# ESTIMATION OF NITROGEN MINERALIZATION FROM SOLID BEEF CATTLE AND LIQUID SWINE MANURES

 $\mathbf{B}\mathbf{Y}$ 

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#### ABSTRACT

Sayem, S. M. M.Sc., The University of Manitoba, April, 2014. Estimation of Nitrogen Mineralization from Solid Beef Cattle and Liquid Swine Manures. Major Professor: Wole Akinremi, PhD

Manures are valuable sources of nitrogen (N); an accurate estimation of N release from manure is essential to maximize manure N use. Mineralization is the process that converts organic N to plant available N and is influenced by environmental and soil factors. Field and laboratory incubation studies were conducted to determine the N mineralization and fertilizer equivalence of locally available manures. Field studies were carried out using anion exchange resin at two sites, on a clay and a loamy sand soil. Laboratory incubation study under various water filled pore space (WFPS) at 14 and 21 °C was conducted using loamy sand soil. These studies used an unamended soil, solid beef cattle and liquid swine manures to estimate the net mineralized N. Amendments including fertilizer were incorporated at 100 kg ha<sup>-1</sup> available N to estimate the fertilizer equivalence of manures. Solid beef cattle manures (SBM) have typically more organic N and less inorganic N (i.e., ammonium N) than liquid manure. Available N from SBM amendments increased as the studies progressed due to mineralization. Liquid swine manure (LSM) behaved similarly to commercial fertilizer with high initial concentrations of ammonium N which declined continuously as the studies progressed, presumably due to ammonia volatilization, denitrification and immobilization, since plants were absent. Anion exchange resin was able to capture nitrate leached on the loamy sand soil whereas it was ineffective on the clay soil. In the field, the maximum mineralized N of 48 and 28 kg ha<sup>-1</sup> was estimated from SBM in 2011 and 2010, respectively. The growing season of 2011 was drier than 2010 and the volumetric water content ranged between 14 to 36 % (35 to 66 % WFPS) while average soil temperature was 22 °C. Manure N mineralization varied significantly (p < 0.01) due to the influence soil moisture, manure types and study period as well as their interaction under laboratory incubation. In the laboratory incubation, the maximum estimated mineralized N was 64 kg ha<sup>-1</sup> from the SBM amended soils incubated under 40 % WFPS at 21 °C. The fertilizer equivalence of SBM in loamy soil under laboratory and field conditions was initially low compared to LSM and finally approached 80 % of ammonium nitrate fertilizer. Slightly lower amounts of mineralized N in the field than laboratory could be explained by fluctuations of soil temperature and moisture in the field during the growing season compared to uniform condition during the laboratory incubation. Rapid loss of inorganic N from the clay soil in the field and from the loamy sand soil at higher soil moisture and soil temperature (i.e., 60 and 80 % WFPS) in the laboratory may be due to denitrification at higher soil moisture. To maximize site-specific manure N use by crops and minimize N losses, N mineralized from manure can be estimated from laboratory incubation study as long as soil moistures and soil temperatures are closer to those in the field. The study demonstrated that soils that are warm, moist and well aerated have the highest manure mineralization capacity. Slower mineralization of manure should be expected when soils are dry or saturated with water. It was also clearly observed that under loamy sand soil the use of anion exchange resin is feasible but it was not a viable method on a clay soil. These studies suggested that manure N mineralization requires emphasis on the initial form of N in manures besides soil moisture, soil temperature, and aeration.

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#### **1. INTRODUCTION**

Considerable efforts have been made to understand the role of soil and environmental factors on in-situ nitrogen (N) mineralization from manures. The objectives of these reviews were to understand the net N mineralization from manures and how it is affected by soil factors including, temperature and moisture variations under natural field conditions in a growing season.

#### 1.1 Manure as a source of plant available N

Plant available N is the sum of nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) and ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) in the soil. The availability of N is critical to crop production because of the large amount of nitrogen that is used by the plant and its sensitivity to loss through different processes (Ma et al. 1999; Stevens et al. 2005).

Manures are natural by-products of livestock production which have great value as a source of plant nutrients (Gilley and Eghball 2002). Manure N comes in both organic and inorganic forms and the major form of manure N varies with manure types as solid manure contains more organic N than inorganic N. Manure nitrogen in the soil is commonly separated into 5 pools: (i) nitrate-nitrogen; (ii) ammonium-nitrogen (iii) organic N compounds which are potentially available and constitute the bulk of the mineralizable substrate; (iv) microbial biomass; and (v) unavailable N which is resistant to microbial attack and mineralization processes (Zaman et al., 1998). Where pool (i) and (ii) would be the main source for the rapid release of nitrate during initial weeks after application, pool (iii) and (iv) serve as the substrate for continuous release of plant available inorganic N through the process of mineralization and nitrification.

#### **1.2 Manure N mineralization**

When manure is applied to the soil, the manure organic N is converted by soil microbes to ammonium nitrogen ( $NH_4^+$ -N) through the process of mineralization (Janson and Persson 1982) and becomes plant available inorganic N (Prescott 2005). Manure organic N needs to mineralize before it can be taken up by the plant, which makes some forms of manure slow sources of plant available N, especially the high organic N containing manure (i.e., solid forms of manure). The organic N fraction in different types of manures can vary between 14 - 99 %, such that 40 - 90 % of the total N in solid manure can be present as organic forms which make manure N more slowly available for plant uptake than fertilizer (MAFF 1994). Moreover, N loss through denitrification may increase in a manure cropping system compared to fertilizer due to the presence of more available C. However, denitrification also varies with soil characteristics and weather conditions (Loro et al. 1997)

The  $NH_4^+$  (ammonium) that is produced during mineralization is nitrified by different soil microbes to  $NO_3^-$  (nitrate) in a process called nitrification. If environmental conditions are not limiting,  $NH_4^+$  is oxidized to  $NO_3^-$  almost as rapidly as it is produced (Schmidt 1982). Thus,  $NO_3^-$ -N is the dominant form of plant available N in most annually cropped agricultural soils (Kaboneka et al. 1997; Liang and Mackenzie 1994; Ferreira et al. 2000). However, net N mineralization is the result of two opposing processes, i) gross

N mineralization which is the total amount of N being mineralized from organic N and ii) gross N immobilization which is the total amount of N being immobilized primarily through microbial assimilation. Net N mineralization is determined by direct measurement of changes in inorganic soil N (Wessel and Tietema 1992). The mineralized manure nitrogen may take several routes: (i) the mineralized N may remain in the soil and increase the inorganic N pool or be taken up by plant; (ii) it could be immobilized by microbes and become part of the microbial biomass pool; and (iii) NO<sub>3</sub><sup>-</sup>-N derived from manure may be lost from soil as nitrous oxide (N<sub>2</sub>O), di-nitrogen (N<sub>2</sub>) or directly as nitrate (NO<sub>3</sub><sup>-</sup>) form. In solid manured cropping systems, mineralization supplies most of the crop N need. An understanding of the mineralization pattern or release of plant available N from manure during the growing season will improve manure management by meeting crop N demand and by minimizing environmental risk associated with the loss of excess nitrogen. This will also help to predict or estimate the net availability of manure N to crops (Sorensen 2001).

Nitrogen mineralization from manure generally exhibits a slow initial rate, which is indicative of a lag period. This is followed by a rapid increase in rate and a subsequent slow N release rate (Chae and Tabatabai 1986). Studies often report varying proportion of mineralized organic N from a variety of sources, such as 56 % from beef feedlot manure (Chang and Janzen 1996), 25 % from dairy cattle manure (Sanderson and Jones 1997), 11 % to 21 % from composted and non- composted manure (Eghball 2000), 56-61 % from blood meals, 41-52 % from alfalfa pellets and 37-45 % from chicken manures amendments during the growing season (Agehara and Warncke 2005). However, the

proportion of mineralized N from organic N sources also varies with the length of the study period. Morven et al. (2006) compared 47 different animal wastes and found that net mineralization of applied organic N ranged from 31 - 51 % after 270 days of incubation. On the other hand, very low amount of only 10 % of organic N mineralized from cattle, poultry and sheep manure in 90 days was reported by Abbasi et al. (2007). There is also evidence of only 15-20 % dairy manure organic N mineralized within the first 21 days of incubation (Pettygrove et al. 2003). However, mineralized N from manure could be taken up by plant, lost from the soil during the first growing season or when the remaining residual organic nitrogen becomes available in the succeeding years. Manure N mineralization studies also observed the disappearance of manure N and reported this as negative mineralization (Lentz and Lehrsch 2012). Manures are highly variable in their N mineralization of 30 % of the organic N (Van Kessel et al. 2000; Cusick et al. 2002; Morvan, et al. 2006).

#### **1.3 Factors affecting manure N mineralization**

During the last five decades, considerable research has been directed toward the development of manure N mineralization assessment methods. Several factors influence manure N mineralization, many of which are inter-related, making it difficult to understand the mineralization of manure N under in-situ field conditions (Zaman et al. 1999). However, manure N mineralization directly depends on microbial activities and the type of manure, which is affected by abiotic factors such as soil temperature, soil moisture and soil aeration, etc. Although a number of studies reported that N

mineralization was influenced by soil environment (Sims 1986; Whalen et al. 2001; Cookson et al. 2002; Zaman and Chang 2004; Agehara and Warncke 2005), the relationships between N mineralization and soil environment is still not well understood (Whalen et al. 2001; Cookson et al. 2002; and Bagherzadeh et al. 2008). A small fraction of manure labile pool serves as an active substrate for N mineralization and it is often influenced by a number of factors including type of manure and the method of manure application, soil organic matter (Chae and Tabatabai 1986; Pierzynski et al. 2000, Thompson and Meisinger 2002), soil moisture and soil temperature (Goncalves and Carlyle 1994; Dalias et al. 2002; Zaman and Chang 2004; Dahlin et al. 2005; and Griffin 2007), soil wetting and drying cycles (kruse et al. 2004), soil micro-organisms and their activity (Whalen et al. 2001; Cookson et al. 2002; and Bagherzadeh et al. 2008), soil physical characteristics (Schjonning et al. 1999; Gordillo and Cabrera 1997), such as soil texture (Torbert and Wood 1992) pore size distribution in aggregates, bulk density, clay content, and soil pore structure (Sleutel et al. 2008), water holding capacity, nutrient use efficiency (Akinremi et al. 2000) and management practices (Zaman et al. 1999; Dharmakeerthi et al. 2005, 2006). Manure N management without consideration of these factors can result in an inefficient N use with detrimental losses to the environment.

From a management standpoint, it is important to understand how chemical composition and soil environment influence the availability of N from manure (Palm et al. 2001 and Juan et al. 2009). However, chemical composition can be controlled to increase the synchronization of nitrogen release with plant N demand, for example, by

manipulating animal diet; whereas climatic factors are beyond our control and so must be taken into account prior to manure application.

#### 1. 3. 1 Soil characteristics

There is no consensus in the literature on the influence of soil types on manure N mineralization. For example, mineralization has been found to proceed faster in coarse textured soil (Jobbagy and Jackson 2000), while Watt et al. (2007) showed that it proceeded at a slower rate in coarse textured soils. Other researchers have shown that there is no significant influence of soil texture on mineralization of manure (Giardina et al. 2001; Whitmore 2007). Fine textured soils containing more organic matter have been shown to increase manure N mineralization (Egelkraut et al. 2003; Watt et al. 2007) or could decrease manure net N mineralization (Jobbagy and Jackson 2000). Nitrogen mineralization studies often conducted by various researchers to relate with a number of soil factors including soil total N, C: N ratio, soluble organic N, particle size distribution, organic matter content, soil moisture and soil pH, etc. The results of those studies often vary with soil type or may be specific to the condition of the experiment, suggesting that more studies are needed at various locations and environments to improve our understanding. Thus, there is a need for further research to understand the N mineralization of manures, particularly in Manitoba soils during the growing season.

#### 1. 3. 2 Manure Characteristics

Manures from various animal species have an impact on net N mineralization (Thompson and Meisinger 2002). This often varies with the chemical characteristics of the manure (Tisdale et al. 1993) such as organic N and inorganic N content as well as C:N ratio of manure (Morven et al. 2006). For example, manures that contain bedding materials or other material rich in carbon can temporarily immobilize available N from the soil and delay its release to the plant. However, studies have observed conflicting results regarding manure composition and its impact on N mineralization. For example, the C:N ratio of manure can cause 40 % variation in N mineralization (Chadwick et al. 2000; Griffin 2007; Goncalves and Carlyle 1994) and conversely, there was no significant contribution to manure N mineralization from variation in organic C (Egelkraut et al. 2003). Furthermore, manure liquid density, total suspended solid and manure organic N influence the net N mineralization of N in liquid manure (Chae and Tabatabai 1986). Comparison of the N mineralization of liquid versus solid manures under field conditions has shown differences in the mineralization rate as mineralization of liquid manures is faster than solid manures (Rochette et al. 2006). Liquid manure usually contains a greater proportion of  $NH_4^+$ -N than solid manure which may provide more available N during the initial stages of mineralization (Schoenau et al. 2000; Calderon et al. 2005).

#### **1. 3. 3** Soil moisture and soil temperature

Soil moisture and soil temperature play a very important role in regulating manure N mineralization or availability of N (Agehara et al. 2005; Dahlin et al. 2005). The maximum microbial activity, which often determines the decomposition rate of organic substances in soil, occurs at 60 % WFPS for a wide range of soils (Linn and Doran 1984). Soil water content above this threshold result in slower decomposition rate as anaerobic conditions limit oxygen availability as well as microbial activity (Linn and Doran 1984).

Excessive soil moisture often creates anaerobic soil conditions, which can slow down mineralization, and increase denitrification (Linn and Doran 1984; Schomberg et al. 1994). Low soil moisture can inhibit microbial activity by reducing diffusion of soluble substrates (Schjonning et al. 2003), microbial mobility (Killham et al. 1993) and intercellular water potential (Stark and Firestone 1995). Thus, at low soil water content N mineralization declines due to declining microbial activity (Zak et al. 1999)

Most microbial processes involved in N transformation are temperature dependent; therefore, increasing soil temperatures in the field and during laboratory incubation often stimulate net manure N mineralization due to increased microbial enzymatic activity (Eghball 2000; Rustad et al. 2001; Cookson et al. 2002; Dalias et al. 2002; Melillo et al. 2002; Wan et al. 2005). The accumulation of net mineralized N during incubation is often linearly related to incubation time at lower temperatures due to the small microbial demand for the readily mineralizable organic N and curvilinear at higher temperatures due to faster consumption of readily mineralized organic matter (Wang et al. 2003). For example, 22, 33, 41, and 60 % of total N in organic residues was mineralized at 2, 5, 10, and 15 °C after 160 days of incubation (Cookson et al. 2002). Decomposition of organic soil amendments is 3.7 times faster at 25 °C than at 15 °C and 13 times faster at 15 °C than at 5 °C (Vigil and Kissel 1995).

