THE LONG-TERM IMPACT OF MANURE APPLICATION ON SOIL MICROBIAL PROPERTIES AND NUTRIENT CYCLING IN MANITOBAN SOILS

BY

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ABSTRACT

Czurak-Dainard, M. M. Sc., The University of Manitoba, December 2005. The long-term impact of manure application on soil microbial properties and nutrient cycling in Manitoban soils. Major Professor, Dr. David Burton.

The impact of long-term manure application on soil microbial properties was studied at ten sites across the south portion of Manitoba. Each site had different management histories, but consisted of adjacent non-amended and manure-amended (hog or cattle) fields. With no long-term manure-amended field plots available in Manitoba, this study provided a survey of the impacts of long-term manure-amendment on range of soil properties. Biological, physical and chemical soil aspects as well as predictive measures of N mineralization (KCl extractable NH_4^+, laboratory incubations) were tested against field N mineralization as influenced by manure treatment.

Site differences dominated most parameters examined; suggesting that approaches to N mineralization prediction must include site-specific characteristics. Many parameters responded differently to manure treatment. In general, manure application stimulated microbial community size and activity as demonstrated by higher levels of microbial biomass C (MBC), microbial biomass N (MBN), glutaminase, urease, and dehydrogenase. These parameters were correlated to extractable organic carbon levels,

which were greater in manure-amended soils. Hence, manure application by increasing the availability of carbon substrate, enhanced the microbial community and increased the mineralization potential of the soil. The influence of manure amendment was most consistently expressed in sites with longer manure management histories (>35 years). Step-wise regression analyses demonstrated distinct relationships between selected variables on manure-amended and non manure-amended sites. The variation in field N mineralization in manure-amended soils was best described by MBN, urease, organic carbon, pH, and sand content ($R^2 = 0.76$, RMSE 1.07).

Manure application did not significantly impact on soil microbial diversity as measured by substrate utilization patterns; however, longer histories of manure application tended to have greater microbial diversity as shown by the Shannon Diversity Index and partial RDA analyses. Texture, current crop and manure type also affect the diversity of the microbial community and other biological and chemical observed in this study.

This study demonstrated that biological parameters are critical to the understanding of nutrient dynamics in manure-amended soil, but no one single measure can be used. Site-specific characteristics and the potential for nitrogen loss via leaching and denitrification also need to be considered to allow estimation of plant available N.

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FOREWORD

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1. INTRODUCTION

The history of use of land for agriculture in Canada is relatively short compared to older agricultural civilizations. Since the turning of the sod on the Great Prairies nearly 100 years ago, we have had a dramatic impact on this soil. As the century progressed, agriculture became more intensive. The economy has become more globally based with a heavily reliance on agricultural export markets. In addition, the numbers of farms have decreased and farm size has increased in response to economies of scale. These larger agricultural operations tend to be more intensive and more specialized. As a result, there is a greater concentration of animal waste production per land base associated with livestock operations. The high water content and low nutrient content of manure makes it expensive to haul and spread. Hence, most producers would prefer to spread manure as close to the operation as possible to minimize costs. However, reapplying high concentrations of manure to the same land area year after year can over load the nutrient storage capacity of the soil and lead to nutrient leaching and runoff into groundwater and surface waters. In Manitoba, regulations have been established to help producers manage their animal manure as a soil nutrient resource. These regulations limit the soil's nitrate levels to no greater than 150 ppm. Phosphate is not currently been regulated in Manitoba, but it is in other provinces and states. In the future, it is anticipated the phosphate levels will be regulated here, as well

Monitoring only the inorganic nutrient content of soil, the pool considered to be immediately available to the plant, can become problematic. Manure amendment also increases the organic nutrient content of the soil and results in an increased nutrient mineralization capacity. Without knowing the soil's mineralization potential, it is difficult to assess the soil's nutrient status for crop growth. Thus, it makes determining the amount of nutrients available to the crop and at risk of environmental impact complicated. There is a need to understand and to be able to measure/predict the mineralization potential in Manitoba's manure- amended soils. Microbial parameters influence nutrient cycling in the soil. Other studies exist that demonstrate these effects, but they tend to take place in regions with different soil forming factors that influence the way soil behaves. The goals of this project were to assess the suitability of different microbial and biochemical parameters in predicting nutrient mineralization and the impact from long-term manure-amended soils in Manitoba's temperate climate.

2. LITERATURE REVIEW

2.1 Manure

Manure is a heterogeneous mixture of partly digested feed, fecal matter, and various inorganic and organic molecules. Its composition is influenced by the livestockspecies, mass, age, food intake, how it was housed, how the manure is collected and stored and what climate this all occurred in (Eghball et al., 2002). The quality of the organic N and the ratio of inorganic:organic N in different types of manure can greatly influence N mineralization. Manure has higher potential N mineralization rates prior to composting as compared to composted manure due to the larger number of stabilized organic N forms formed during composting (Tyson and Cabrera, 1993). Manure nutrient status is highly variable, not just at the regional level, but also at the livestock operational level (Davis et al., 2002). Predicting nutrient mineralization of soil amended with manure from different types of livestock is also difficult (Chang and Janzen, 1996; Van Kessel and Reeves, 2002).

The mineralization of organic nutrients into inorganic forms is not only an integral part of nutrient cycling; it is essential for crop growth and yield. It's estimated that between 1 and 4% of the soil's organic N undergoes mineralization into inorganic N forms during a growing season (Tisdale et al., 1993). The importance of this nutrient source to plants, in terms of both its size and the timing of its release, and the variability of mineralization

from site to site and from year to year emphasizes the need to develop tools that allow for site-specific assessment. For example, the mineralizable N pool varied depending on the crop rotation from 137 mg N kg^{-1} for soils cropped with continuous soybean to >500 mg N kg^{-1} in soils cropped with meadow-based rotations (Deng and Tabatabai, 2000).

