85% WFPS, as NH_4^+-N concentrations did not increase in these treatments over the incubation. One possible interpretation is that the NI inhibitor was ineffective under these conditions. This is unlikely however as at this high water content reduced oxygen content would limit nitrification. A more probable interpretation is that the decreased NH_4^+ content reflects N immobilization induced by the increase in C substrate availability, yet the soil C:N ratio was below 25:1. Denitrification rates were generally higher as water content increased across both carbon amendments. The high carbon intensity soil exhibited greater rates of denitrification, due to increased substrate availability under these anaerobic

conditions allowing for increased denitrifying activity. Higher respiration rates were observed in the high carbon intensity soil, which indicated that increases in substrate and water content increased microbial activity.

Again, the NI method was able to simultaneously measure net N mineralization and denitrification rates in these two soils of contrasting carbon amendment across the full range of water contents considered. Further study would be required to determine the immobilization of N in these soils by using the ^{15}N isotope to trace the fate of N in these soils.

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Table A.1. Analysis of variance (ANOVA) for nitrous oxide (N_2O) concentration over a 30-minute sampling period in CL- and SL-textured soil during a 28-day incubation in experiment 2. Treatments of soil texture (ST) and water content (WC) shown.

Treatment	Day	Prob > F
ST	0	<0.0001*
WC		<0.0001*
ST x WC		0.0256*
ST	14	<0.0001*
WC		<0.0001*
ST x WC		<0.0001*
ST	28	<0.0001*
WC		0.0617
ST x WC		0.0089*

- ** significantly different*

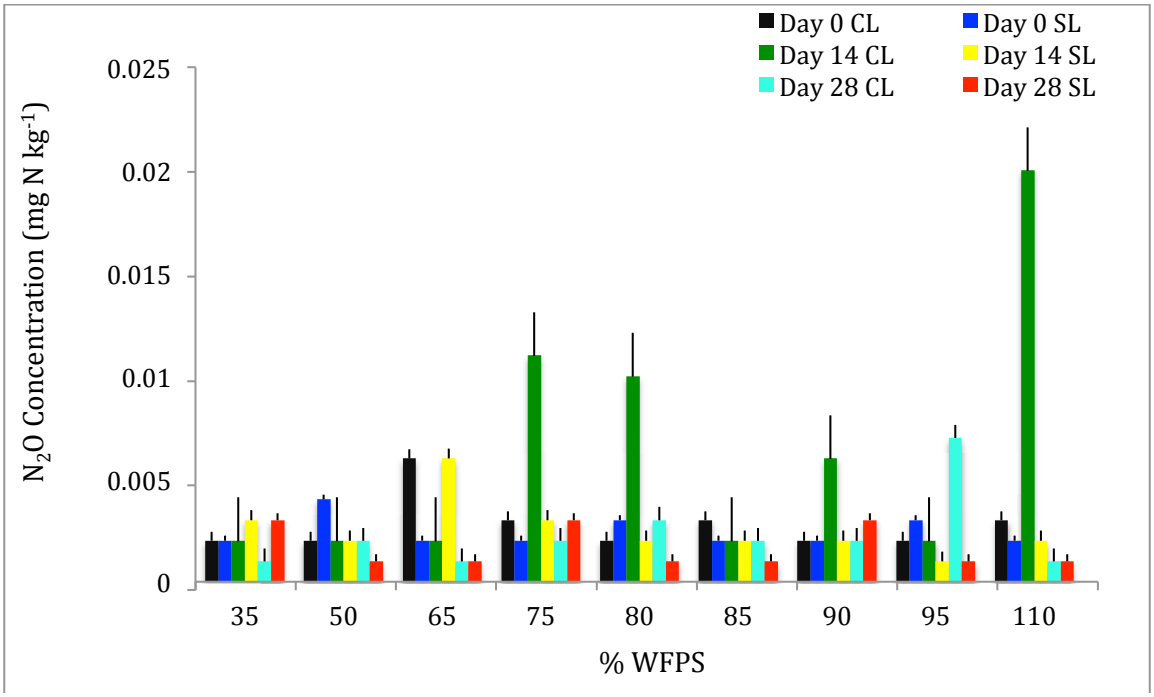

Figure A.1. Concentration of nitrous oxide (N_2O) at nine water contents in CL- and SL-textured soil over a 30-minute sampling period on days 0, 14 and 28 in experiment 2 (least squares means).

Table A.2. Analysis of variance (ANOVA) for respiration rate (slope) in CL- and SL-textured soil over a wide range of water contents during a 28-day incubation in experiment 2. Treatments of soil texture (ST) and water content (WC) shown.