Studies have reported a significant interaction effect of soil moisture and soil temperature on N mineralization from manures (Knoepp and Swank 2002), as well as microbial activities (Grant and Rochettel 1994) and therefore soil respiration (Akinremi et

al. 1999). Hence, besides soil temperature, adequate soil water is required for mineralization to proceed at an optimal rate (Prescott 2005). Optimum N mineralization occurs when soil temperature is between 25 and 35 °C (Nicolardot et al. 1994; Stark and Firestone 1996) and soil moisture is near field capacity (Stanford and Epstein 1974; Prescott 2005).

#### 1. 4 Synchronizing manure N mineralization with plant N demand

The aim of manure N management is to synchronize manure N mineralization with plant N demand (Campbell et al. 1995; Murwira and Kirchmann 1993). However, accurate methods for accurately estimating credits for mineralized N in manure are not presently available (Ma et al. 1999; Gurlevik et al. 2004; Van Kessel and Reeves 2002; Wang et al. 2001). In general, annual crops tend to take up N slowly during their initial establishment, quickly as they develop, and then more slowly as they mature (Crohn 2004). In a manured cropping system, it is essential to supply adequate amounts of plant available N during periods of substantial plant N uptake, whereas minimal amounts of N should be present during periods of lower plant N demand (Honycutt et al. 2005) and when there is a high risk of plant available N loss through denitrification, leaching and volatilization (Jayasundara et al. 2010).

#### **1.5 The fertilizer value of manure N**

The fertilizer equivalence of manure is the amount of fertilizer N required to achieve the same yield and crop N uptake achieved with manure. Farmers may increase the utilization of manure for crop production if they have a realistic estimate of its fertilizer value. The amount of N mineralized from manures and its value as a fertilizer in a growing season is an important variable to be considered prior to the recommendation of manure application rate for better crop production (Logan 1990; Haney et al. 2001; Tilman et al. 2002; Gutser et al. 2005; Yousif and Mubarak 2009). Estimation of fertilizer equivalence is very critical and often varies with forms of initial N and application rate of manures (Tien 2010). The fertilizer equivalence of injected liquid swine manures is about 60 % (Kyveryga et al. 2005). Schoenau et al. (2000) also reported that the availability of N from LSM containing 50 % ammonium was 50 to 70 % effective as urea.

#### 1. 6 Methods used to measure manure N mineralization

A number of laboratory and field procedures have been developed for estimating mineralized N from manure and other organic amended soils (Keeney 1982; Douglas and Magdoff 1991; Hart et al. 1994; Cabrera et al. 1994; Gordillo and Cabrera 1997; Gilmour and Skinner 1999). Most of these have proven inadequate because they do not measure the mineralization of N during the growing season (Campbell et al. 1995). Moreover, there is no proof that one approach is consistently better than the other for predicting net N mineralization during the growing season (DeWilligen 1991; Benbi and Richter 2002). Laboratory methods often use chemical extraction, microbial biomass (Horwath and Paul 1994), microbial activities (Dahnke and Johnson 1990), and electro-ultra filtration method (Nemeth 1982) under controlled environmental or an ideal condition which differ greatly from those observed under in-situ field condition (Wienhold 2007). To understand net N mineralization of manure under field condition a number of methods have been used including buried bag, covered cylinder, ion exchange resins, and soil testing such as

residual profile  $NO_3$ -N test, pre-sidedress  $NO_3$ -N test (PSNT), use of stable isotope nitrogen-15 (<sup>15</sup>N) (Barraclough 1991; Barraclough and Puri 1995) etc. Nevertheless, results are often variable with site-specific field condition (Beauchamp et al. 2004; Curtin and Macalluin 2004).

Various methods exist to measure N mineralization in soil. Of these, the anion exchange resin can provide realistic measures of nitrogen that is released from manure during the growing season (Raison et al. 1992). The ability of anion exchange resin to capture NO<sub>3</sub><sup>-</sup>-N ions has made this method to be popular and promising (Griffin et al. 2008; Honeycutt et al. 2005; Brye et al. 2002) as it often simulates natural soil condition (Eghball 2000; Brye et al. 2002; Honeycutt et al. 2005; Griffin et al. 2008). The resin technique has been used in various studies such as in grassland (Gibson et al. 1985; Hook and Burke 1995), forests (Binkley and Matson 1983; Binkley et al. 1986; Smeturst and Nambiar 1989), deserts (Lajtha 1988), dry land agroecosystems (Kolberg et al. 1997, 1999), moist, fertilized agricultural soil (Brye et al. 2002), with manure, compost and organic soil amendments (Eghball 2000; Hanselman et al. 2004) and in arctic soils (Giblin et al. 1994).

In spite of the many advantages of using ion exchange resins, there are disadvantages which may create significant variability in the measured mineralized manure N in the field (Hanselman et al. 2004). For example, soil water content of the soil containing ion resin could be greater than in the natural field soils (Hanselman et al. 2004) which also can hamper the ability of resins to adsorb available N ions (Kjonaas

1999a, 1999b). Ion resins could react with low molecular humic substances or organometal complexes which could reduce exchange capacity of resins (Krause and Ramlal 1987) and the development of roots from surrounding plants can also severely influence the estimate of mineralized N, since roots and microbes may use the nitrate on the resin as a nutrient source (Hart el al. 1994).

There is uncertainty regarding the extrapolation of commonly derived mineralization values (Heumann and Bottcher 2004; Griffin et al. 2008) to the field because N mineralization process can be affected by a number of dynamic and soil specific factors (Khan et al. 2007). Moreover, much less research is available on how these values can be extrapolated to other sites with differing environmental conditions and soil characteristics (Klausner et al. 1994). Thus, various studies have tried to relate N mineralization to a number of factors, including soil total N, C:N ratio, soluble organic N, presence of nitrate, particle size distribution, organic matter content, soil moisture, soil temperature and soil pH (Berendse et al. 1994; Groot and Houba 1995; Hassink 1994; Troelstra et al. 1995). However, these factors often have contradictory effects on mineralization; as such, the developed relationships may apply to a small range of soil types only or to the specific circumstances under which they were derived (Schaffere 2000). Quantitative information on the mineralization of manure N during the growing season is needed for a better estimate of the application rate of manure. Limited information exists on the mineralization of manure N in Manitoba soils. More detailed information on the pattern of manure N mineralization during the growing season relies on matching the mineralized N release to crop demand (Ma et al. 1999). Moreover, determining the fertilizer value of manure will act as a tool for more appropriate soilspecific recommendation to maximize crop benefit and avoid detrimental effects on plant and environment (Beraud, et al. 2005).

Because of the numerous factors affecting mineralization, it remains difficult to accurately predict mineralization patterns during the growing season in the field (Brye et al. 2002). It is well recognized that mineralization is a microbial mediated process which is not only influenced by substrate characteristics but by soil climate, primarily soil temperature, soil moisture, and soil aeration status (Griffin and Honeycutt 2000). A better understanding of the major influencing factors such as soil temperature, soil moisture availability, and their interactions on net N mineralization in various soils will facilitate our understanding of manure N availability (Luxhøi et al. 2006; Sorensen 2001). Hence, there is a need to understand soil N mineralization pattern under field conditions during the growing season to provide more precise estimates of mineralized manure N.

Thus, this study was undertaken with a view to estimate net N mineralization from locally available manures in Manitoba. To achieve the objectives, two years of in-situ resin study and a laboratory incubation study were conducted using standardized protocols (Griffin et al. 2008 and Honeycutt et al. 2005).

Chapter 2 of this thesis describes the findings from studies conducted during two growing seasons in 2010 and 2011 using anion exchange resin and four manure types. Chapter 3 reports on findings from a companion laboratory incubation study at four soil water contents and two soil temperatures to simulate a range of growing season's soil moisture and soil temperature conditions. The fertilizer equivalence as the amount of net available N was estimated to compare net mineralized N obtained from manures and the amount of available N obtained from fertilizer at each sampling period.

Finally, chapter 4 is a general synthesis of the field and laboratory incubation study findings (i.e., estimated manure net N mineralized N and its fertilizer value). It attempts to reconcile the measured manure N mineralization in the field with those from laboratory incubation. Therefore, the specific objectives of this study were to: I. Monitor inorganic nitrogen release and fertilizer equivalence of locally available manures in two typical agricultural soils and to II. Quantify the effects of manure type, soil moisture and soil temperature on the release of mineralized N and its fertilizer equivalence.

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# 2. FIELD MEASUREMENT OF NITROGEN RELEASE FROM SOLID BEEF AND LIQUID SWINE MANURES

# 2.1 Abstract

Manures are valuable sources of nitrogen (N); an accurate estimation of N released from manures is essential to maximize N use efficiency and minimize adverse impact of manure application to the environment. Mineralization determines the release of organic N from manures and is driven by local soil environment, especially soil moisture and soil temperature. The objective of this study was to determine the release of inorganic N and fertilizer equivalence of locally available manures N. The study was carried out during two consecutive growing seasons in 2010 and 2011. Study treatments were arranged in a randomized complete block design (RCBD) with four replicates at two sites on a heavy clay and loamy sand soil in Manitoba (MB), Canada. There were six treatments consisting of three solid beef cattle manures (SBM), one liquid swine manure (LSM), a commercial N fertilizer (urea or ammonium nitrate) and a control. Amendments were incorporated into soil cylinders at a rate of 100 kg ha<sup>-1</sup> of available N (i.e., estimated as  $NH_4-N$  + organic N \*0.25) to evaluate the fertilizer equivalence and compared to an unamended soil. Polyvinyl chloride (PVC) columns were installed in the field under fallow condition in order to eliminate plant N uptake and allow the resin to retain leached nitrate-N (NO<sub>3</sub><sup>-</sup>N). Soil moisture and soil temperature were continuously recorded using in-situ soil probes. Soil cylinders with anion exchange resin cloth bags at the bottom were retrieved at 0, 7, 14, 28, 42, 56, 70, 98 and 126 day (d) intervals. The volumetric soil

moisture and soil temperature ranged from 13 to 51 % and 2.5 to 27.4 °C in clay soil and from 14 to 36 % and 8.1 to 27.6 °C in loamy sand soil in 2010. During the 2011 growing season volumetric moisture and soil temperature of loamy sand soil ranged from 21.6 to 32.2 % and 4.8 to 26.7 °C respectively. Initially, large amounts of  $NH_4^+$ -N were measured from Fertilizer and LSM, with almost 100 % recovery of added inorganic N. Within 21d, the  $NH_4^+$ -N disappeared due to nitrification, and  $NO_3^-$ -N became the main inorganic N form in the soil. The initial available N from SBM amendments was small and thereafter increased as the growing season progressed due to mineralization of organic N. The maximum net inorganic N (NIN) of SBM was measured between day 42 and day 70 and, in loamy sand soil this ranged between 37 and 39 kg ha<sup>-1</sup> in 2010 and between 55 and 68 kg ha<sup>-1</sup> in 2011. Inorganic N from Fertilizer and LSM decreased as the season progressed probably due to ammonia volatilization, denitrification and immobilization of soil nitrogen. The maximum mineralized N of 28 kg ha<sup>-1</sup> was measured from ABM amendments in loamy sand at 2010. Mineralized N of SBM amendments ranged from 41 to 48 kg ha<sup>-1</sup>, which was equivalent to 56 to 73 % of the added organic N under loamy sand in 2011. The fertilizer equivalence of SBM increased from 30 to 80 % as the growing season progressed due to the release of inorganic N through mineralization. In contrast, the fertilizer equivalence of LSM decreased from 88 to 60 % in amended loamy sand soils. The recovery of N from amended clay soil in 2010 was very poor as inorganic N decreased steadily, probably due to denitrification caused by very wet and waterlogged soil. This study showed that changes in soil moisture as influenced by the amount of precipitation, soil temperature and soil texture coupled with manure type, influenced the pattern of inorganic N release from manure.

#### **2.2 Introduction**

It is important to determine the amount of N that is supplied by manures for optimum crop production and maintenance of adjacent environment. Nitrogen (N) mineralization is the process of converting organic N to inorganic plant available N. Efforts are currently under way to develop a model for estimating available N in manure amended soils (Honeycutt et al. 2005). There are different components of these efforts to predict field based N mineralization data following addition of different types of animal manures. Thus, it is important to understand how various factors in the field influence the availability of N from manure (Palm et al. 2001 and Juan et al. 2009).

There is a need to provide farmers with accurate information to support them in selecting appropriate rates and time of using manures as a fertilizer to meet crop N needs while minimizing negative environmental impacts on soil and water quality. The rate of N mineralization could be defined as the fraction of the initial organic N that will become available within a particular time. If we could predict the amount of N release from manure it will serve as an efficient tool for manure management that will synchronize application with crop demand, thereby reducing the risk of N deficiency or environmental harm (Beraud et al. 2005).

To make the optimum use of manure as a fertilizer replacement we must know its fertilizer value during a growing season. If the amount of N release exceeds plant demand, then the N becomes susceptible to various pathways of loss, i.e. volatilization, denitrification, leaching and immobilization (Goulding 2004; Peoples et al. 2004). In some instances, manures can cause excess accumulation of  $NO_3^-$  (nitrate) in the soil (Khalil et al. 2005) with potentially detrimental effects on the environment. Conversely, if amount of N release is too slow, then crop yield may be reduced.