2.2 N mineralization

Mineralization is defined as the breakdown of organic compounds as a result of the process of decomposition primarily to release energy (Paul and Clark, 1996). This breakdown is a result of the enzyme activity. The majority of these enzymes act intracellularly, releasing energy to metabolism. Some of the enzymes active in decomposition and the mineralization of inorganic constituents act external to the cell, frequently acting on complex organics either too large or too toxic to be metabolized intracellularly. Thus, the enzymes mediating mineralizing reactions occur both intracellularly and extracellularly. In addition to enzymes associated with the living biomass, nutrient-mineralizing enzymes may occur in a free state or adsorbed to soil colloids (Rao et al., 1996; Klose and Tabatabai, 2000).

Deaminization and ammonification are the primary reactions in converting organic nitrogen compounds, such as proteins into amines, amino acids and urea into ammonium. Ammonium is converted to nitrite and then nitrate by nitrification. Various populations of microorganisms carry out these processes. Consequently, the same environmental pressures that influence the activity of microbial populations in general, also affect mineralization rates (Paul and Clark, 1996). Adequate moisture and oxygen levels,

higher temperatures and an abundance of substrate are the major influences on the microbial community and its capabilities to mineralize organic matter (Paul and Clark, 1996). Soil N mineralization research typically employs incubation studies conducted over periods ranging from hours to weeks, occurring either *in situ* or in the laboratory (Stanford and Smith, 1972). Nitrogen mineralization is commonly described using a first-order reaction rate (2.1):

$$(N_t) = (N_0)\ (1\text{-}e^{-kt}) \qquad (2.1)$$

Where N_0 represents the amount of mineralizable N at time 0, N_t represent the amount N mineralized at time t, and k is the mineralization rate constant (Stanford and Smith, 1972). This equation is temperature dependent and requires steady-state environmental conditions (moisture and temperature). This equation directly emphasizes the role of the mineralizable pool (substrate quality and quantity) and the metabolic capacity of the soil microbial community in determining the rate of N mineralization. The role of environment variables (temperature, moisture) is indirectly reflecting in the change in mineralization rate constant under different environmental conditions.

Another approach is the use of a mass balance method that measures changes in inorganic nitrogen stocks over a specific time frame (Hadas et al., 1986; Hook and Burke, 1995). This approach is more laborious, time consuming and not easily generalizable, prompting researchers to seek more fundamental understanding of this process that would permit generalization. Attempts have also been made to describe mineralization through the characterization of the size of the mineralizable N pool utilizing different physical,

chemical and biological fractions of soil organic matter as a measure of soil N mineralization potential (Whalen et al., 2000; Mulvaney et al., 2001).

Manure application further complicates nutrient mineralization prediction by altering the quality and quantity of the mineralizable pool of nitrogen and influencing the composition of the microbial population. Chang and Janzen (1996) cite that half of manure applied N is readily available the current year of application. The remainder is mineralized slowly in subsequent years. With repeated applications of manure, the quality and quantity of mineralizable N becomes increasingly distinct from non-amended soils, thus increasing the challenge of and need for an effective means of predicting mineralization (Whalen et al., 2001). Furthermore, mineralization potentials are also impacted by soil properties and biological quality, field management practices, and environmental conditions.

2.3 KCl extractable NH_4^+ for predicting N mineralization

Gianello and Bremner (1986a) designed a simple chemical method, extraction of ammonium using a heated 2M KCl solution as a means of determining the soil's nitrogen mineralization potential. One of the benefits of this method is that it can be used with air-dried soil and the soil is not affected by air-dry storage. In addition, the method is rapid and involves limited sample manipulation and the results are not affected by varying particle sizes (Gianello and Bremner, 1988). The method is based on the difference between the amount of NH_4^+ extracted with 2M KCl, heated on in a block digestor at

100°C for 4 hours and the NH_4^+ extracted with 2M KCl at room temperature being related to the amount of plant available, mineralizabled N. Gianello and Bremner (1986a, b) referred to hot-KCl extractable NH_4^+ as the difference between the hot and cold measured ammonium extracts and attributed it to the amount of ammonium-N released from the organic portion of the soil N. Although most of the literature refers to hot-KCl extractable NH_4^+ as the difference between hot- and cold-KCl extractable NH_4^+, Jalil et al., (1996) examined each of phase of extraction process (cold-KCl extractable NH_4^+, hot-KCl extractable NH_4^+ and the total-KCl extractable NH_4^+ (the hot-KCl extractable NH_4^+ without subtracting the NH_4^+ extractable by cold NH_4^+) for their relative abilities to predict the size of the mineralizable pool. For our purposes and to minimize confusion the unheated KCl extractable NH_4^+ will remain also cold-KCl extractable NH_4^+. The heated KCl extractable NH_4^+ will be referred to as total KCl extractable NH_4^+. The difference between total- and cold-KCl extractable NH_4^+ will be called hot-KCl extractable NH_4^+.

In an Iowa study, hot-KCl extractable NH_4^+ had a strong positive correlation with nitrate and nitrite-N produced during 14 day aerobic laboratory incubations (r=0.92***) with typical values ranging from 5.2 to 48.9 μg N g^{-1} (Gianello and Bremner, 1986a). However, in a study with Saskatchewan soils, Jalil et al. (1996) found hot-KCl extractable NH_4^+ to be weakly correlated to nitrogen mineralized during a 24 week incubation (N_{min}) ($r^2 = 0.43$). The hot- and the cold-KCl extractable NH_4^+ alone had higher correlations with nitrogen mineralized over the 24 week period (N_{min}) respectively ($r^2 = 0.79$, $r^2 = 0.69$). A similar method, utilizing an 1 hr hot 1M KCl extraction of NH_4^+

was also found to be highly correlated to nitrogen plant uptake by potted rye grass (r^2= 0.85), oats (r^2= 0.79) and barley (r^2= 0.64) (Smith and Li, 1993). Whitehead (1981) had similar success with predicting plant uptake utilizing a 1M KCl extractant during an one hour heating period. Groot and Houba (1995) found soil texture influenced both N mineralization rates and hot-KCl extractable NH_4^+, with coarse-textured soil with higher organic matter content having higher correlations than poorer quality loam soils in their study.