Treatment	Day	Prob > F
ST	0	0.1409
WC		0.6120
ST x WC		0.0571
ST	14	0.0193*
WC		0.3367
ST x WC		0.9776

- ** significantly different*

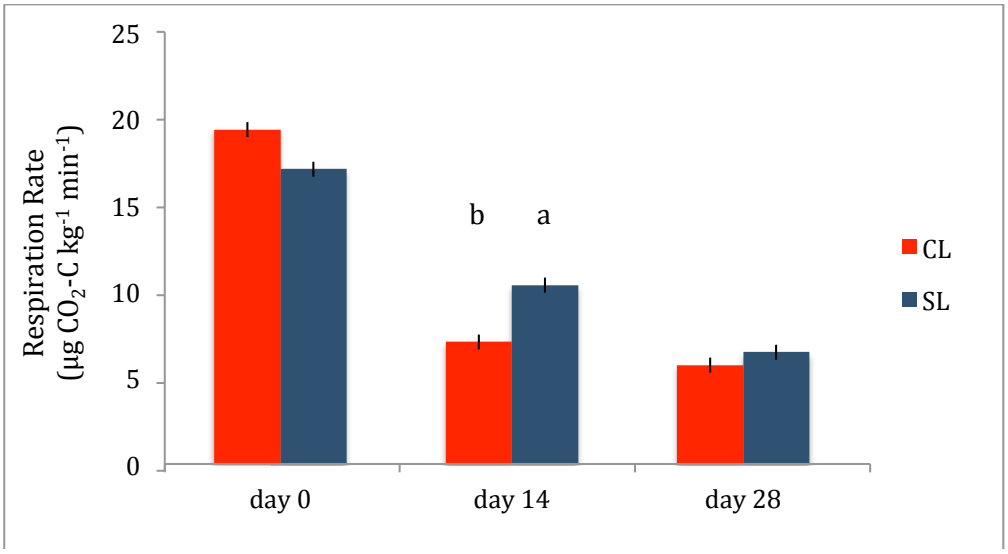

Figure A.2. Respiration rate (slope) in CL-textured and SL-textured soil on day 0, 14 and 28 (least squares means) in experiment 2. Treatments with same letter not significantly different ($p > 0.05$) based on Student's t test.

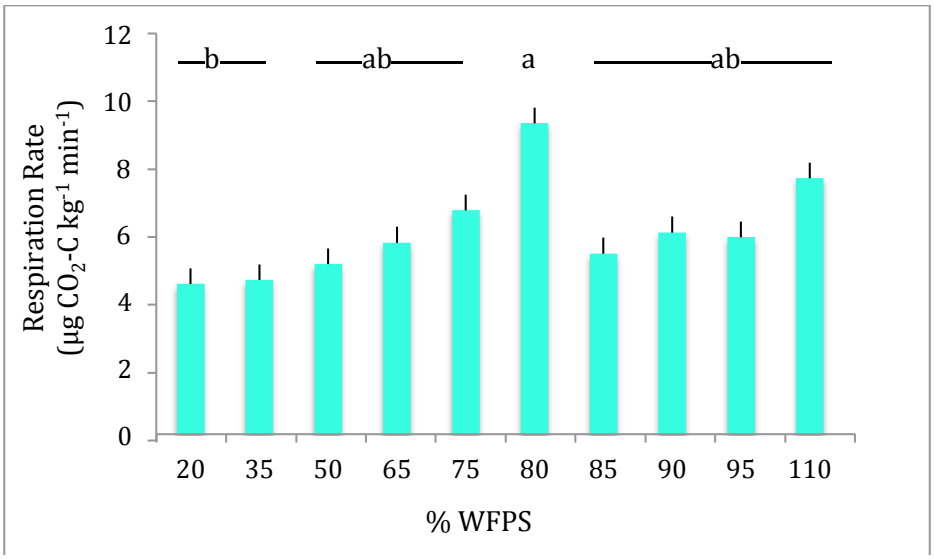

Figure A.3. Respiration rate (slope) over a wide range of water contents on day 28 (least squares means) in experiment 2. Treatments with same letter not significantly different ($p > 0.05$) based on Tukey's HSD test.

Table A.3. Net N mineralization rate (slope) in low (L) carbon intensity and high (H) carbon intensity soil between 20 and 80% WFPS during a 28-day incubation in experiment 3. Analysis of variance (ANOVA) for treatments of carbon amendment (CA) and water content (WC).

Treatment	N Mineralization Rate (mg N kg^{-1} d^{-1})
CA	
L	0.043
H	0.075
ANOVA	**Prob > F**
CA	0.2287
WC	0.0150*
CA x WC	0.0115*

- ** significantly different*

Table A.4. Analysis of variance (ANOVA) for dissolved organic carbon (DOC) concentration in low carbon intensity and high carbon intensity soil between day 0 and 28 in experiment 3. Treatments of carbon amendment (CA) and water content (WC) shown.