Mineralization of manure N is affected by several factors such as composition of manure (i.e. initial inorganic N, C:N ratio etc), soil temperature and soil water content, drying and rewetting events (Mooleki et al. 2004; Kruse et al. 2004), soil texture, and soil characteristics (Gordillo and Cabrera 1997). For example, liquid and solid manures vary in composition and thus may vary in their rate of release of available N (Rochette et al. 2006). Solid beef manure has a higher percentage of organic N which needs to be mineralized before crop use, slowing the release of available N. Conversely, liquid manure has a greater proportion of inorganic N which may provide readily available N for crops or may be susceptible to rapid loss right after application. A large contribution of mineralized N from manure may be subjected to various losses, depending upon soil type and soil climate. For example, in a coarse textured soil (i.e. sandy soil) excess precipitation may lead to N loss predominantly through NO<sub>3</sub><sup>-</sup> leaching due to low water holding capacity and high hydraulic conductivity. However, in a fine textured (i.e. clay soil), N loss may be through denitrification due to higher water retention capacity and low hydraulic conductivity of clay soil. Several studies have observed differences in N releases from different soils due to local soil environment (Chae and Tabatabai 1986; Collins et al. 1992; Whitmore and Groot 1997; Gordillo; Cabrea 1997; Thomson and Olsen 2000) and have therefore recommended that the local soil environment, especially

soil moisture and soil temperature, be considered when estimating the release of available N from manure or for increasing N-use efficiency (Hubbard et al. 2008; Watts et al. 2007). Manitoba's current recommendation to farmers that the amount of plant available N in manure is equal to its ammonium-N plus 25 % of its organic N (MAFRI 2005); ignoring any of the factors that influence mineralization is questionable.

A number of techniques have been used to estimate N mineralization from manures under field conditions (Travis et al. 2004). The most popular and updated method of anion exchange resin has been very helpful in improving our understanding of N manure mineralization under natural field conditions despite of its cost and labour intensiveness (Eghball 2000). Resin technique also allows rainfall and leaching to proceed naturally under field conditions (Bhogal et al. 1999). Thus this study aimed to use the promising resin technique to track N mineralization pattern (Griffin et al. 2008 and Honeycutt et al. 2005) and to study the feasibility of this technique in agricultural soils of Manitoba (MB).

Although some research results of mineralized manure N during specific periods in several soils are available in Canada (Drury et al. 2003; Beauchamp et al. 2004; Dharmakeerthi et al. 2005), these results cannot be directly applied to Manitoba conditions due to differences in soil, manure type and climate. This study was undertaken with a view to measure the inorganic N release and fertilizer equivalence from various locally available manures during the growing seasons in two typical soils in Manitoba. Study results should aid farmers to estimate more accurately the application rate of

manures based on available N during the growing season and their efficiencies compared with chemical fertilizer.

Abbreviations: ABM, Argyle beef manure; Fert, Fertilizer; AN, Ammonium Nitrate; CBM, Carman beef manure; DOY, Day of year; FC, Field Capacity; LSM, Liquid swine manure; MBM, Morden beef manure; NIN, Net inorganic nitrogen; NMN, Net mineralized nitrogen; SBM, Solid beef manure; TIN, Total inorganic nitrogen; WFPS, Water filled pore space.

#### 2.3 Materials and Methods

# 2.3.1 Amendments

Five soil amendments were used in this study: four types of manure (three solid beef cattle manure, liquid swine manure and fertilizer, i.e., urea or ammonium nitrate), including a control (unamended) treatment. Therefore, a total of six treatments were included in this study. The three solid beef cattle manures (SBM) were collected from various farms in Manitoba at Carman (CBM), Morden (MBM), Argyle (ABM) and a liquid swine manure (LSM) from Steinbach.

#### 2.3.2 Site information and soil characteristics

The study was carried out at two sites located at the University of Manitoba agricultural research farms at Glenlea and Carman. Routine analysis of soil samples at establishment included soil pH, soil texture, soil nutrient status (total N, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, total-C, and Olsen P etc.) and soil bulk density (Table 2.1). The soil pH was determined in 0.01 M CaCl<sub>2</sub> solution using a soil to solution ratio of 1:5. The soil particle size distribution was determined by the pipette method (Gee and Bauder 1996). All soil and amendment analyses were performed on a minimum of triplicate samples.

The chemical and physical properties of the soils used in this study as well as the amendments and their application rates are presented in Table 2.1 and Table 2.2.

Characteristics	Carman soil	Glenlea soil
Soil Series	Orthic Black Chernozem	Rego Black Chernozem
Soil pH	5.8	7.1
Soil texture	Loamy sand (Sand, Silt and	Clay (Sand, Silt and Clay-
	Clay-86.75%, 5.02% and	5.28%, 21.86% and 72.87%,
	8.23%, respectively)	respectively)
Bulk density	1.2 (15 cm depth)	1.0 (15 cm depth)
WHC	High (33 % on volume basis)	Very high (39 % on volume
		basis)
Drainage	Moderately to slow	Slow permeability
	permeability	
Organic matter	6.52%	7.7%
Runoff	Slow surface runoff	Slow surface runoff
Olsen P (mg kg <sup>-1</sup> )	20	21

Table 2.1 Characteristics of loamy sand at Carman and clay soil at Glenlea

Table 2.2 Manure characteristics and application rates at 2010 and 2011

Year-2010								
Treatment	Available N as NH <sup>+</sup> 4 (kg ha <sup>-1</sup> )	Available N as organic N (kg ha <sup>-1</sup> )	Total available N (kg ha <sup>-1</sup> )	Total C (%)	Total N (%)	C:N	Moisture (%)	
CBM	36.4	63.6	100	33.7	1.3	25.9	68	
MBM	32.2	67.8	100	25.7	1.2	21.4	60	
ABM	24.6	75.4	100	39.3	1.5	26.2	72	
LSM	96.4	3.6	100	35	4.8	7.3	98	
Year-2011								
CBM	34.1	65.9	100	34.9	2.7	13.1	81	
MBM	31.5	68.5	100	20.8	1.5	13.9	60	
ABM	26.1	73.9	100	36.8	1.7	22.3	79	
LSM	83.3	16.7	100	23.2	3.5	6.6	96	

#### 2.3.3 Amendment characterization

Total N in manure was determined by the Kjeldahl method. Organic N in manure was obtained by subtracting the ammonium N that was measured using the auto analyzer (Technicon Ind. Syst., Tarrytown, NY.) from the total Kjeldahl N. Total carbon was determined using high temperature combustion by Dohrmann Dc-190 carbon analyzer (Rosemount Analytical Inc., Orrville, OH) (Table 2.2)

# 2.3.4 Microplot cylinder construction and resin bag preparation

Cylinders were constructed from 3.8 cm radius PVC pipes with a wall thickness of 0.4 cm and a length of 17 cm. One end of each cylinder was marked as the top and measured down 2 cm from that end along the inside of the cylinder. Marks were placed at this point around the inside circumference of the cylinder to denote the top level of the soil after insertion. Anion exchange resin [J.T. Baker: A-554, Cl<sup>-</sup> from, Type 2, Beads (16-50 mesh)] bags were made out of polyester fabric, that was cut into round pieces each of which was 12.7 cm in diameter (Figure 2.1). Two pieces of polyester fabric were partially sewn together to form an open-ended bag with an inner diameter of 8.9 cm. Each bag was filled with 25 g (dry weight) of anion exchange resin and finally the small open end of each bag was sewed and the bags were stored in deionized water.



Figure 2.1 Diagram of in-situ soil PVC column

# 2.3.5 Cylinder installation

A block of wood was placed on top of each cylinder and driven into the soil with a hammer. The top edges of the PVC cylinder were positioned 2 cm above the soil surface to avoid capturing surface runoff. This space also allowed enough room for repacking the soil after incorporating the amendments. Cylinders were placed in blocks by sampling date, as this made it easier to know which cylinders to remove during sampling. Cylinders were installed in the field at both sites for about a month before application of amendments and installation of resin bags. This allowed the soil in the cylinder to settle following installation.

# 2.3.6 Resin bag installation

In the field, cylinders were excavated from each site to install resin bags at the bottom and to incorporate amendments on top of the soil. Cylinders were lifted carefully from the ground using a shovel to prevent spillage. The cylinder was inverted by placing a plastic board on top of the cylinder to install the resin bag. To install the resin bags 2 cm of soil from the bottom of each cylinder was removed and deionized water-saturated resin bag were placed in each cylinder. The purpose of the anion resin beads was to capture NO<sub>3</sub><sup>-</sup>-N that was leached through the soil column. A double layer of rubber band was used to hold the soil and resin bags in place at the bottom of each cylinder. Finally, cylinders were immediately returned to the same hole and inserted firmly, so that the cylinder base contacted the soil below to promote drainage. Soil around the cylinder was packed tightly to ensure good contact between outside cylinder wall and the surrounding soil.

#### 2.3.7 Incorporation of amendments

Manure was manually incorporated to ensure uniformity on June 14, 2010 (DOY 165) at Glenlea and on May 27, 2010 (DOY 147) and June 24, 2011 (DOY 175) at Carman, respectively. After the installation of resin bag, approximately 5 cm of surface soil was removed from the top of each cylinder into a ziploc bag containing appropriate amount of amendment to provide an application rate of 100 kg ha<sup>-1</sup> of available N (i.e., estimated as NH<sub>4</sub>-N + organic N \*0.25). The ziploc bag was sealed and the soil and the amendment were thoroughly mixed. Finally, the soil and amendment mixture was returned to the top of each cylinder. The soil from unamended cylinders was also excavated into ziploc bag, mixed and then returned to the cylinder in the same manner to create similar disturbance.

#### 2.3.8 Cylinder maintenance and monitoring soil temperature and soil moisture

The inside as well as surrounding of the cylinders was maintained in a fallow condition over the study period to avoid plant uptake of N and water. Fallow condition was maintained by applying herbicide (glyphosate), frequent inspection and handweeding of the column when necessary. Soil temperature and soil moisture content at 15 cm depth from designated cylinders were continuously monitored using two in-situ Stevens Hydra Probes (TM) at each site.

#### **2.3.9** Collection and processing of cylinder soil samples and resin bags

To estimate the available N in soil and resin, cylinders were collected from June 14 to October 18, 2010 (i.e., Day of year{DOY} 165 to 291) at Glenlea, May 27 to September 30, 2010 (i.e., DOY 147 to 276) and from June 24 to October 28, 2011 at Carman (i.e., DOY 175 to 301). The nine sampling dates following experiment initiation were 0, 7, 14, 28, 42, 56, 70, 98, and 126 days. These correspond to 0, 1, 2, 4, 6, 8, 10, 14, and 18 weeks after manure application. At each sampling period, a shovel was used to dig around each cylinder to carefully lift it from the ground. Care was taken to avoid dislodging the resin bag at the bottom of the cylinder, or throwing out any soil from top of the cylinders. The entire PVC cylinder with resin bags was covered with a ziploc bag at both ends to serve as a vapour barrier to minimize drying. The soil on the outside of the cylinder was carefully removed using a spatula and the cylinders were placed in an upright position in a cooler containing ice packs and transported to the lab for processing.

The resin bags were removed from the bottom of each cylinder and kept in a freezer until they were processed. Soil from the cylinder was removed and mixed thoroughly. A 50 g sub-sample of the mixed soil was taken for the measurement of soil inorganic N and gravimetric water content. Soil inorganic N concentration was measured by shaking 6 g of field moist soil for 30 min with 50 mL 2 M KCl on a wrist-action shaker. After filtering the extract using Whatman no. 42 filter paper, the NO<sub>3</sub>-N, NO<sub>2</sub> and NH<sup>+</sup><sub>4</sub>-N in the extract were determined by automated spectrophotometric method using Technicon auto analyzer II (Technicon Ind. Syst., Tarrytown, N.Y.). Gravimetric water content was determined using approximately 20 g of soil from each cylinder at 105 °C for 48 h. The resin bag was cut open and its content was emptied into a 500 mL container. The resin was extracted by shaking with 250 mL of 2 M KCl for 60 minutes. After filtering with Whatman no. 42 filter paper the extract was analyzed following the same procedure that was used for soil extracts. As this was an anion exchange resin, the extract was analyzed together for NO<sub>2</sub> and NO<sub>3</sub> When immediate extraction of the soils and resin was not feasible the intact columns were kept in a fridge at 4° C and then extracted within one week of sample collection. The initial sample taken at day 0 right after final installation of amended soil cylinders was processed immediately in the lab. There was no resin bag used for the cylinders from the first sampling date (day 0).

# 2.3.10 Calculation of net inorganic N (NIN), net mineralized N (NMN) and fertilizer equivalence of manures

(i) Net inorganic N (NIN), or available N was obtained as the sum of  $NH_4^+$ -N plus  $NO_3^-$ - $/NO_2^-$ -N in each soil after deducting the measures of inorganic N from the unamended soil at each sampling time.

(ii) Net N mineralization in amended soil was calculated using the following equation (Travis et al. 2004):

Where  $N_{min}$  refers net mineralized N in kg ha<sup>-1</sup>; (0) refers to initial time; t refers sampling time; A refers amendments; C refers control or unamended soil and IN refers inorganic N  $(NH_{4}^{+} + NO_{3}^{-} plus NO_{2}^{-})$  in soil plus resin in kg ha<sup>-1</sup>.

(iii) To express mineralization as a percentage of amendment organic N, the quantity of N mineralized at each extraction time was divided by the initial amount of organic N and the result was multiplied by 100.

Thus, %  $N_{min} = [N_{min(t)} / ON] * 100$ -----(2)

Where ON is the added organic N from manure

(iv) To obtain the fertilizer equivalence of manures, the net inorganic N (amendments inorganic N deducted from unamended soils) from manure amended soils was divided by the amount of net inorganic N from fertilizer amended soils at each sampling this was then multiplied by 100 to convert it to percentage. i.e.,

 $MN_{eq} = [NIN_m/NIN_f] * 100$ ------(3)

Where,  $MN_{eq}$  denotes manure nitrogen fertilizer equivalence,  $NIN_m$  and  $NIN_f$  denotes net inorganic N from manure and fertilizer, respectively.

Negative values indicated the disappearance of available N (i.e., volatilization, denitrification and or immobilization) among the amendments.