Thus, many researchers have identified hot-KCl extractable NH_4^+ as a method for predicting N mineralization and plant available N. Jalil et al. (1996) further stated that the temporal consistency of measured hot-KCl extractable NH_4^+ in a soil over a three to five year period might reduce the need for annual soil testing, especially if coupled with soil and climatic properties. Thus, if hot-KCl extractable NH_4^+ is able to predict mineralizable N rates in manure-amended soils, then this value can be reanalyzed every four years to estimate soil N supply, allowing the addition of other nitrogen sources to be adjusted accordingly.

2.4 Soil biological properties

2.4.1 Soil microbial biomass

The soil microbial community catalyses the process of nutrient mineralization and therefore is an important regulatory factor in nutrient cycling. The soil microbial community represents not only an important catabolic agent in soil, it is also a very labile

pool of organic nutrients. It is commonly held that the larger the community size, the greater the diversity, and the greater the potential for nutrient mineralization. There are many measures of microbial community size. Perhaps the most direct measure of the microbial component of soil is the measure of the biovolume. This method is seldom used by researchers as it is extremely laborious, tedious and somewhat subjective. It involves the volume measurement of the various microbes and then counting the number of microbes in a sample. Since it relies upon visual identification of microbial cells, it is also a somewhat subjective approach and there is the potential for bias. Thus, more researchers are turning toward biological, chemical or physical measures of the microbial biomass measure as a means to quantity the microbial community. This involves measuring chemical components of the soil that comprise the "microbial biomass" (Paul and Clark, 1996).

The most common methods for assessing the microbial biomass include two techniques that measure of the compounds released as a result of soil exposure to $CHCl_3$. The $CHCl_3$ fumigation-incubation (FI) method, developed by Jenkinson and Powlson (1976), involved fumigating one set of soil samples with $CHCl_3$ for 24 hours to cause the rupture of microbial cell walls and the release of the cytoplasmic constituents into the soil. The surviving microbial community then decomposes these constituents resulting in the release of CO_2 and the mineralization of organic nitrogen. The unfumigated and fumigated samples are incubated for a period of time (usually for 10 days). An alkali solution (KOH or NaOH) is used to collect CO_2 and the accumulated inorganic N is extracted with a salt solution (KCl, $CaCl_2$, or K_2SO_4). In this method, the microbial

biomass is calculated by the difference of the CO_2 evolved and mineral N released between the fumigated (additional substrate due to the $CHCl_3$ induced rupture of microbial biomass) and the unfumigated samples. The level of released carbon dioxide and mineral N represents the amount of metabolized microbial community killed during the fumigation. It is then divided by a constant, k_c (as determined by Jenkinson, 1988) to calculate the amount of microbial biomass C and k_N for microbial biomass N. This method is very dependent on establishment of proper soil conditions for the experiment to be effective.

The most popular method for biomass measurement is the fumigation direct extraction (FE) method developed by Brookes et al. (1985). Rather than relying on the microbial metabolism of the carbon and nitrogen compounds released during $CHCl_3$ fumigation, this method relies upon extraction in 0.5 M K_2SO_4 and chemical determination of the organic C and N compounds released. Chemical determination generally involves the automated digestion and determination of mineral constituents (CO_2, NH_4^+, PO_4^{3-}). Soil microbial biomass C and N are determined by calculating the difference between the fumigated and the unfumigated samples for their respective constituents. This difference is then divided by the extraction coefficient constant, k_C or k_N.

Anderson and Domsch (1978) developed a physiological method for assessing the size of the microbial biomass referred to as the substrate-induced respiration (SIR) method. This method assumes that the metabolic capacity of the soil is a function of the size of the microbial community. As a result when an excess of substrate is added to the soil the

level of carbon dioxide respired is a measure of its metabolic capacity which is related to the size of microbial biomass. With SIR, the amount of CO_2 produced from the sucrose addition is calculated as the difference from the sample minus a control.

In general, additions of organic amendments increase soil microbial biomass initially and gradually decrease after 30 days (Zaman et al., 1999b). The addition of organic amendments provides an energy source that stimulates microbial growth. With increased microbial biomass, the catalytic potential of the community increases including nitrogen mineralizing enzymes. These increases were significantly correlated with each other, microbial biomass carbon (MBC) and gross N mineralization (Zaman et al, 1999a). Zaman et al. (1999a) also showed that microbial biomass C and N were the best indicators of gross nitrogen mineralization during their short-term study. Organic amendments increased MBC (276 μg C g^{-1}) on long-term beef manure-amended soils as compared to the control (168 μg C g^{-1}) (Fauci and Dick, 1994). Short-term incubations noted a 210% increase in microbial biomass C over a 306-day period following beef manure addition. After 17 applications of liquid hog manure, MBC was significantly higher in surface soils (0-15 cm), and most notably at the 90 m^3 ha^{-1} application rate (248 μg C g^{-1} MBC, opposed to untreated 129 μg C g^{-1}) (Lalande, et al., 2000). Higher rates of application (120 m^3 ha^{-1}) did not result in a corresponding increase in microbial biomass and its activity, suggesting that the growth and activity of the microbial biomass is not only limited by substrate addition.

2.4.2 Soil Enzymes

In the past several decades, there has been an increasing interest in the study of soil enzymes as an indicator of the response of microorganisms to their environment. Many researchers have found that the addition of energy sources such as manure or other C sources can increase the activity of these enzymes in soil (Fauci and Dick, 1994; Zaman et al., 1999a, b; Lalande et al., 2000). In this project, urease and glutaminase were examined as potential indicators of the impact of long-term manure application on nitrogen mineralization. Alkaline monoesterphosphatase was also utilized as an indicator of changes in microbial biomass.