Treatment	Day	Prob > F
CA	0	<0.0001*
WC		0.2242
CA x WC		0.0232*
CA	14	<0.0001*
WC		0.0059*
CA x WC		0.9996
CA	28	0.0003*
WC		<0.0001*
CA x WC		0.5009

- ** significantly different*

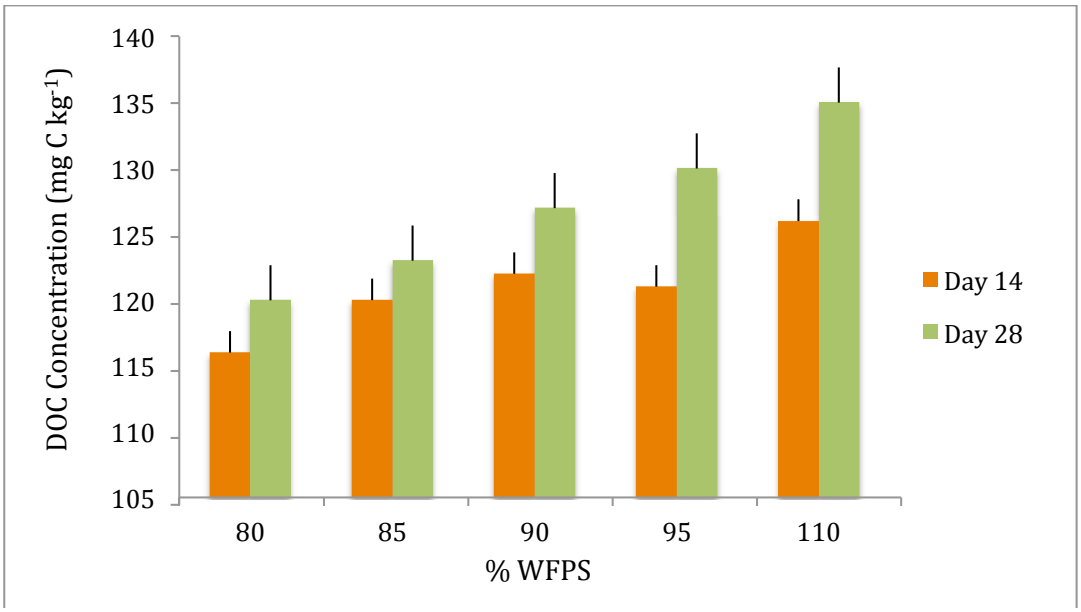

Figure A.4. Dissolved organic carbon (DOC) concentration in both carbon amendments as influenced by water content at 80 - 110% WFPS on day 14 and 28 (least squares means) in experiment 3.

Table A.5. Analysis of variance (ANOVA) for dissolved organic nitrogen (DON) concentration in low (L) carbon intensity and high (H) carbon intensity soil on over a wide range of water contents during a 28-day incubation in experiment 3. Treatments of carbon amendment (CA) and water content (WC) shown.

Treatment	Day	Prob > F
CA	0	<0.0001*
WC		0.0009*
CA x WC		0.3022
CA	14	<0.0001*
WC		0.0059*
CA x WC		0.9996
CA	28	0.0003*
WC		<0.0001*
CA x WC		0.5009

- ** significantly different*

Table A.6. Dissolved organic nitrogen (DON) concentration in low (L) carbon intensity and high (H) carbon intensity soil on day 14 and 28 in experiment 3.

Treatment	Day	DON Concentration (mg N kg^{-1})	
		WC (% WFPS)	
WC	0	80	17 a
		85	12 b
		90	20 a
		95	17 ab
		110	19 a
CA x WC	14		
L		80	39 def
		85	37 ef
		90	36 f
		95	42 def
		110	57 bc
H		80	50 cd
		85	49 cde
		90	64 b
		95	48 cdef
		110	79 a
CA x WC	28		
L		80	28 f
		85	29 f
		90	26 f
		95	34 f
		110	48 e
H		80	59 d
		85	67 cd
		90	77 bc
		95	83 b
		110	102 a

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and Tukey's HSD test and * significantly different*

Table A.7. Analysis of variance (ANOVA) for nitrous oxide (N_2O) concentration in low carbon intensity and high carbon intensity soil during a 28-day incubation in experiment 3. Treatments of carbon amendment (CA) and water content (WC) shown.

Treatment	Day	Prob > F
CA	0	0.0368*
WC		0.3845
CA x WC		0.0371*
CA	14	0.1963
WC		0.0002*
CA x WC		0.0023*
CA	28	0.8648
WC		0.0804
CA x WC		0.0024*

- ** significantly different*

Table A.8. Respiration rate (slope) in low (L) carbon intensity soil and high (H) carbon intensity soil as influenced by carbon amendment on days 0 and 14 in experiment 3. Analysis of Variance (ANOVA) for treatments of carbon amendment (CA) and water content (WC) on days 0, 14 and 28.

Treatment	Day	Respiration Rate ($\mu g\ CO_2\text{-}C\ kg^{-1}\ min^{-1}$)
CA		
L	0	15.7 b
H		23.9 a
L	14	7.9 b
H		11.9 a
ANOVA		**Prob > F**
CA	0	<0.0001*
WC		0.0001*
CA x WC		0.1042
CA	14	0.0177*
WC		0.6559
CA x WC		0.9361
CA	28	0.1221
WC		0.6892
CA x WC		0.5693

- *Treatments (least squares means) with same letter not significantly different (P > 0.05) based on Student's t test and * significantly different*