### 2.3.11 Experimental design and statistical analysis

Five soil amendments and a control unamended treatment were included in this study. Glenlea and Carman field studies were conducted as a RCBD with six treatments and four replicates for a total number of 24 experimental units. Microplot cylinder treatments consisted of 1 soil-site X 6 treatments X 4 replications X 9 sample dates for total of 216 experimental units. The same number of experimental units was maintained for both sites. Data from the field studies was analyzed using the PROC MIXED of SAS. The analyses of variance (ANOVA) were done with a factorial arrangement to examine the main effect of manure type, sampling period and their interaction on NIN, NMN and fertilizer equivalence. Manure type and sampling time were fixed variables whereas replication used as random variable. To test treatment factors main effects and interactions was accomplished using Fisher's significant difference (LSD) technique. All results were considered significantly different at P < 0.05 unless noted otherwise.

# 2.4 Results

Mineralization is a relatively slow microbial process that is influenced by a number of factors. The major challenge of using manure as fertilizer is the uncertainty of its fertilizer equivalence. In Manitoba, manure is generally applied based on the amount available N assuming that the inorganic N and 25 % of organic N will be available in the year of application. The study included two soil types and locally available manures to estimate the fertilizer value of manures in order to allow us to examine the effect of soil type and manure characteristics on the release of inorganic N from manures.

# 2.4.1 Precipitation and soil moisture

At Glenlea in 2010, measurable precipitation occurred on 38 days out of the 126 days of the study (Figure 2.2). A major precipitation event occurred on 243 DOY (August 31) when 51 mm of precipitation was recorded. The frequent precipitation in 2010 and slow infiltration rate of the clay soil at Glenlea caused extensive waterlogged conditions for most of the study period, except during the initial week. During the study period, Glenlea soil received 370 mm of precipitation. As such, the volumetric water content was in the range of 36 to 51 % and was, for the most part, greater than the field capacity of 39 % during the study period (Table 2.3). The greatest volumetric water content of 51 % was measured in the laboratory on 263 DOY (September 20, 2010) following a number of precipitation events including the greatest amount of precipitation in a single day 51 mm (i.e., 243 DOY).



Figure 2.2 Soil moisture and precipitation at Glenlea during the 2010 growing season

Rainfall in 2010 was more frequent at Carman than at Glenlea, as measurable precipitation occurred at Carman on 65 days during the 126 day study period (Figure 2.3). Two major precipitation events were recorded on 232 and 249 DOY (i.e., August 13 and August 30) when more than 55 mm of precipitation was received, causing the soil to become waterlogged until the end of the study. Total precipitation during the study period at Carman was 526 mm and measured volumetric water content ranged between 14 to 36 % (Table 2.3).

Sampling day	Glenlea-2010		Carman-2010			Carman-2011			
					%			%	
	Θ	% FC	WFPS	Θ	FC	WFPS	Θ	FC	WFPS
0	35.5	91.0	60.69	25.7	77.9	47.0	43.3	131.2	79.1
7	47.6	122.0	81.38	20.5	62.2	37.5	32.3	97.8	59.0
14	45.13	115.7	77.16	32.6	98.7	59.5	29.4	89.0	53.6
28	44.21	113.3	75.58	25.7	77.7	46.9	26.3	79.6	48.0
42	46.4	119.0	79.33	28.7	87	52.5	25.2	76.4	46.0
56	41.19	105.6	70.42	19.1	57.7	34.8	23.4	70.8	42.7
70	44.29	113.5	75.72	14.0	42.2	25.5	21.6	65.5	39.5
98	51.44	132.0	87.95	20.4	61.7	37.2	23.7	72.0	43.4
126	47.12	120.8	80.56	35.95	109.0	65.7	24.6	74.5	45.0

Table 2.3 Measured volumetric moisture content ( $\Theta$ ), percentage of field capacity (% FC) and percentage water filled pore space (WFPS) at each sampling time

Note: Field capacity of clay soil at Glenlea and loamy soil at Carman was 39 and 33 % (on volume basis), and the bulk density of the top 15 cm soil was 1.1 and 1.2 g cm<sup>-3</sup> respectively.



Figure 2.3 Soil moisture and precipitation at Carman during the 2010 growing season

The study at Glenlea was very difficult to handle throughout the growing period due to waterlogged clay soil caused by a combination of a wet season and a soil with low infiltration rate. Thus, the study was repeated on only the loamy sand soil at Carman in 2011. The mean measured volumetric water contents at different sampling dates in relation to soil water at field capacity and water filled pore space are presented in Table 2.3

The year 2011 was drier than 2010 at Carman as measurable precipitation occurred on only 29 days and the total amount of precipitation during the study period was 169 mm (Figure 2.4). Only one major precipitation event of 68 mm was recorded on DOY 263 (i.e. August 29, 2011). The maximum volumetric moisture content of 43 % was recorded initially, due to a precipitation of 44 mm immediately after amended cylinder installation and for the rest of the study period volumetric water content ranged from 22 to 32 % (Table 2.3).



Figure 2.4 Soil moisture and precipitation at Carman during the 2011 growing season

In summary, the clay soil at Glenlea had higher soil moisture and it rose above the FC during the whole study period in 2010. The soil moisture of the Loamy sand at Carman ranged between 42 to 98 % of FC during the growing season of 2010. Although there were more precipitation events at Carman in 2010, nevertheless, the loamy sand soil experienced dry conditions a few times during the growing season. On the other hand, in 2011 at Carman, the soil moisture was always below FC except at the initial sampling day due to a precipitation event right after the incorporation of the amendments which raised the initial soil moisture above FC.

# **2.4.2 Soil Temperature**

Daily mean soil temperature at Glenlea ranged from 2.5 to 27.4 °C during the growing season in 2010. Soil temperature in the initial 80 days ranged between 12 to 27 °C with temperature remaining above 20 °C during a 30 day period. Thereafter soil temperature declined to 2.5 °C by fall (Figure 2.5a).

Mean soil temperature at Carman during the study period of 2010 ranged from 8 °C to 28 °C (Figure 2.5a). The initial soil temperature ranged between 12 to 18 °C; thereafter, it stayed within a range of 10 to 25 °C for about two months and then declined steadily to 5.4 °C at the last sampling period. In 2011, the mean average soil temperature ranged between 5 to 27 °C, with soil temperatures exceeding 20 °C during the initial 70 days. Thereafter, soil temperature declined steadily into the fall season. However, at Carman in 2011 soil temperature was less variable compared to year 2010.



Figure 2.5 Mean soil temperature at depth of 15 cm measured by soil probes during the study period in 2010 (a) and 2011(b)

#### 2.4.3 Net inorganic N release in clay soil at Glenlea in 2010

Though the estimated amount of available N applied to each treatment was equivalent for all the amendments the amounts of  $NO_3^-N$ ,  $NO_2^-N$ ,  $NH_4^+-N$  and organic N were different due to compositional differences of the amendments. The NIN and

NMN of manure amendments was significantly affected by manure type but not affected by sampling period. However, the interaction effect of sampling period and manure type was significant (Table 2.4). High proportions of NH<sub>4</sub><sup>+</sup>-N were measured initially for each amendment compared to the other sampling dates. Thereafter, very small amount of accumulated NH<sub>4</sub><sup>+</sup>-N were measured over the study period in heavy clay at Glenlea. Mean NH<sub>4</sub><sup>+</sup>-N concentrations of AN and LSM amended soils were initially greater than those of the SBM amended soils (Figure: A 2.2). Net NO<sub>3</sub><sup>-</sup>-N concentrations in the amended soil at Glenlea were small initially and increased to a maximum of 6 kg ha<sup>-1</sup> for the SBM amended soils at the 6th sampling period (i.e. DOY 207, Figure: A 2.2). The mean NO<sub>3</sub><sup>-</sup>-N concentrations were greatest in the soil amended with AN and thereafter LSM amended soils at each sampling period.

Mean NIN concentrations were initially greater in the soils that received AN and LSM than those that received SBM. Thereafter, by 14d after treatment application (DOY 179) there was a rapid decrease in the mean NIN of AN and LSM amended soil and a further decline during the next four weeks (up to DOY 207) before reaching a plateau till the end of the study period (Figure 2.6a).

Effect	Degree	Net	Net	Fertilizer
	of	inorganic	mineralized	equivalence
	freedom	N, NIN	N, NMN	(Pr > F)
	(DF)	(Pr > F)	(Pr > F)	
Glenlea-2010				
Manure amendments (M)	3	<.0001	0.0003	<.0001
Sampling period (T)	8	0.0039	0.0015	0.3167
M*T	24	0.0032	0.0013	0.0212
Carman-2010				
Manure amendments (M)	3	<.0001	0.0048	<.0001
Sampling period (T)	8	<.0001	0.0049	<.0001
M*T	24	0.0008	<.0001	0.0019
Carman-2011				
Manure amendments (M)	3	0.0007	<.0001	0.0301
Sampling period (T)	8	<.0001	0.0002	<.0001
M*T	24	<.0001	<.0001	0.0005

 Table 2.4: Type 3 Tests of Fixed Effects on NIN, NMN and fertilizer equivalence of manure amendments during the field studies



Figure 2.6 Net inorganic N (a) and net mineralized N (b) at Glenlea during the 2010 growing season

The inorganic N declined continuously during the growing season at Glenlea such that at the end of the study there was less than 20 kg ha<sup>-1</sup> of NIN in these treatments (Figure 2.6a).

At the end of the study period only 14 and 7 kg ha<sup>-1</sup> of NIN was measured from AN and LSM amended soils whereas only 1 to 3 kg ha<sup>-1</sup> of inorganic was measured in the SBM amended soils (Figure 2.6a). The calculated NMN values were negative due to the smaller amount of NIN during the growing season in comparison to the initial amount of NIN. The NMN of -19, and -45 kg ha<sup>-1</sup> was estimated for SBM and LSM amended soils, respectively at Glenlea (Figure 2.6b), representing a loss of nitrogen from the soil system through immobilization or more likely through denitrification. However, the calculated NMN values from SBM amended soils were positive at initially but it was negative at the end of the study period from among the amended soils.

# 2.4.4 Net inorganic N release in loamy sand at Carman in 2010

The NIN and NMN of manure amendments significantly affected by manure type, sampling period and their interaction (Table 2.4). The mean concentration of  $NH_4^+$ -N in amended and unamended soils was different within the first 3 weeks but was not much different between 4 to 8 weeks (i.e., DOY 175 to 217). Mean concentration of  $NH_4^+$ -N in amended soil was greater than unamended soil from 10 to 14 weeks of the study period (Figure: A 2.3). Initially, there were less mean NO<sub>3</sub>-N concentration among the amended soils but the unamended soil had greater NO<sub>3</sub>-N concentration than the unamended soils. Thereafter, a dramatic accumulation of  $NO_3^-$ -N was observed in the amended soils indicating the conversion of the added ammonium to nitrate by nitrification (Figure: A 2.3). Maximum net  $NO_3^-$ -N concentration was obtained at the 6th week (DOY 196) from SBM amended soils and the greatest accumulation of 74 kg ha<sup>-1</sup> mean net  $NO_3^-$ -N was obtained from

Urea amended soils. Among the SBM the greatest amount of 38 kg ha<sup>-1</sup> mean net  $NO_3$ -N was measured from MBM amended soils.



Figure 2.7 Net inorganic N (a) and net mineralized N (b) at Carman during the 2010 growing season

In the first 4 weeks of the study period, mean NIN concentrations were greater where LSM and Urea were applied than those of SBM amended soils. This was due to the  $NH_4^+$ -N fraction of TIN being greater where LSM and Urea were incorporated. However,

the greatest NIN (38 kg ha<sup>-1</sup>) was measured at week 6 among the SBM amended soils (Figure 2.7a). The NIN from SBM initially increased during the first week but declined in the second week, thereafter, NIN increased steadily and peaked at 196 DOY. The highest amount of 28 kg ha<sup>-1</sup> mineralized N was measured from ABM at week 8, which was about 37 % of the organic N. The NMN from LSM was always negative due to the higher amount of initial inorganic N (Figure 2.7b).

#### 2.4.5 Net inorganic N release in loamy sand at Carman in 2011

The NIN and NMN of manure amendments significantly affected by manure type, sampling period and their interaction (Table 2.4). Greater amounts of NH4+-N was measured in the amended soils than in the unamended soils during the study period. The initial mean concentration of NH4<sup>+</sup>-N was greater in AN and LSM amended soils than SBM amended soils during the first 6 weeks of the study period. Although there were no initial differences in the concentration of NH4+-N among SBM amended soils within initial 6 weeks, remarkable differences were observed from 8 to 14 weeks of the study period. In general, mean  $NH_4^+$ -N concentrations in the amended soils declined during the study period (Figure: A 2.4). However, mean NH<sub>4</sub><sup>+</sup>-N concentrations in AN and LSM amended soils declined throughout the study period (Figure: A 2.4) similar to the results obtained at Carman and Glenlea during the 2010 growing season. The maximum amount of net NO<sub>3</sub><sup>-</sup>N at week 6 was 65 kg ha<sup>-1</sup> from AN and 60 kg ha<sup>-1</sup> at week 10 from SBM amended soils. However, the smallest amount of net  $NO_3^{-}N$  (52 kg ha<sup>-1</sup>) was measured in LSM amended soil (Figure: A 2.4). The TIN and NIN were dominated by the amount of NO<sub>3</sub><sup>-</sup>N and followed the same pattern as observed for the net NO<sub>3</sub><sup>-</sup>N accumulation. The greatest amount of NIN from SBM was measured after 10 weeks of the study and it ranged between 55 to 68 kg ha<sup>-1</sup> for ABM and CBM amended soils, respectively, thereafter, NIN declined steadily to the end of the study period (Figure 2.8a). Similar to the results obtained in 2010, estimated NIN from AN and LSM amended soils declined throughout the study period from an initial value of 83 and 75 kg ha<sup>-1</sup>, respectively to a final value of about 32 kg ha<sup>-1</sup>.

Net mineralized N from SBM amended soils ranged from 41 to 48 kg ha<sup>-1</sup> at week 10 in ABM and CBM amended soils. The NMN from SBM was about 56 to 73 % of the added organic nitrogen. The NMN from SBM was not greater from that of the LSM during the initial 6 weeks. Thereafter, mineralized N was greater in the SBM than in the LSM amended soils (Figure 2.8b). However, the net mineralized N from LSM was always negative considering the initially high amount of inorganic N similar to AN amended soil. For the LSM and AN amended soils, negative net mineralization was observed indicating that the rapid conversion of ammonium N to nitrate was followed by immobilization or denitrification of available nitrogen as the inorganic N declines throughout the growing season in LSM and AN.