2.4.2.1 Urease. Urease or urea amidohydrolase (EC 3.5.1.5.) is an enzyme that specifically catalyzes urea hydrolysis (Hasan, 2000). This enzyme breaks urea down into ammonia and carbon dioxide. An excellent review of soil urease activity was provided by Hasan (2000). There is a wide range of microorganisms that produce urease, including various fungi, actinomycetes and bacteria. Furthermore, the mechanism and regulation of urease production can vary among different microbial species (Mobley and Hausinger, 1989). Urease is found to be responsive to soil quality changes. Urease activity increases with the incorporation of inorganic (Goyal et al., 1999), organic (Falih and Wainwright, 1994; Zaman et al., 1999a, b) or combination of organic and inorganic amendments (Goyal et al., 1999) to soil and decreases with soil degradation (Garcia et al., 1994). Dick (1984) found that long-term N fertilizer application could also suppress soil urease activity.

The main factors regulating the production of urease are temperature, pH, carbon source and the concentration of the substrate (urea) and product (ammonia) (Hasan, 2000). Depending on the microorganism, the optimum pH range can vary from 2 to 8. Initial increases in urea concentration have shown to increase the rate of urea hydrolysis. However, as the end product, ammonia, accumulates and the pH increase, it has been speculated to slow down and even reduce the production of urease and hence, reduces the rate of urea hydrolysis (Hasan, 2000). McCarty at el. (1992) noted that although C-amended soils showed increased urease production in the presence of ammonium or nitrate, by-products of urease production can suppress urease production. They found that early potential by-products of ammonium assimilation such as L-isomers of certain amino acids suppress urease production. Thus, urease production is regulated by a feedback mechanism.

Many researchers have found that immediate incorporation of organic amendments can increase urease content (McCarty et al., 1992; Zaman et al., 1999a, b; Hasan, 2000). Hasan (2000) stated that it could take between 2 to 6 days, depending in the species for a bacterial population to reach it peak urease production after urea addition. Zaman et al. (1999b) noted significant increases in urease activity immediately after dairy shed effluent (DSE) were applied to soil (~69 μg N g^{-1} h^{-1}). Although urease content appeared to subside after 10 days, DSE amended soils still remained higher (~40 μg N g^{-1} h^{-1}) for up to 30 days, in comparison to inorganic treatments (~30 μg N g^{-1} h^{-1}). A sugar beet amendment also increased urease content during a 28 day incubation period (Falih and Wainwright, 1996). Thus, increases in urease responses are related to the organic

substances and nutrients in amendments that help stimulate ureolytic microorganism activities (Falih and Wainwright, 1996; Zaman et al, 1999b). Once these resources are used, urease content declines. Most experiments involving urease had short-term application histories. The study by Goyal et al. (1999) had the longest amendment histories of 11 yrs when comparing urease levels. Inorganic amendments had lower levels of urease (55-64 $\mu g\ N\ g^{-1}\ h^{-1}$) and a combination of organic and inorganic amendments had greater urease levels (65-88 $\mu g\ N\ g^{-1}\ h^{-1}$). Goyal et al. (1999) found straw incorporation and farmyard manure (both also applied with inorganic fertilizer) increased soil urease content by 52% and 35%, respectively While there are some contradictions as to whether inorganic fertilizers have a negative or positive influence on soil urease content, organic amendments clearly increase urease content (McCarty et al., 1992). Furthermore, Klose and Tabatabai (2000) found that 46% of soil urease content was associated with the microbial biomass. The remaining 54% percentage was extracellular in nature. More research is needed to show how these amendments are increasing different soil enzyme contents and how these amendments are impacting potential soil nitrogen cycling.

2.4.2.2 Glutaminase. L-Glutaminase (L-glutaminase amidohydrolase EC 3.5.1.2) hydrolyses glutamine into L-glutamic acid and NH_3 (Frankenberger and Tabatabai, 1995). L-Glutaminase is ubiquitous in nature. In soil, microorganisms are the main sources for L-glutaminase production. This enzyme is found to decrease with soil profile depth and is correlated with organic C ($r=0.79$, $P<0.01$) and total N ($r = 0.76$, $P<0.01$) in 25 surface soils (Frankenberger and Tabatabai, 1995). L-Glutaminase appears not to be

influenced by pH or texture (Frankenberger and Tabatabai, 1995; Monreal and Bergstorm, 2000). Unfortunately, most soil quality researchers have overlooked the potential of this enzyme. Monreal and Bergstrom (2000) found glutaminase to be a sensitive indicator of soil quality and potentially useful as an indicator of soil N mineralization model as it was responsive to changes in the land use and management. In their study, the level of tillage disturbance influenced glutaminase content., as the less invasive the land use, the higher the levels of glutaminase (~78-423 $\mu g\ N\ g^{-1}\ h^{-2}$) with the highest average being approximately 332 $\mu g\ N\ g^{-1}\ h^{-2}$ for undisturbed soil. The addition of organic amendments resulted in increased glutaminase content in soils of limited productivity, such as saline soils (Pathak and Rao, 1998).

2.4.2.3 Phosphatase. Phosphatase catalyses an important reaction in the phosphorus cycle, the mineralization of organic phosphorus. One of the primary reactions in the mineralization of phosphorus is catalyzed by a general class of enzymes referred to as phosphatases. One specific type of phosphatase enzyme cleaves the ester bond in various organic compounds containing phosphorus resulting in the release of phosphate (Duff et al., 1994). In a model proposed by McGill and Cole (1981), the presence of compounds containing organic phosphorus induces phosphatase production and P mineralization to occur. Many researchers have found that phosphorus mineralization and phosphatase production are controlled by a negative feedback mechanism (Clarholm, 1993; Sinsabaugh et al., 1993; Tadano et al., 1993). The accumulation of ortho-phosphate, the product of the phosphatase mediated reaction, results in the inhibition of the phosphatase enzyme and suppression in the synthesis of phosphatase enzymes. Negative feedback

mechanisms are considered a method for plants and microbes to control mineralization activities to regulate nutrient supply levels. Other researchers have made opposing conclusions about the existence of a negative feedback mechanism (Adams, 1992; Harrison, 1983). Hence, the accumulation of inorganic phosphorus may not be the agent limiting phosphatase production in all cases.