Figure 2.8 Net inorganic N (a) and net mineralized N (b) at Carman during the 2011 growing season

# 2.4.6 Fertilizer equivalence of manures

The fertilizer equivalence of manure amendments during the growing season 2010 in clay soils at Glenlea was significantly affected by manure type but not affected by sampling period. However, the interaction effect of sampling period and manure type affected significantly (Table 2.4). Thus, the estimated fertilizer equivalence also showed significant variation particularly between the LSM and among the SBM initially during the growing season (Figure 2.9). However, the variations become not significant as study progressed till 193 to 235 DOY, thereafter the fertilizer equivalence becomes varied significantly between LSM and SBM amended soils.



Figure 2.9 Fertilizer equivalence of applied manures at Glenlea during the 2010 growing Season (Standard error, SE bars equal  $\pm$  9.14)

The fertilizer equivalence in Glenlea at each sampling period was below 50 % indicating that the added manure N was not as available as urea-N. Sold beef manure (SBM) is the combination of feces, manure, bedding materials, wasted feed and water. The average C: N ratio of SBM was 24.5, the fertilizer equivalence of SBM increased from an average of 10 to 40 % over the duration of the study. In contrast, the fertilizer

equivalence of LSM (i.e., C: N ratio of 7.3) decreased from an initial value of 78 % to a final value of 52 % over the study period (Figure 2.9).

The fertilizer equivalence of manure amendments at Carman during the growing season 2010 and 2011, significantly affected by manure type, sampling period and their interaction (Table 2.4). In 2010, there was a significant difference in fertilizer equivalence of SBM and LSM amended soils initially but the difference becomes not significant as the study progressed (i.e., after 189 DOY or 6 wk after incorporation of amendments). The fertilizer equivalence of SBM followed an increasing pattern, with an initial average value of 15 % which increased steadily to a maximum of 77 % between 189 to 245 DOY (Figure 2.10). The LSM amended soils showed opposite pattern of decreasing fertilizer equivalence which was 77 % initially and declined to 58 % at the end of study (Figure 2.10).



Figure 2.10 Fertilizer equivalence of applied manures at Carman during the 2010 growing season (SE bars equal  $\pm$  9.77)

In 2011 at Carman, there was a significant difference in fertilizer equivalence of SBM and LSM amended soils initially but the difference becomes not significant as the study progressed except 245 DOY or 10 wk after incorporation of amendments (Figure 2.11). The fertilizer equivalence of SBM (Figure 2.11) during the growing season at Carman in 2011 followed an increasing trend, with an initial value of 26 to 34 % which increased to above 80 % from ABM and MBM amended soils. However, LSM amended soils showed the opposite pattern of decreasing fertilizer equivalence which was 88 % at initially and declined to 63 % at the end of the study period.



Figure 2.11 Fertilizer equivalence of applied manure during the 2011 growing season at Carman (SE bars equal  $\pm$  9.21)

# **2.5 Discussion**

During the 2010 growing season, there was almost no any available N either in the clay soil or resin at Glenlea due to excessive wetness. The excessive wetness of the clay soil in 2010 probably increased denitrification leading to loss of all nitrate. From the results of our study on the clay soil at Glenlea during the wet 2010 growing season we concluded that anion exchange resin method may not be feasible for measuring mineralization on heavy clay soils. Therefore, in 2011, the study was limited to the loamy sand soil at Carman. Although the discussion considers all three site years, emphasis will be placed on the two consecutive field seasons in 2010 and 2011 using loamy sand soil at Carman.

# 2.5.1 Precipitation and soil moisture

Fluctuations in soil moisture content were observed corresponding to multiple rainfall events, which has been not defined statistically but the physical explanation has been discussed in this section for this phenomenon. Comparison of the precipitation patterns between the two sites showed that the Glenlea soil had received moderate rainfall events in 2010 and a greater amount of rainfall at Carman in 2010 than in 2011 (Figure 2.2, Figure 2.3 and Figure 2.4). However, despite less rainfall at Glenlea, the slower infiltration rate of the clay soil caused greater volumetric moisture contents throughout the study period. Conversely, the loamy sand soil at Carman had a faster infiltration rate which caused drier surface soil conditions at Carman than at Glenlea. However, both sites

were relatively wet in 2010 due to greater amount of precipitation than at Carman in 2011.

From the above comparison, one of the remarkable causes of greater mineralization on the loamy sand at Carman during the growing season in 2011 compared to 2010 may be due to the moderate soil water content which rarely exceeded its FC (i.e., for most of the study period soil water ranged between 66 to 80 % FC). Coppens et al. (2006) reported that the difference in decomposition between surface and incorporated organic residues is largely determined by the water content of the residues and mineralization of organic materials generally increases with increasing moisture content up to field capacity and then declines. However, soil moisture content can also significantly impact nitrification and NMN (Sierra 1997; Paul et al. 2003; Agehara et al. 2005), with the maximum NMN occurring when soil moisture is near its field capacity (Stanford and Epstein 1974).

The results of our study agree with those reported by Alexander (1977) that the optimum soil moisture content ranged from 55 to 70 % of field capacity due to influence soil water on mass transfer rates of oxygen and substrate degradation. Linn and Doran (1984) and Franzluebbers (1999) reported that maximum aerobic microbial activity occurred at soil moisture levels between 50 and 70 % of water holding capacity (WHC). On the other hand, low soil moisture inhibits microbial activity by reducing diffusion of soluble substrates (Schjønning et al. 2003), microbial mobility (Killham et al. 1993) and intracellular water potential (Stark and Firestone 1995). Stottlemyer and Toczydlowski

(1999) also reported higher gross N mineralization rates at around 100 % FC than in treatments with less soil water content due to the optimum microbial activity with good soil aeration.

#### **2.5.2 Soil Temperature**

The study was conducted during summer, and there were frequent fluctuations in soil temperature in both years at Carman and Glenlea (Figure 2.5). The fluctuation in soil temperature has been not defined statistically but the physical explanation has been discussed for this phenomenon in this section. The soil temperature at Glenlea during the initial two months (Calendar day 165 to 235) was optimal for mineralization processes; this was followed by temperatures below 20 °C from 70 to 126d (217 to 301 Calendar days) due to long period of water logged condition. The final two months had low soil temperatures due to water logged soil as well as the onset of fall. This probably accounted for the low mineralization due to slow decomposition of the added manures at Glenlea under low soil temperature (Paul 2001). At Carman, soil temperature fluctuations were greater in 2010 than in 2011. The soil temperature distribution at Carman in 2011, especially from 6 to 10 weeks was suitable for mineralization and this was reflected in the maximum mineralized N of 48, 45 and 41 kg ha<sup>-1</sup> from CBM, MBM and ABM amended soils, respectively.

Part of the lower estimated NIN in the clay soil at Glenlea as compared to the loamy sand soil at Carman soil could have been related to lower temperatures, since microbial activity increases with soil temperature. Increasing temperature enhances mineralization (Eghball 2000) by stimulating microbial activity and accelerating diffusion of soluble substrates in soil (Nicolardot et al. 1994; MacDonald et al. 1995; Zak et al. 1999). Griffin and Honeycutt (2000) incubated soil amended with dairy, poultry, and swine manures for 112 d, and found that increasing temperature from 10 to 24 °C significantly accelerated the mineralization rate. Stark (1996) reported 30 °C to 35 °C as the range for maximal nitrification rates, while Grundman et al. (1995) found optimal temperatures to be 20 °C to 25 °C. No such consistent statements can be made for net mineralization or ammonification (Dalias et al. 2002). Net mineralizations (Cookson et al. 2002; Dalias et al. 2002). Consequently, elevated temperatures manipulated with different warming facilities in the field are reported to simulate NMN in various biomes across the world (Rustad et al. 2001; Shaw and Harte. 2001; Melillo et al. 2002).

The overall NMN patterns in our field studies are similar to that reported by Wang et al. (2003) who showed that the patterns of mineral N accumulation is linear as observed in clay soil and curvilinear as was observed in loamy sand soil. The same study also reported that at higher temperatures the readily mineralizable organic matter was consumed faster and N mineralization gradually slowed down due to limiting substrate availability.

# 2.5.3 Net inorganic N release in clay soil at Glenlea-2010

The gradually decrease of inorganic N in amended clay soils during the study period may be due to the rapid loss of  $NO_3$ -N through denitrification. There was small

amount of accumulated NH<sub>4</sub><sup>+</sup>-N measured during the whole study period especially from SBM amended soils probably due to mineralized N. Thomsen and Olsen (2000) also reported that net mineralization of organic N after 266 days of aerobic incubation was positive of 16 % and during anaerobic incubation, the values were negative due to 30 % of applied inorganic N was immobilized from various animal manures. It was difficult to calculate the NMN from the wet clay soil at Glenlea due to greater loss compared to initial inorganic N in the amended soils (Figure 2.6). The reason for this was probably the combination of heavy clay soil with lower infiltration that restricted NO<sub>3</sub>-N movement to the resin. Moreover, wet conditions of the soil at Glenlea probably have promoted denitrification resulting in the disappearance of nitrate. A number of studies have shown that there is an important interaction between temperature and water content with regard to N mineralization (Goncalves and Carlyle 1994; Sierra 1997; Knoepp and Swank 2002). It is critical to study interactions of temperature and soil moisture on NMN especially with the concurrent variations in soil temperature, precipitation, source of N and time in the field (Agehara and Warncke 2005).

#### 2.5.4 Net inorganic N released in loamy soil at Carman-2010

The observed pattern of NIN release from SBM amended soil was characterized by an initial period of NMN, followed by immobilization, and then, by a period of increasing net N mineralization (Figure 2.7). Moderately high soil temperature (above 20 °C) during the middle of growing season (6 to 10 weeks) and moderate soil moisture (25 to 53 % WFPS or 42 to 87 % FC, Table 2.3) under loamy soil at Carman influenced NMN from SBM amended soils. However, the decrease in NIN from SBM through immobilization may be due to the utilization of labile carbon in the beef manure amended soil. Similar pattern of N release was also observed during aerobic incubation of manure amended soils under laboratory conditions (Calderon et al. 2004; Probert et al. 2005).

#### 2.5.5 Net inorganic N released in loamy soil at Carman-2011

Initially there was an immobilization phase or loss of N in the SBM amended soil and rest of the study period showed a similar trend as observed during the growing season of 2010 (Figure 2.8). The initial decreasing phase of inorganic N at Carman during the 2011 growing season could be due to the 40 mm precipitation that was received immediately after incorporation of amendments (Figure 2.4), which might have resulted loss of NO<sub>3</sub>-N by denitrification. The disappearance of NH<sub>4</sub><sup>+</sup>-N was followed by an increase in NO<sub>3</sub>-N especially from SBM amended soils signified mineralization. The NMN in 2011 was greater than that measured in 2010. This was due to a more conducive environment for mineralization in 2011 than in 2010 as the soil was warmer and not excessively wet and warmer in 2011 compared to 2010. The maximum NMN among SBM amended soils between 6 to 10 weeks after addition of amendments, coincided with periods of warm temperatures (>20 °C) and moderate moisture contents (39.5 to 59 % WFPS). This is consistent with the results obtained by Griffin et al. (2002) who found that optimum mineralization and nitrification occurred between 20 to 30 °C and soil moisture content at 50 to 60 % WFPS. The release of NMN from the SBM is consistent with the study by Van Kessel et al. (2000) who reported that the proportion of manure organic N mineralized during an aerobic incubation varied widely, from -29 % (net immobilization) to 55 % (net mineralization). Other studies have also shown that mineralization of organic N from cow manure in the first year of application is highly variable, ranging from 0 to 50 % (Kirchman and Lundvall 1993; Paul and Beauchamp 1994; Serna and Pomares 1991). Another important factor influencing the greater mineralized manure N in 2011 could be the lower C: N ratio of SBM in 2011 compared to 2010 (Table 2.2). As Chadwick et al. (2000) showed that manure C: N ratio accounted for 40 % variation in the amount of mineralized N. Other studies also reported that as the C: N decreases, the percentage of lignin also typically decreases, and the amount of manure that is rapidly decomposed in soil increases (Ajwa and Tabatabai 1994; Van Kessel et al. 2000). The N dynamics with the addition of SBM is characterized by the initial disappearance or loss of inorganic N perhaps due to immobilization followed by a gradual increase of inorganic N due to NMN and finally a net immobilization or denitrification toward the end of the growing season as the inorganic N declined following the midseason peak.

# 2.5.6 Fertilizer equivalence of manures

The study included fertilizer for comparison with manure treatments and to calculate the fertilizer equivalence of manures. Calculated values of fertilizer equivalence from SBM amended soils was initially small, an evidence of an initial immobilization or a lag in mineralization. As the growing season progressed the fertilizer equivalence of SBM increased with increasing release of inorganic N through mineralization (Figure 2.9, 2.10 and 2.11). Overall, the maximum fertilizer equivalence of about 80 % as ammonium nitrate or urea was measured in the SBM amended soils between 6 to 10 weeks of the growing season, reflecting increased net mineralization during that period. Mineralized N

from LSM was difficult to detect compared to decline in available N at each sampling period. However, the initial fertilizer equivalence of LSM was above 80 % and this declined as the season progressed to a final value of about 60 %. As such, the study showed that net mineralization of nitrogen from LSM is questionable in these soils. This information is also useful in understanding the potential rate of mineralization and dynamics of N from applied LSM under different climatic condition. The initial fertilizer recovery of LSM and fertilizer at the time of application was below 100 %. This may be due to a loss of ammonium by volatilization and/or immobilization from LSM (i.e., presence of organic N fraction). The easily decomposed carbon can also stimulate denitrification of nitrate or immobilization of both nitrate and ammonium. These findings support earlier study that N release depends on soil type and chemical composition of the manure (Mubarak et al. 2010).

#### **2.6 Conclusions**

Reliable estimates of net N mineralization of manure during the growing season are needed to improve manure recommendation and minimize leaching loss to ground water. Field studies were affected by excessive precipitation during the study period in 2010 due to a number of major rainfall events during the study period which caused waterlogged conditions for most of the study period in the clay soil at Glenlea and a part of the growing season at Carman. In 2011 the loamy sand soil was comparatively dry due to lower precipitation and the volumetric soil moisture content was lower than in 2010.

Timing of maximum NMN in the loamy sand at Carman occurred at the same time in both years but its magnitude differed due predominantly to soil moisture and soil temperature differences. In 2010, only 10 to 28 kg ha<sup>-1</sup> of NMN was measured and which was equivalent of 15 to 40 % of the supplied organic N from SBM amendments. The maximum NMN from SBM was obtained between 6 to 10 weeks, which ranged between 41 to 46 kg ha<sup>-1</sup> or 56 to 73 % of the added organic nitrogen in the loamy sand at Carman in 2011. In the clay soil in 2010, none of the amendments including urea accumulated significant amount of either nitrate or ammonium most likely due to intense denitrification under wet conditions with available C for the denitrifiers supplied by manures. The NMN from LSM was negative and followed the same trend as fertilizer due to continuous loss of inorganic N. More importantly the study confirmed that fertilizer equivalence of LSM was initially above 80 % but declined to about 60 % due to the loss of available N from LSM. The fertilizer equivalence of SBM amendments was increased as the growing season progressed but there is concern of initially low N availability from SBM amended soils. The result from Glenlea was not surprising because precipitation was frequent and sustained period of waterlogged condition was observed during the study. It may be concluded from the study that there is a high probability that NMN will be significantly slower in clay soils than on loamy sands. The study observed that in combination with soil texture, soil moisture and soil temperature differences are responsible for observed differences in NMN between the two soils and farmers need to take this into consideration when planning manure applications. The study results raised an important question on the current method for calculating available N from manures as it showed that the present application rate of manure is not equivalent to nitrogen fertilizer. Moreover, the application rate of SBM could supply reasonable amount of available N initially. There is thus a need to revise the traditional application rate of manure for better crop production.

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# 3. RELEASE OF NITROGEN FROM SOLID BEEF AND LIQUID SWINE MANURES DURING LABORATORY INCUBATION

# **3.1 Abstract**

Manure nitrogen (N) comes in both organic and inorganic forms. The inorganic N, ammonium  $(NH_4^+)$  and nitrate  $(NO_3^-)$ , are readily available to plants while the organic form has to be converted to an inorganic form through the process of mineralization before it can be used by the plant. However, the organic and inorganic N fractions of manures vary with manure type (i.e. solid and liquid). Farmers need to know the amount of available nitrogen in manure and the commercial fertilizer equivalence of manure for proper rate and timing of manure application. Although the potential influence of soil temperature and moisture and their interactions on manure mineralization is recognized. there is conflicting evidence in the literature concerning the role of these two environmental factors. There is therefore a need to improve our understanding of N mineralization from various manures and how this is influenced by soil temperature and moisture as a means for making better recommendation and attaining better synchrony between manure available N supply and crop demand. A laboratory incubation study was therefore conducted to test the influence of soil temperature and soil moisture on N mineralization of different types of manure. The study was a split-split plot design using amendments of three solid beef manures (SBM), liquid swine manure (LSM), ammonium nitrate (AN) and an unamended soil. Air dried loamy sand surface soil (0-15 cm) was incubated after mixing with manures and fertilizer at an equivalent rate of 100 kg ha<sup>-1</sup> of available N (i.e., estimated as NH<sub>4</sub>-N + organic N \*0.25). The amended soils were incubated under 20, 40, 60 and 80 % water filled pore space (WFPS) at 14 and 21 °C to represent typical soil moisture and soil temperature conditions during the local growing season. Soil moisture content, NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N concentrations of amended and unamended soils were determined at weekly, biweekly and monthly intervals up to 126 days from treatment application. Soil moisture, manure type and incubation period and their interaction significantly (p<0.01) influenced net inorganic N (NIN), net mineralized manure N (NMN) and fertilizer equivalence of manures. The greatest amount of mineralized N as well as fertilizer equivalence of manures was obtained at 21 °C under 40 % of WFPS in SBM amended soils.

Accumulation of NIN increased under 20 and 40 % WFPS and declined at 60 and 80 % WFPS. Though there were no significant differences among SBM amended soils, the maximum amounts of net mineralized N were 64, 52, and 50 kg ha<sup>-1</sup> from MBM, CBM, and ABM amendments, respectively. This corresponded to a release of 73, 56, and 54 % of organic N in these manures, respectively. The fertilizer equivalence of SBM's increased from 3 % immediately after additions to 82 % at the peak of manure mineralization whereas the fertilizer equivalence of LSM decreased from 88 to about 50 % over the same period.

# **3.2 Introduction**

Although manure contains N in inorganic and organic forms, N assimilated by plants is derived only from inorganic N pools of ammonium  $(NH_4^+)$  and nitrate  $(NO_3^-)$ . However, inorganic N is also generated through the biological process of mineralization from organic N pools. The amount of inorganic and organic N as well as mineralized N varies with manure types. For example, manure containing 50 % of total N as ammonium N might have, on average, 65 % of fertilizer (i.e. urea) equivalent N (Schoenau et al. 2000). The pattern of net N release through mineralization of manure and its fertilizer value in a growing season is often overlooked.

For solid manure, a substantial part of N is in the organic form that mineralizes slowly and gradually over several years (Sorensen et al. 1994). Liquid manure contains a large fraction of N as  $NH_4^+$ -N that may rapidly supply the plant available N or be subjected to significant immobilization following application due to the presence of easily decomposable organic compounds (Sorensen and Jensen 1995). This phase of net immobilization may last for several months. However, most of the routine soil tests used to estimate available N from previously applied manure have proven inadequate to estimate the mineralizable N over the growing season (Campbell et al. 1995). An accurate estimate of plant-available N in manure is needed to maximize benefit to the crop and to minimize undesirable losses from soils.

Laboratory manure N mineralization experiments are often conducted under ideal conditions (Dalias et al. 2002; Collins and Allinson 2002; Zinati et al. 2007) and are therefore often poorly correlated with mineralization in the field (Wienhold 2007). Quantitative information on the mineralization of manure is fundamental for a better understanding of available N supply, to synchronize soil N mineralization to plant demand, and to develop sound recommendations for efficient management of manure (Campbell et al. 1995; Haney et al. 2001; Tilman et al. 2002; Gutser et al. 2005; Yousif 2009; Ma et al. 1999). Furthermore, estimation of mineralized N can ensure an accurate rate and time of manure application avoiding the detrimental effects on both plant and environment.

The independent effects of soil temperature and soil moisture content on manure N mineralization in soil have been widely studied (Goncalves and Carlyle 1994; Agehara and Warncke 2005; Griffin 2007) often with conflicting results that vary with manure type, soil and season (Wong and Nortcliff 1995; Eneji et al. 2002; Deenik 2006). Most of the studies confirmed that the optimum conditions for N mineralization occurs at a soil temperature between 25 and 35 °C (Nicolardot et al. 1994; Stark and Firestone 1996) and when soil moisture is near field capacity (Stanford and Epstein 1974). However, little is known about the influence of the interactions of soil temperature and moisture content on the N mineralization process (Agehara and Warncke 2005). A better understanding of the effects of soil temperature, soil water availability, and their interactions on net N mineralization in soils will facilitate matching the release of manure N to crop demand

with environmentally and agronomically sound management in agriculture (Sorensen 2001; Beraud et al. 2005).

There is limited information on manure N mineralization and its fertilizer value in MB soils. This dearth of information warrants detailed study using different local manures to examine N mineralization as affected by local soil types and environments. Such information will help to match crop N demands with supply of N from manure (Beauchamp et al. 2004) and to manage N more efficiently under MB soil and environmental conditions. However, fluctuation in soil moisture and temperature is typical under natural field soil environment. Hence, to measure the response of manure N mineralization to soil moisture and soil temperature, a split-split plot incubation study was carried out using various local manures at two different temperatures of 14 and 21 °C and at 20, 40, 60, and 80 % WFPS.

The specific objectives of this study were to estimate: I. The available N release from locally available manures in a typical soil of MB and its fertilizer value II. Net effects of manures on N mineralization under controlled laboratory conditions with an approach that combines effects of soil temperatures and soil moistures.

# **3.3 Materials and Methods**

#### **3.3.1 Amendment and soil characterization**

The laboratory incubation study was conducted using a loamy sand soil (Orthic Black Chernozem soil) collected from the field site of The University of Manitoba's Ian N. Morrison Research Farm at Carman, MB. Six treatments including three of solid beef manures (SBM) collected from Argyle (ABM), Carman (CBM), Morden (MBM) and additional treatments included liquid swine manure (LSM) collected from Steinbach, ammonium nitrate (AN) and a control without amendment. The nutrient composition of the manure samples was analyzed by the wet oxidation method of Akinremi et al. (2003). The concentration of NH4<sup>+</sup>-N and NO3<sup>-</sup>-N in manures were determined automated spectrophotometric method using auto analyzer (Technicon Ind. Syst., Tarrytown, NY.). Total carbon and nitrogen were determined using high temperature combustion by Dohrmann Dc-190 carbon analyzer (Rosemount analytical Inc., Orrville, OH). Routine analysis of baseline soil characteristics included soil pH, soil texture, soil bulk density and soil nutrient status (total N, NH4<sup>+</sup>-N, NO3<sup>-</sup>-N, and total C status, etc.). Soil pH was determined in 0.01 M CaCl<sub>2</sub> solution using a soil to solution ratio of 1:5. Soil particle size distribution was estimated by the pipette method (Gee and Bauder, 1996). Soil and manure samples were analyzed at least in triplicate (Table 3.1 and Table 3.2).

Soil Series	Orthic Black Chernozem
Soil pH	5.8
Soil texture	Loamy sand (Sand, Silt and Clay-86.75%, 5.02% and 8.23%, respectively)
Bulk density	$1.2 \text{ g cc}^{-1}(15 \text{ cm depth})$
WHC	33 % on volume basis
Drainage	Moderately to slow permeability
Organic matter	6.52%
Runoff	Slow surface runoff

Table 3.1 Characteristics of loamy sand soil at Carman

Table 3.2 Freeze dried manure characteristics and application rates

Treatment	N as NH <sup>+</sup> 4 (kg ha <sup>-1</sup> )	Organic N (kg ha <sup>-1</sup> )	Total N (kg ha <sup>-1</sup> )	C (%)	N (%)	C:N	Organic C:N	Moisture (%)
CBM	7.3	92.7	100	34.9	2.7	13.1	13.9	96.7
MBM	13.2	86.8	100	20.8	1.5	13.9	16	97.7
ABM	8.8	91.2	100	36.8	1.7	22.3	23.7	95.7
LSM	84.9	15.1	100	23.2	3.5	6.6	43.9	68.0

# **3.3.2 Experimental design**

The study was a split-split plot design, where the main plots were two soil temperatures of 14 and 21 °C. Main plots were replicated three times in six incubators. Studies often reported that 60 % WFPS is optimum for maximum microbial activities; 25 to 30 % WFPS is below optimum but often exists during the growing season and 70 - 80 % WFPS usually favours denitrification (Linn and Doran, 1984; Honeycutt and Potaro, 1990; Honeycutt et al. 2005; Griffin, Honeycutt and He, 2002; Honeycutt, Griffin and He, 2005b). Thus to represent dry, moist, optimum and excessive soil moisture under natural field conditions the study used four moisture levels of 20, 40, 60 and 80 % WFPS. Within each temperature, the four moisture levels were designated as sub-plots. Six treatments

including manure amended and unamended soils were designated as sub-sub plot, and each combination of sub plot with sub-sub factors was assigned to a total number of 144 experimental microcosms.

# 3.3.3 Construction of soil container

The soil containers were constructed using PVC tube with a height of 2.5 cm, an inside diameter of 7.6 cm, and 113.4 cm<sup>3</sup> in volume. Each container was filled with 136 g (dry wt.) of soil which translates into a bulk density of 1.2 gcm<sup>-3</sup>. The 144 PVC containers were constructed for both temperatures on February 1, 2011 and the bottom end of each container was sealed using a thin plastic board to hold the soil in place. Finally, each container was allocated a number which was matched to its empty weight.

# **3.3.4 Soil collection and amendment procedure**

A soil auger was used to collect the soil from a depth of 15 cm. The soil was collected from the field site that was used for a companion field study on in situ manure mineralization. Plant debris and coarse materials were removed and the soil was air-dried and ground to pass through a 2 mm sieve. The soil was stored in covered plastic containers at room temperature until required for the experiment. A week prior to the incubation study the gravimetric water content of the soil was measured in 24 replicates. Manures were collected from various farms and freeze dried over a period of two weeks using a Modulyod Freeze Dryer (Thermo Electron Corporation, Milford, MA, USA.).

# **3.3.5** Amendment application

Freeze dried manures were ground and the appropriate amount was weighed to provide 100 kg ha<sup>-1</sup> available N according to the standard formula used in Manitoba (i.e., inorganic N,  $NH_4^+$  + organic N \*0.25) on February 14, 2011. The weighed freeze dried manure was added to 3265 g dry soils, which was sufficient to fill 24 containers for each amendment (4 moisture levels x 2 temperatures x 3 replicates). Unamended soil was used as the control and was also mixed in a similar manner as the amended soils. The thoroughly mixed amended and unamended soils were placed into ziploc bags of each treatment, which were separated into four groups and each group was adjusted to the designated soil WFPS by adding the appropriate amount of water to the soil in the ziploc bag. Nitrogen mineralization is mediated by soil microorganisms and their activities are controlled by the supply of nutrient substrates and soil environment. Researchers have suggested that the water filled pore space (WFPS) is a superior to gravimetric soil water content as a means of accounting for the influence of soil texture, bulk density, soil porosity and air filled pore space (100 - WFPS), on aeration dependent biological process of N mineralization (Doran et al. 1990; De Neve and Hofman 2002). It is therefore of interest to determine the influence of WFPS on manure N mineralization. Thoroughly remixed amended and unamended soils at different WFPS plastic bags were then packed into the PCV container (Figure 3.1). Finally, the soil containers were placed into six different incubators maintained at their designated temperature.