The presence of abiontic, stable enzymes adsorbed onto organic matter or clay mineral surfaces can result in persistent phosphatase activity, independent of phosphorus status (Svensson and Pell, 2001). It is interesting that many researchers have found that N addition tends to increase phosphatase activity and production (Olander and Vitousek, 2000). This may be due to the role of N in biosynthesis and enzyme production and that N may increase both plant and microbial productivity. If plant and microbial productivity increases, the demand for P may further stimulate phosphatase production.

The activity of phosphatase enzymes is pH-dependent. Consequently, they are classified and studied according to pH at which optimal activity occurs (acid, neutral or alkaline phosphatases). Plants, earthworms, fungi, and bacteria can synthesize phosphatase (Oberson at al., 1996; Olander and Vitousek, 2000). Both plants and microorganisms produce acid phosphatase (Tabatabai, 1982) and are dominant in soils with low pH (Juma and Tabatabai, 1988). By contrast, microorganisms are the only producers of alkaline phosphatase and are associated with soils of higher pH typically found in arable land. Since most southern Manitoban soils are arable and alkaline, alkaline phosphomonoesterase was chosen for this study. This type of enzyme can be used

intracellularly or released in to the surrounding environment to form stable active phosphatase enzyme complexes.

Long-term manure application can increase organic P fractions in soil compared to untreated soils (Motavalli and Milnes, 2002). In a long-term study by Parham et al. (2002), inorganic fertilizers increased P accumulation over manure-amended soils. However, phosphatase was significantly higher in the manure-amended soils. They also found that manure derived P was more mobile and plant available and were concerned about phosphate leaching into water bodies. Livestock manure itself contains various levels of phosphatase, the highest being in hog manure (43% phosphatase/total organic P) opposed to cattle manure (15% phosphatase/total organic P) (He and Honeycutt, 2001). In another study, the addition of inorganic fertilizer was found to not to increase the phosphatase content of soils, but differences in cropping systems were found to influence phosphatase content (Lalande et al., 2005). The effect of cropping systems (Dick et al., 1988), and tillage practices (Deng and Tabatabi, 1997) has been studied. The addition of legume residue increased soil phosphatase content (Dick et al., 1988). The effect of tillage on phosphatase depended on the type of phosphatase studied; however, no difference was seen between no-till and mold-board plow for alkaline phosphatase (Deng and Tabatabai, 1997). With manure applications increasing enzyme activity (Dick et al. 1988), organic P fractions, and crop yield, more studies are needed to assess the correlation of phosphatase content to mineralization of phosphorus and the possibility of utilizing phosphatase content a means of predicting P mineralization potential.

2.4.3 General Measures of Soil Biological Activity

2.4.3.1 Dehydrogenase. Soil microbial community activity can be measured by analyzing dehydrogenase enzyme content (Alef, 1995a). As organic substances are oxidized, they generate energy in the form of NADH or NADPH. Dehydrogenase is a membrane-bound enzyme that collects and funnels electrons from NADH through an electron transport chain, generating energy fir the cell. These electrons are shifted to the cytochrome system where they are oxidized by O_2, the final electron acceptor. This process is coupled with oxidative phosphorylation to produce ATP. Since no free active dehydrogenase is not expected or has been reported, dehydrogenase is commonly used as an indicator of biological activity in soil (Frankenberger and Dick, 1983). Thus, the greater the measured dehydrogenase contents in a soil sample, the greater the overall aerobic microbial activity.

Dehydrogenase is greatly influenced by organic amendments (Ritz et al., 1992; Albiach et al., 2000; Lalande et al., 2000; Parham et al., 2002). During the fourth year of study, Albiach et al. (1992) found soil to which ovine manure had been applied had significantly higher dehydrogenase activities (approximately 6.5 μg TPF g^{-1} dry soil g h^{-1}) than control treatments (approximately 4.5 μg TPF g^{-1} dry soil g h^{-1}). Hadas et al. (1996) also found dehydrogenase increases after manure application. However, they found that after an initial increase over 10 days, dehydrogenase activity decreased and returned to pre-amendment levels. Hadas et al. (1996) felt that nitrogen mineralization levels were more related to the properties of the residues being decomposed than to properties of soil

microbial community (including soil dehydrogenase) in soils treated for 30 years with cattle manure. Others have consistently shown the long-term manure application increases dehydrogenase activities regardless of the time after application (Lalande et al., 2000, Parham et al., 2002). Eighteen years of liquid hog manure (LHM) treatment increased potential dehydrogenase activity 130% from 1.8 μg TPF g^{-1} dry soil g h^{-1} for control treatments to 4.2 μg TPF g^{-1} dry soil g h^{-1} for 90 m^3 LHM ha^{-1} treatment in surface soils (Lalande et al., 2000). Parham et al. (2002) studied a century-long field experiment first amended with cattle manure in 1899, and found that with manure application occurring one year prior to the study's sampling, soil dehydrogenase levels were still significantly higher than control plots. Hence, the effects of repeated manure applications were still able to stimulated dehydrogenase responses after they were discontinued.