Figure 3.1 Soil container after the incorporation of designated amendment and moisture (WFPS) being packed at bulk density of 1.2 gcm<sup>-3</sup>

# 3.3.6 Maintenance of soil columns

The PVC columns were kept inside Rubbermaid (TM) containers (15cm in height) with water at the bottom and covered with lid to reduce moisture loss during incubation. Thus, each of experimental unit was kept at their designated temperature in a humid environmental chamber for a period ranging from 0 to 126 days. For each incubator, temperature was monitored daily with a thermometer kept inside the incubator and from the incubator display. Most of the time, the temperatures did not vary more than  $\pm 1$  °C from their designated temperature. A 2 L beaker filled with deionized water was placed at the bottom of the incubation chamber to maintain humidity inside the incubators. Plants or weeds within the containers were removed manually whenever noticed. Each container was weighed every 3 days and moisture loss was replaced by adding distilled water with a plant misting bottle to maintain the designated WFPS. This process of weighing also ensured adequate aeration of the soil twice a week.

The use of WFPS was a solution to the logistical problem of maintaining different soils at given water potential and is widely applicable across soils for establishing optimal aerobic condition for microbial activity and minimizing denitrification losses. The WFPS was calculated as:

WFPS = (Soil gravimetric water content x bulk density/ [1 - (bulk density/particle density)]) (Honeycutt et al. 2005 and Sistani et al. 2008).

Comparison of approximate water content at the designated WFPS, gravimetric and volumetric soil water content and percent field capacity basis is shown in Table 3.3.

WFPS (%)	Gravimetric water content (%)	Bulk density (g/cc)	Volumetric water content (%)	Percentage of FC (%)
20	9.1	1.2	11	33
40	18.2	1.2	22	66
60	27.3	1.2	33	100
80	36.5	1.2	44	133

Table 3.3 Comparison between WFPS, gravimetric or volumetric moisture content and percentage field capacity

# **3.3.7 Sample Collection**

Soil samples were collected by scooping soil (about 16 g wet weight) from each designated soil containers at 0, 7, 14, 28, 42, 56, 70, 98 and 126 days after initiating the incubation. The mass of soil remaining after sub sampling was recorded at each sampling period so as to allow for subsequent proper moisture readjustment. The initial samples of

soil were sampled immediately after packing into the container and before they were placed into incubators (i.e. time = 0).

#### **3.3.8** Soil sample analysis

At each sampling period, 16 g (wet basis) of soil was scooped out and thoroughly mixed in a plastic cup. The gravimetric water content was determined using 10 g of soil placed in an oven at 105 °C for 24 hrs. The remaining 6 g of moist soil was extracted with 25 mL of 2 M KCl and was shaken for 30 min using a horizontal end to end shaker. The extract was filtered through Whatman no. 42 filter paper and analyzed for  $NO_3^--N$ ,  $NO_2^--N$ , and  $NH_4^+-N$  by automated spectrophotometric method using Technicon auto analyzer II (Technicon Ind. Syst., Tarrytown, NY.).

# 3.3.9 Calculation of net inorganic N (NIN), net mineralized N (NMN) and fertilizer equivalence of manures

(i) Net inorganic N (NIN), or available N was obtained as the sum of  $NH_4^+$ -N plus  $NO_3^-$ / $NO_2^-$ -N in each soil after deducting the measures of inorganic N from the unamended soil at each sampling time.

(ii) Net mineralized N (NMN) in the amended soil was calculated by:

Subtracting the amount of inorganic N extracted from control soil from the inorganic N extracted from amended soils, and subtracting the initial amount of inorganic N that was applied in the amendments from the different sampling i.e.:

 $N_{\min}(t) = (IN_{a(t)} - IN_{a(0)}) - (IN_{u(t)} - IN_{u(0)})$ 

Where  $N_{min}$  refers mineralized N in kg ha<sup>-1</sup>; 0 refers to initial time; t refers to sampling time; a refers to amendments; u refers to unamended soil and "IN" refers to inorganic N in the soil.

(iii) To express mineralization as a percentage of amendment organic N, the net quantity of N mineralized at each extraction time was divided by the initial amount of organic N present in the amendment and then multiplied by 100. Organic N in each amendment was obtained as the difference between total N and inorganic N (ammonia N plus nitrate N).

Thus, %  $N_{min} = [N_{min(t)} / ON] *100$ 

Where ON is the added organic N from manure in the same unit of kg ha<sup>-1</sup> as the  $N_{min (t)}$ .

(iv) To calculate the fertilizer equivalence (FEQ) of manures, the NIN from manure amended soils was divided by the NIN from fertilizer amended soils at each sampling time and then multiplied by 100 to express FEQ in percentage.

i.e.,  $FEQ_{MN} = [NIN_m / NIN_f] * 100$ 

Where,  $FEQ_{MN}$  denotes manure nitrogen fertilizer equivalence,  $NIN_m$  and  $NIN_f$  denotes net inorganic N from manure and fertilizer, respectively.

The NIN was calculated on an oven-dry weight basis and converted to kg ha<sup>-1</sup> at each sampling period. Nitrogen losses through ammonia volatilization, immobilization and denitrification during the incubation periods were not taken into account. Negative values indicated the disappearance of available N (i.e., volatilization, denitrification and or immobilization) among the amendments.

#### **3.3.10** Statistical analysis

Data were analyzed using the PROC GLM program of SAS. The analysis of variance (ANOVA) was done with a split-split plot arrangement of treatments. Amendment types, soil moisture, soil temperature, and sampling time were fixed variables whereas replication was tested as a random variable. To test manure amendments main effects and interactions, means separation for each main effect and subsequent interaction was determined by ANOVA and Fisher's significant difference (LSD) technique. All results were considered significantly different at P < 0.05 unless noted otherwise.

#### **3.4 Results**

# 3.4.1 Effects of soil moisture, amendment types and incubation period on NIN, NMN and fertilizer equivalence of manures

Addition of amendments increased the inorganic N concentration in soil, the net magnitude of the increase varied with soil moisture, amendment types, incubation period and their interactions. The NIN, NMN and fertilizer equivalence was significantly influenced (P<0.0001) by interaction of incubation period, soil moisture and amendment types (Table 3.4). The largest amount of NIN during the incubation period of 70 to 126 days were 95, 54, 55, 71.2 and 52.3 kg ha<sup>-1</sup> at 21°C and 40 % WFPS from AN, LSM, CBM, MBM and ABM amendments, respectively (Figure 3.2 to Figure 3.6). Thus, the net inorganic N varied at that period in the order of AN > LSM > SBM amendments.
There were no significant differences in the mineralized N from SBM amendments incubated at similar soil moisture. The maximum amounts of NMN from MBM, CBM, and ABM amended soils, were 64, 52 and 50 kg ha<sup>-1</sup>, respectively. In general, four phases of mineralization were observed at both temperatures among the SBM amended soils incubated at the soil moisture content of 20 and 40 % WFPS (Figure 3.7 and Figure 3.8). About 17 kg ha<sup>-1</sup> of NMN was measured in the first week in the SBM amended soils incubated under 20 and 40 % WFPS. This was followed by slower NMN up to day 42. Thereafter, there was a sharp increase of NMN between the periods of 42 to 70 days. This was again followed by a slow NMN to the end of the study period (Figure 3.7 to Figure 3.10). Net N mineralization under 60 and 80 % WFPS increased until 42 days of incubation and then declined over time at both soil temperatures to levels near or below zero (Figure 3.9 and Figure 3.10). As LSM contained higher concentrations of NH<sub>4</sub><sup>+</sup>-N (i.e. 85% of total N) initially, it was very difficult to estimate NMN from LSM and values of mineralized N were negative at each sampling period (Figure 3.7 to Figure 3.10). The SBMs released inorganic N from the amendments incubated at 20 and 40 % WFPS and LSM lost inorganic N at all WFPS as incubation progressed. The largest amount of NMN (60 kg ha<sup>-1</sup>) was obtained from MBM amended soil at 21 °C under 40 % WFPS (Figure 3.8).

The fertilizer equivalence of SBM amendments incubated at 20 and 40 % WFPS increased from 3 to 70 % relative to ammonium nitrate nitrogen during the period of incubation (Figure 3.11 and Figure 3.12). The fertilizer equivalence of LSM was initially high and declined continuously as the incubation progressed. The fertilizer equivalence of

LSM decreased from 89 to about 40 % of ammonium nitrate nitrogen at the end of the incubation under 20 and 40 % WFPS at both temperatures (Figure 3.12). In general, the study showed that LSM had initially high fertilizer equivalence which declined progressively with incubation period. A rapid declined of fertilizer equivalence was measured in LSM amended soil that incubated under 60 and 80 % WFPS.

Table 3.4Analysis of variance table indicating significant and non-significant<br/>effects on the various compartment of manure amendment N during<br/>incubation

Source of variation	Degrees of freedom (DF)	Net inorganic N (NIN)	Net mineralized N (NMN)	Fertilizer equivalence
Temperature (T)	1	0.3139	0.0566	0.1567
WFPS (W)	3	<.0001	<.0001	<.0001
T * W	3	0.1269	0.3446	0.0979
Amendment type (A)	3	<.0001	<.0001	<.0001
T * A	3	0.2836	0.6324	0.1464
W * A	9	<.0001	<.0001	<.0001
T * W * A	9	0.1112	0.5589	0.0147
Incubation period (I)	8	<.0001	<.0001	<.0001
I * T	8	0.8016	0.6202	0.6118
I * W	24	<.0001	<.0001	<.0001
I * T * W	24	0.9342	0.7576	0.7547
I * A	24	<.0001	<.0001	<.0001
I * T * A	24	0.9998	0.9988	0.999
I * W * A	72	0.0002	<.0001	<.0001
I * T * W * A	72	0.9999	0.9995	0.9995



Figure 3.2 Changes of net inorganic N (NIN, after correcting for the unamended soil) in CBM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure 3.3 Changes of NIN (after correcting for the unamended soil) in MBM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure 3.4 Changes of NIN (after correcting for the unamended soil) in ABM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure 3.5 Changes of NIN (after correcting for the unamended soil) in LSM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure 3.6 Changes of NIN (NIN, after correcting for the unamended soil) in AN amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS





Figure 3.7 Net mineralized N (NMN) amendments incubated at 14 °C (a) and 21 °C temperature and 20 % WFPS



Figure 3.8 NMN amendments incubated at 14  $^{\circ}C$  (a) and 21  $^{\circ}C$  (b) temperature and 40 % WFPS



Figure 3.9 NMN amendments incubated at 14 °C (a) and 21 °C (b) temperature and 60 % WFPS



Figure 3.10 NMN amendments incubated at 14  $^{\circ}C$  (a) and 21  $^{\circ}C$  (b) temperature and 80 % WFPS





Figure 3.11 Fertilizer equivalence of CBM (a) and MBM (b) amendments incubated under various WFPS at 14 °C and 21 °C temperatures



Figure 3.12 Fertilizer equivalence of ABM (a) and LSM (b) amendments incubated under various WFPS at 14 °C and 21 °C temperatures

# 3.4.2 Effects of soil moisture, soil temperature and amendment types on fertilizer equivalence of manures

The three way interaction effect of soil moisture, soil temperature and amendment types was statistically significant for fertilizer equivalence of manures N but was not significant for NIN and NMN (Table 3.4). The fertilizer equivalence declined from LSM amended soils at all WFPS and largest amount of 29 to 43 % fertilizer equivalence was estimated from LSM amendments incubated at 20 and 40 % WFPS. There was an increased fertilizer equivalence of 7 and 14 % obtained from the LSM amended incubated at 21 °C than 14 °C under 40 and 20 % WFPS, respectively. The initial time lag in SBM amendments fertilizer equivalence was 4 weeks at 14 °C but was shortened to 2 weeks at 21 °C at 40 % WFPS (Figure 3.8). The estimated fertilizer equivalence among the amendments incubated under 60 and 80 % WFPS was very low (< 10 %) at both temperatures. The fertilizer equivalency of SBM's was greater in the amendments incubated at 21 °C than 14 °C. For example, the fertilizer equivalence of CBM amended incubated at 20 and 40 % WFPS was 13 and 28 % higher at 21 °C than 14 °C, respectively. There was about 7 and 9 % less fertilizer equivalence at 14 °C than at 21 °C in the MBM amended soil incubated under 40 and 20 % WFPS, respectively.

#### **3.5 Discussion**

### 3.5.1 Effects of soil moisture, amendment types and incubation period on NIN, NMN and fertilizer equivalence of manures

The concentration of inorganic N was always significantly greater in the amended soils than unamended soil, which was similar to the results of the study reported by Honeycutt et al. (2005a). Initially LSM had the lowest C:N ratio (6.6) and synthetic fertilizer (AN) had the highest proportion of inorganic N. Conversely, the SBMs with high C:N ratios (13 to 22) had initially low proportion of inorganic N. LSM used in this study essentially had higher proportion of inorganic N than organic N which behaved like a commercial fertilizer (Figure 3.11). The concentrations of inorganic N for LSM declined continuously as the incubation progressed probably due to volatilization, denitrification and immobilization. The decline in NIN from LSM amendments incubated at 20 and 40 % WFPS was smaller than for LSM amendments incubated at 60 and 80 % WFPS. Overall decline in NIN for LSM was greater than for fertilizer probably due to carbon induced immobilization or denitrification with LSM (Sorensen and Jensen 1995). A small amount of NIN was initially measured in SBM amended soil. As the incubation progressed the inorganic N in SBM amended and unamended soils increased but the amount of NIN supplied by SBM exceeded the background levels of inorganic N in soil due to mineralized N.

The NMN increased with incubation period for soils that were incubated with SBM under 20 and 40 % WFPS at both temperatures (Figure 3.7 to Figure 3.10). Other studies

also reported increasing accumulation of inorganic N with incubation time (Cookson et al. 2002; Knoepp and Swank 2002; De Neve et al. 2003; and Amador et al. 2005). During the initial phase of mineralization, there was a slow accumulation of inorganic N obtained from SBM amended soil. The high carbon content of amended soil could explain the slow initial release of mineralized N (Figure 3.2 to Figure 3.6). Similar results were obtained by Tyson and Cabrera (1993) and Whalen et al. (2001) during the first week of incubation. However, the rapid increase in NMN after 42 days in SBM amended soil is similar to the result from the study conducted by Tyson and Cabrera (1993), who observed the second steepest increase of NMN after 30 days of incubation.