2.4.3.2 Respiration. Respiration can be defined as a catabolic process where electrons are transferred from organic compounds through the electron transport chain to oxygen. The measurement of CO_2 production or O_2 consumption from respiration allows for assessment of the soil's aerobic metabolic activity. It is one of the oldest parameters used to assess soil microbial activity (Alef, 1995b). Basal respiration is the respiration in the absence of any added organic substrate. This differs from substrate-induced respiration that measures respiration in response to the addition of an organic substrate, which is used to estimate soil microbial biomass (Anderson and Domsch, 1978). Soil respiration can be measured using incubation vessels with gas samples absorbed in alkaline traps and detected chemically, or by detection of accumulated CO_2 in the headspace using gas

chromatographs or infrared spectroscopy. Carbon dioxide measurements are often used to calculate the mineralization of soil organic matter. Respiration can be influenced by soil texture, structure, substrate availability, nutrient availability, soil moisture and temperature (Alef, 1995b). Soils amended with manure are shown to have increased CO_2 production during incubation (Castellanos and Pratt, 1981; Paré et al., 2000; Dao and Cavigelli, 2003). Soils with higher organic carbon content also tend to have greater CO_2 fluxes (Dao and Cavigelli, 2003). Furthermore, Castellanos and Pratt (1981) found significant correlations between N mineralization and CO_2 production from short incubation periods of one to four weeks. Correlations between plant available N and respiration reflect the common dependence on the quality of organic matter in various manures. In addition, composted manure had greater organic matter stability, consequently resulting in a reduction in N mineralization and respiration. Strong correlations were demonstrated between respiration from soils incubated under laboratory conditions and field plant N uptake in soils that have received manure (Haney et al., 2001).

2.4.4 Soil Diversity

The abundance and activity of soil microbes are influenced by a variety of environmental variables, such as soil type, nutrients, pH, etc. (Grayston et al., 1998). When looking at the impacts of manure on microbial population, soil diversity should be analyzed to determine how changes in the population might influence metabolic capacity and ultimately the mineralization processes. As previously discussed, application of organic manure can increase microbial activity in general. Manure application is also shown to

increase soil organic C, microbial biomass and soil microbial diversity (Peacock et al., 2001). However, the current literature lacks examples of how long-term manure applications impact soil diversity and its relation to nutrient cycling. Typically, when describing a community's diversity, the types of organisms present are analyzed; the number of each population within the community is enumerated; and niche descriptions are evaluated. Unfortunately, the diversity of the soil microbial communities is more difficult to describe than other terrestrial communities because of their small size, the magnitude of population numbers, and the extreme diversity of habitat over very small distances (< 1 mm). Several methods have been used to describe microbial community structure and diversity. These include plating isolates, most probable number techniques (MPN), substrate utilization profiles, phospholipid fatty acids composition profiles (PLFA), and various methods of assessing the DNA content of the soil community (Øvreås and Torsvik, 1998; Gamo and Shoji, 1999; Pankhurst et al., 2001).

Community substrate utilization profiles have been assessed using Biolog plates (Biolog Inc., Hayward, California) containing 96 wells and 95 separate carbon sources and one water blank well. Each well has a redox indicator dye, tetrazolium red that turns purple to give a positive reaction to indicate microbial growth. Originally, plates designed for the identification of gram-negative bacterial species were used because of the predominance of Gram negative, non-spore forming, rod-shaped bacteria such as Pseudomonas, Agrobacterium and Achromobacter in soils (Maire et al., 1999). Recently, a more generic microbial community assay plate has been manufactured that encompasses many of the substrates found in both the gram negative and gram positive

bacterial identification plates with more emphasis on the former constituents. There are many positive and negative aspects of using metabolic diversity profiles, also known as sole carbon source utilization profiles, to describe soil microbial community diversity (Zak et al., 1994; Haack et al., 1995; Howard, 1997). This methodology does not directly describe diversity, but rather it is implied by the functional capacity of the sample as demonstrated by the number of substrates utilized. The greater and range of substrates used, the greater the implied diversity soil microbial community. However, this approach does not account for the potential for functional redundancy across a range of bacterial species.

The community's diversity as measured by the diversity in metabolic function has been shown to correspond to genetic diversity (Franklin et al., 2001). In addition, metabolic diversity profiles have been found to respond of the varying availability of soil carbon sources (Grayston et al., 1998). Some of the drawbacks of using metabolic diversity profiles are that they are selective for culturable microbial populations. This is also a concern for other microbial diversity methods, such as MPN and direct count. Inoculum cell density and rate of color development need to be considered (Howard, 1997). High cell densities are sought after because of the better representation and avoiding lower dilution effects. However, high cell inoculum's densities could include large amounts of biodegradable organic matter, thus causing high background color levels. In addition, faster growing organisms may "mask" slower growing organisms. Even if the slower organisms only use these carbon sources, the inoculated microbial community also appears to change during the incubation. Community changes are also observed during

soil manipulation for the assay set-up. The positive aspects of using Biolog plates to demonstrate community diversity is they are reproducible (Bossio and Scow, 1995; Haack et al., 1995), their strong correlations to other diversity analyses (Widmer et al., 2001), and their ability to distinguish the effects of different crops and other management influences. Metabolic diversity profiles are also very successful in differentiating different types of plant communities, species and changes during the growing season (Zak et al., 1994; Grayston et al., 1998; Schutter et al, 2001; Petersen et al., 2002).

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3. THE IMPACT OF LONG-TERM MANURE APPLICATION ON MICROBIAL PROPERTIES IN TEN SITES ACROSS SOUTHERN MANITOBA

3.1 Abstract

The increase number of intensive livestock operations (ILOs) in Manitoba has many individuals concerned about the environmental impact of these operations (Gibson, 2002). Unfortunately, long-term manure-amended plots have not been established in Manitoba. In the absence of long-term controlled research plots, research trials on farms that have a history of manure use provide the best opportunity to study the impacts of manure addition on soil biological properties. Microbes are an essential part in the soil nutrient cycle and are often used as indicators of soil quality (Gregorich et al, 1994). Our study examined the effects of manure application on selected microbial parameters. Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), respiration, dehydrogenase, urease, glutaminase, and alkaline phosphatase were measured at ten sites across southern Manitoba. Each site contained two adjacent fields one with a history of manure amendment and one that does not. These sites had varying soil characteristics, manure sources (hog and cattle) and farm management practices. Consequentially, season, crop, texture, manure types and the length of the manure application history impacted the selected microbial parameters to varying degrees. Site effects were seen in all parameters, thus indicating that site-specific effects on nutrient cycling processes should not be ignored. Soil MBC, glutaminase, urease and dehydrogenase contents were

the most sensitive to manure amendments and appear to potential indicators of manure management history.

3.2 Introduction

In the past, farmyard manure was not generally considered a waste product, but an important nutrient source for crop production. The specialization of agricultural industry has caused a separation of animal production and crop production. Hence, manure production does not necessarily occur in proximity to crop production. Further, other nutrient sources are considered to be more efficient for use in crop production. With the growing number of intensive livestock operations (ILOs) in the province (Gibson, 2002), land application is increasingly seen as a way of managing organic waste. If manure from ILOs is managed prudently, ecological impacts can be minimized while still benefiting crop production.

Application of organic amendments is known to increase the size and activity of microbial populations (Dormaar and Chang, 1995; Filip et al., 1999; Goyal et al., 1999; Parham et al., 2002). Microbial and biochemical parameters are studied because of their critical role in nutrient cycling (Fauci and Dick, 1994; Svesson and Pell, 2002). In this study, long-term manure amended soils throughout the agricultural zone of Manitoba were monitored and compared to adjacent sites with little or no history of manure application. These sites included a range of livestock and crop production systems, differences in land application and manure management practices of individual farmers

and soil characteristics. The response of biological parameters to repeated manure application was assessed for its ability to provide greater understanding of soil nutrient cycling. Microbial parameters selected for study included microbial biomass C + N, microbial respiration, and the content of dehydrogenase, urease, glutaminase and phosphatase enzymes in soil. If these microbial parameters are impacted by long-term manure application, nutrient cycling within the soil will also be impacted.

3.3 Objective of the Study

The objectives of the study were to examine how long-term manure application affect selected microbial parameters at different sites in Manitoba and which parameters are the most sensitive to manure amendment.

3.4 Material and Methods

3.4.1 Experimental Setup and Site Description

All sites were located in southern Manitoba and the soil samples collected in late spring (May) and at harvest time (August) in 2000. Plant samples were collected in August at the same time as the soil samples. The experimental design was a randomized complete block with ten blocks and two treatments. Each block represented a site and each site contains two treatments fields (manured field and non-manured field). These two fields were separate, but adjacent. Because the sites were located in various areas in Manitoba, each field was under the management of different producers and had different soil types

reflecting the influence of the area's soil forming factors (Table 3.1). The crops grown varied among the different fields (Table 3.2). Climatic information from the nearest weather station and more detailed site histories can be found in the Appendix I.

Four soil samples, each being a composite of ten soil cores collected to a depth of 15 cm, were randomly selected from each field treatment. The ten sub-samples of soil cores were collected from a 4 m^2 area. For row crops, five soil samples were taken in-row and five samples between-rows and combined for one composite sample. Samples were stored in polyethylene bags at 4 °C until analysis. Gravimetric moisture content was determined for each soil sample, and for each sample period. Bulk density of each sample was measured in August and used to determine volumetric moisture content prior to any soil analysis (Table 3.2). A portion of the refrigerated soil was air-dried and sieved through a 2mm mesh high-speed grinding mill for soil chemical analyzes. For the remainder of the soil, the soils that were below 70% field capacity were adjusted to 70% field capacity by adding distilled water. This portion of the soil sample was used to determine biological quality. Only soils at sites 4, 6, 8, 9, and 10 were moisture adjusted. The soils were then incubated at room temperature for a 7-day period in a humidified chamber before performing the selected microbial tests.

Table 3.1 Soil classification and past manure management.

Site	Dominant Soil series		Canadian soil classification		Livestock Manure	Manure history First application; frequency	
	Non-manured	Manured	Non-manured	Manured	Type	Non-manured	Manured
1	Taggart	Taggart	Gleyed Carbonated Rego Black	Gleyed Carbonated Rego Black	Feed Cattle	Never	1930's; every three years
2	Marquette	Marquette	Gleyed Rego Black	Gleyed Rego Black	Hog	1960; possibly 3 or 4 yrs apart	1960's; every year
3	Bower	Bower	Gleyed Black	Gleyed Black	Feed Cattle	Never	1986; every three years
4	Osborne	Scanterbury	Rego Humic Gleysol	Gleyed Black	Hog	Never to knowledge	1910's; every year
5	Red River	Red River	Gleyed Rego Black	Gleyed Rego Black	Dairy Cattle	Never	1930's; every year
6	Agassiz	Agassiz	Orthic Black	Orthic Black	Dairy Cattle	Never	1965 or earlier; every two year
7	Osborne	Scanterbury	Rego Humic Gleysol	Gleyed Black	Hog	Only once in 1998	1950's; every year
8	Ramada	Joyale	Orthic Black	Gleyed Rego Black	Hog	Never to knowledge	1970's; every three years
9	Niverville	Dencress	Gleyed Carbonated Rego Black	Gleyed Rego Black	Dairy Cattle	1985; every three years	1920's; every year
10	Aneda	Glenhope	Orthic Dark Gray	Gleyed Carbonated Rego Black	Hog/ Feed cattle	Never	1982; every year

Table 3.2 Standing crop and soil physical properties. Statistical significance can be seen in Appendix D.