The maximum fertilizer equivalence of SBM amendments incubated at 20 and 40 % WFPS was 70 % in relation to ammonium nitrate and is similar to the results from other studies where the 100 kg of solid manure N resulted as equivalents of 60 - 80 kg of fertilizer N (Lorenz et al. 1997). Decreased fertilizer equivalence of LSM indicated that there was no mineralization or the experimental method was unable to detect if there was any mineralized N from the organic fraction of LSM. However, if there was a possibility for the mineralization of organic N from LSM, its magnitude was too small to be of practical importance during laboratory incubation. The low fertilizer value of LSM during incubation was similar to the results reported by Kyveryga et al. (2005) from injected liquid swine manure study which showed that LSM was only about 60 % as effective as N from fertilizer.

## 3.5.2 Effects of soil moisture, soil temperature and amendment types on fertilizer equivalence of manures

The fertilizer equivalence of SBM amended soil was slightly greater at 21 °C than at 14 °C indicating the effect of temperature in enhancing fertilizer equivalence in the soil. The increased soil temperature enhanced microbial activities which promoted accumulation of inorganic N at higher temperature during the incubation period (Andersen and Jensen 2001; Cookson et al. 2002; and Russel et al. 2002). It could be due to microbial communities that are favoured at high temperature and water content near the field capacity. This implies that substrates were not fully utilized at higher or lower soil moisture content (i.e., 20, 60 and 80 % WFPS) or at lower soil temperatures of 14 °C than 21°C. Although not significant but greater amount of NMN was estimated from SBM amendments at 21°C than 14 °C under 40 % WFPS may be the result of more active soil microorganisms at soil moisture content near FC (>60 % of FC) with higher temperature at 21°C (Figure 3.7 to Figure 3.10). However, the result of overall highest NMN at 21°C among the SBM amendments under 40 % WFPS could suggest that there could be an interaction effect of soil temperature, soil moisture and amendments on mineralized manure N thereby increased fertilizer equivalence (Figure 3.11 and Figure 3.12). As such the greater amount of estimated SBM amendments mineralized N at 21 °C in this study was very close to the study conducted by Watts et al. 2007. Quemada and Cabrera (1997) reported that the effect of moisture on microbial activities was enhanced as temperature increased. A number of studies have shown that soil moisture has a smaller effect on N mineralization than soil temperature and suggested that soil moisture is less limiting than temperature for the microorganisms responsible for mineralization (Sierra 1997 and Goncalves et al. 1994). Previous studies have also reported significant effect of soil temperature, soil moisture and manures interaction on microbial activities (Grant and Rochette 1994; Nurman et al. 1992 and Akinremi et al. 1999) and on manure N mineralization (Frazer et al. 1990, Knoepp and Swank 2002 and Wang et al. 2006). Thus, may influence the fertilizer equivalence of manures.

#### 3.6 Conclusions

The addition of SBM and LSM increased the inorganic N in manure amended soils. Manure type, soil moisture, incubation period and their interactions played an important role on mineralization and fertilizer equivalence of manures. Results indicate N availability or release of inorganic N was influenced differently depending on soil moisture, manure type and incubation period. Manure amendments used in this study demonstrated contrasting results in their N release patterns at different moisture contents. In general, the amendments incubated at 20 and 40 % WFPS showed similar pattern of NIN accumulation. Conversely, amendments incubated at 60 and 80 % WFPS showed similarity in the disappearance of NIN at both temperatures throughout the study period due to denitrification. In the relatively dry soil at 20 % WFPS, N mineralization was low because soil microorganism activity may have been limited by water availability. Conversely, lack of oxygen in the wet soils at 60 and 80 % WFPS limited NMN because only the soil microorganisms that can survive under anaerobic conditions were active and denitrifiers were promoted by limited oxygen availability. An enhanced N release was observed among the SBM amendments at 40 % WFPS at 21°C, which could be the

favourable soil moisture and soil temperature to promote maximum NMN and fertilizer equivalence of SBM. The study showed that the fertilizer equivalence of LSM was about 50 % whereas although SBMs had initially low fertilizer equivalence, this increased to 80 % as the study progressed. Study suggested that there is a need to review the traditional formula used to estimate fertilizer equivalence and appropriate rates for manure application. Manure N management needs special consideration in wet soil condition because less N can be expected to be released. Conversely, greater amount of manure N may be expected to release near at field capacity.

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#### **4. GENERAL SYNTHESIS**

Manure is an important source of inorganic and organic nitrogen (N) to the soil (Palm et al. 2001). It would be great if one kilogram of manure N was equivalent to one kilogram of fertilizer N. However, the presence of organic fractions in manure which require mineralization makes manure a slow release source of plant available N depending on the proportions of C and N in the manure. Mineralization of manure N is important since it transforms the organic N to plant-available N ( $NO_3^-$ -N and  $NH_4^+$ -N) as products of activities undertaken by soil microorganisms. The microbial activities are often described as a function of soil temperature, soil moisture, soil texture and manure characteristics (Knoepp and Swank 2002; Crohn 2004; Zaman and Chang 2004; Agehara and Warncke 2005; Griffin 2007). In a manured cropping system, it is necessary to fulfill N requirements of crop by matching N release (or mineralized N) with plant demand (Tilman et al. 2002; Van Kessel et al. 2000). This is very difficult to do due to varying temporal microclimate in the field (Kristofor et al. 2002) and concentration of not immediately available organic N in manure (Ontario Ministry of Agriculture and Food 2003).

The understanding of manure N mineralization in a growing season is essential to make more efficient use of manure by maximizing its benefit to the crop and minimizing undesirable losses (Tilman et al. 2002). This study was an attempt to develop a better estimate of available N from locally available manures, taking into consideration site specific soil temperature and soil moisture. Thus, a two-year field study using soil plus

anion exchange resin in two common agricultural soils and a laboratory incubation study matching typical field conditions of soil moisture and temperatures were conducted to fulfill the objectives.

Incorporation of solid beef manure (SBM) into a sandy loam soil supplied a maximum of 56 and 68 kg ha<sup>-1</sup> of net inorganic N during growing seasons in 2010 and 2011, respectively, whereas in a laboratory incubation study it was 71 kg ha<sup>-1</sup>. Following the incorporation of manures, the availability of N from SBM amendments increased due to mineralization while it decreased with LSM probably due to loss of available N through volatilization, denitrification, and/or immobilization. The mineralized N from SBMs ranged from 28 - 48 kg ha<sup>-1</sup> which was about 37 - 70 % of the added organic N in the field during the growing season. Similarly, 50 - 64 kg ha<sup>-1</sup> of mineralized N equivalent to 54 - 73 % of the added SBM's organic N was estimated under laboratory incubation. Solid beef manure amendments showed increased available N following incorporation up to day 70 while the opposite was the case for LSM amendments. This is similar to the 56-day period required to reach maximum mineralization of N following manure application that was observed by Andrews and Foster 2007 and Gale et al. 2006. The maximum amounts of net mineralized N in the laboratory incubation were similar to those observed by Prasad and Power (1997) where moisture level was slightly below field capacity and temperature increased from 10 to 24 °C significantly accelerating the mineralization of manures. The fertilizer equivalence of SBM increased from 38 to 80 % under field conditions and a sharper increase of 3 to 82 % was observed under laboratory incubation due to mineralized N. However, with LSM, fertilizer equivalence showed a decreasing trend from 81 to 57 % during the growing season in the field and 88 to 50 % during laboratory incubation due to loss of inorganic N. The negative mineralized N from LSM could be explained as immobilization of N (Van Kessel and Reeves 2002) and/or may be due to gaseous loss of available N through ammonia volatilization and denitrification.

Manures are heterogeneous materials (Van Kessel et al. 2000) containing different forms of N which are subject to diverse destinies in the soil. The ammonium form is available for plant uptake, microbial immobilization, clay fixation or nitrification, while the organic forms can be partly mineralized and thereby increase the inorganic N in soil with time (Van Kessel et al. 2000), and partly stored in the soil for periods of variable duration (Sørensen 2004). Thus, diverse amounts and fractions of N in SBM and LSM also vary in their efficiency to supply inorganic N during the growing season. Therefore, if applied at equivalent total N rates, SBM will supply inorganic N slowly but for a longer period compared to LSM which has a greater probability for rapid supply or loss of inorganic N initially. Field and laboratory studies showed lower concentrations of inorganic N for LSM compared to SBM for most of the study period, suggesting that the loss of inorganic N for LSM was much greater than for SBM. Thus, the study showed that initially the fertilizer equivalence of SBM was less than LSM and increased as the study progressed. Finally, the maximum supply of mineralized N from SBM in the field was 16 kg ha<sup>-1</sup> less than in the laboratory incubation. The laboratory incubation with its constant soil water content and temperature probably provided a more favorable environment for microbial mineralization of organic manure N. This is in contrast to field soil water and

temperature that are in constant state of flux and may not be as favorable as that in the laboratory, and hence the amount of N mineralization may be greater under laboratory than field conditions. However, the fallow condition in both studies at field and under laboratory incubation may also contrast to the natural field soil condition by increasing soil moisture, soil temperature and, therefore more favourable condition for manure N mineralization (Rochette et al. 1992 and Akinremi et al. 1999).

Using anion exchange resin was ineffective for trapping leached NO<sub>3</sub><sup>-</sup>-N from clay soil than loamy sand soil. This also implies that caution should be taken in using resin in clay soil. However, the failure of the resin technique in the clay soil could be due to low permeability which limited the transmission of water and nitrate to the depth where the resin was. This, coupled with the high water content of the clay soil, probably promoted denitrification before the nitrate could reach the resin at the bottom of the soil column. The larger amount of NMN in loamy sand soil at Carman than clay soil at Glenlea can also be explained by the wet clay soil that slowed down NMN or may be lack of NMN due to greater losses and warmer and dryer loamy sand soil that promoted the mineralization of manure N (Crohn 2004). The study also suggested that coarse textured soil might help to ensure favourable soil moisture, temperature and aeration for microbial activities (Gordillo and Cabrera 1997). Thus, the study confirmed that manure form and soil type had great impact on manure N mineralization and its fertilizer equivalence during the growing season.

#### 4.1 Conclusions and Recommendations

Field studies often do not provide consistent estimates of manure N mineralization due to confounding effects of several other uncontrolled processes in the field (Balkcom et al. 2009). However, in this study, there is the potential to combine field and laboratory studies to produce reliable information about the potential for N mineralization from different manures. Variation on the amounts of NMN from manure with varying weather conditions in both growing seasons confirmed that mineralized N from manures depends on the weather and therefore may vary between years. The high microbial activity immediately after manure application or during the rapid growth of plants might not be enough to supply adequate available N or inorganic N to meet crop requirements. Thus, farmers who rely solely on manure, especially SBM, may have N deficiency during that period and need to supplement with highly available N (i.e., fertilizer or LSM). However, to maximize manure N use by plant and minimize environmental N losses, side dressing application of liquid swine manure could be recommended. One should account for sitespecific soil and environment conditions prior to adding manures and also refrain from adding manures to sites prone to losses or sites in sensitive areas (i.e., wet clay soil especially under fallow conditions). Finally, the studies have shown that when manures are applied at equivalent rate of available N using Manitoba's traditional formula, both LSM and SBM supplied less N compared to fertilizer. Thus, the study results suggested that there is urgent need to revise the formula that is used to estimate available N from manure to improve manure utilization efficiency.

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## **5. APPENDICES**





Figure: A 2.1 Accumulation of net resin NO<sup>-</sup><sub>3</sub>-N at Carman during the 2011growing season



Figure: A 2.2 Changes in net NH<sub>4</sub><sup>+</sup> and net NO<sub>3</sub><sup>-</sup> among the amendments at Glenlea during the 2010 growing season



Figure: A 2.3 Changes in net NH<sub>4</sub><sup>+</sup> and net NO<sub>3</sub><sup>-</sup> among the amendments at Carman During the 2010 growing season



Figure: A 2.4 Changes in net NH<sup>+</sup><sub>4</sub> and net NO<sup>-</sup><sub>3</sub> among the amendments at Carman During the 2011 growing season



Figure: A 2.5 Gross inorganic N released from unamended soils at Carman during the 2011 growing season



Figure: A 3.1 Changes of net NH<sub>4</sub><sup>+</sup>-N in CBM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.2 Changes of net  $NH_4^+$ -N in MBM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.3 Changes of net NH<sub>4</sub><sup>+</sup>-N in ABM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.4 Changes of net  $NH_4^+$ -N in LSM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.5 Changes of net NH<sub>4</sub><sup>+</sup>-N in AN amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.6 Changes of gross  $NH_4^+$ -N in unamended soils incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.7 Changes of Net  $NO_3$ -N in CBM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.8 Changes of net NO<sub>3</sub><sup>-</sup>N in MBM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.9 Changes of net NO<sub>3</sub><sup>-</sup>-N in ABM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.10 Changes of net NO<sub>3</sub><sup>-</sup>-N in LSM amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.11 Changes of net NO<sub>3</sub><sup>-</sup>N in AN amendment incubated at 14 °C (a) and 21 °C (b) under various WFPS



Figure: A 3.12 Changes of gross  $NO_3$ -N in unamended soil incubated at 14 °C (a) and 21°C (b) under various WFPS



Figure: A 3.13 Changes of gross inorganic N in unamended soils incubated at 14 °C (a) and 21 °C (b) under various WFPS

Table: A 2 1: Analysis of variance table indicating significant and non-significant
rable. A 5.1. Analysis of variance table indicating significant and non-significant
treatment effects on Net $NH_4^+$ -N and Net $NO_3^-$ -N of manure amendments
during laboratory incubation

Source of variation	Degrees of freedom	Net NH4 <sup>+</sup> -N	Net NO <sub>3</sub> -N
Temperature (T)	1	0.0002	<.0001
WFPS (W)	3	<.0001	<.0001
T * W	3	<.0001	<.0001
Amendment type (A)	3	<.0001	<.0001
T * A	3	0.0026	0.4799
W * A	9	<.0001	<.0001
T * W * A	9	<.0001	0.9669
Incubation period (I)	8	<.0001	<.0001
I * T	8	0.0016	<.0001
I * W	24	<.0001	<.0001
I * T * W	24	<.0001	<.0001
I * A	24	<.0001	<.0001
I * T * A	24	0.6975	0.8349
I * W * A	72	0.0084	<.0001
I * T * W * A	72	0.2403	1