Date Treatment Site	Crop		Texture		Volumetric H_2O (%) May		Volumetric H_2O (%) August		70% Field Capacity %		Bulk Density kg m^{-3}	
	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured
1	Wheat	Barley	L	L	27.1	32.5	29.0	34.8	18.7	22.5	1.16	1.07
2	Barley	Canola	C	C	34.2	38.6	35.5	29.4	23.1	24.1	1.05	0.83
3	Winter Wheat	Canola	C	CL	31.0	31.7	25.2	26.2	23.1	22.0	1.04	1.04
4	Wheat	Wheat	HC	HC	49.5	53.2	47.0	38.2	35.5	39.8	0.86	0.78
5	Canola	Corn	HC	HC	45.9	45.6	40.4	40.1	33.2	35.8	0.93	0.86
6	Canola	Corn	SL	SL	18.3	25.2	14.6	22.3	17.9	20.3	1.24	1.01
7	Canola	Corn	HC	HC	50.3	41.6	35.4	34.6	38.3	28.9	0.75	0.89
8	Barley	Wheat	SiL	L	31.4	30.2	21.8	17.8	27.5	20.8	0.84	0.94
9	Barley	Corn	C	C	29.4	30.6	28.8	24.3	27.3	26.7	0.96	0.91
10	Barley	Barley	SCL	SCL	17.7	24.2	14.6	17.5	20.1	16.4	1.19	1.09

3.4.2 Soil analysis

Detailed summary of procedures used for selected biological assays can be found in Appendix II. The chloroform fumigation-extraction method was used to determine the soil's microbial biomass carbon and nitrogen. This method and calculations are outlined by Voroney et al. (1993). Extractable organic C from the unfumigated samples was also included (Table 3.3) as a measure of available organic carbon. Glutaminase and urease soil enzyme contents were determined using a modified method of Frankenberger and Tabatabai (1995) where a NH_4^+ electrode was used to measure ammonium-N. Dehydrogenase and alkaline phosphatase contents were determined using the methods of Tabatabai (1982). Basal respiration was determined by incubating 100 g of moist soil in a 500 mL sealed mason jar. A 15 mL headspace sample was removed at 0, 4 and 8 hours and stored in 10 mL glass vacutainer (Becton Dickenson #366530). Samples were analyzed on a gas chromatograph (Varian 3800, Walnut Creek, CA), calculated and expressed as $\mu L\ CO_2\ g^{-1}$dry soil hr^{-1}. Air-dried soil samples were analyzed by Norwest Labs, Winnipeg, MB for used to measure pH, EC (both 1:2 soil:water ratio), nitrate (dilute $CaCl_2$ extraction, analyzed by cadmium reduction), sulphfate (dilute $CaCl_2$ extraction), potassium (modified Kelowna), phosphorus (modified Kelowna), and % organic carbon contents (modified Wakely Black). Organic carbon, total carbon, and total nitrogen were analyzed using dry combustion (LECO Corporation, St. Joseph, MI). Some of these values are shown in Tables 4.3 to 4.8. Four manure samples were collected from each site in the fall of 2000 to represent typical manure quantities qualities that may be applied to each of the fields under study. Their analysis by Norwest Labs, Lethbridge, AB included moisture, EC (1:10 water), pH (1:10 water), ammonium (dilute $CaCl_2$ extraction, analyzed colorometrically) , organic nitrogen (buffered distillation process), total nitrogen (dry combustion), nitrite + nitrate (dilute $CaCl_2$ extraction,

analyzed by cadmium reduction), phosphorus (0.5 M acetic acid solution extraction, analyzed by inductively coupled plasma emission spectrometry (ICP)), Complete results for manure and soil samples are presented in the Appendix III and IV, respectively. All relevant analyses were expressed on an oven dry basis.

3.4.3 Statistical analyses

A two-way analysis of variance was conducted between site x treatment (no-manure or manure application) and selected soil biochemical and microbial parameters using JMP IN 5 software (© 2005 SAS Institute, Inc.). Where site x treatment effects were significant, an one-way analysis of variance was done. All significantly different means for selected parameters were tested for Least Significant Difference (LSD) with the Tukey-Kramer Honestly Significant Difference (HSD). Results' tables are organized from highest to lowest mean values. Statistical analysis of general soil characteristics are found in Appendix IV. All values are means from four replicate soil samples. Texture, manure application history, type of manure applied and current crop were included for the selected biochemical and microbial parameters to allow observation of possible trends. Sites 2, 4, 5, 6, 7, and 9 were considered to have longer manure application histories (> 25 years of manure application). The current owner of site 6 suspects the manure-amended field as having more than 25 years of manure application. When this farming operation was purchased in 1965, it was already a well-established operation. Texture classes were also simplified to fine and coarse textured soil. Sites 1, 6, 8, and 10 were considered coarse textured soils (clay < 30%). Sites 2, 3, 4, 5, 7, and 9 were treated as fine-textured soils (clay > 30%). Sites 1, 3, 5, 6, and 9 received cattle manure, and the remaining sites received hog manure.

Table 3.3 Soil physical and chemical properties from the top 15 cm of soil. Statistical significance can be seen in Appendix D.

Treatment	Soil Organic C (%)		Soil Total N (%)		pH		EC (dS m^{-1})		Extractable organic carbon (EOC) μg C g^{-1} soil			
									May		August	
	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured
1	3.28	3.70	0.352	0.375	6.9	7.4	0.6	1.0	40.0	62.4	41.8	64.1
2	3.94	4.35	0.381	0.401	7.4	7.6	1.2	1.3	55.8	69.2	42.5	60.6
3	3.76	3.67	0.368	0.398	7.5	7.7	0.9	2.7	52.7	66.5	43.4	75.4
4	5.66	6.44	0.537	0.444	6.5	6.6	2.0	2.6	29.6	63.5	12.8	35.6
5	5.89	6.16	0.479	0.542	6.9	7.4	1.3	1.5	45.5	111.7	29.9	54.7
6	2.28	3.34	0.338	0.273	7.6	7.2	1.7	1.6	19.8	25.7	35.7	63.3
7	5.34	5.10	0.444	0.423	7.5	7.1	2.6	1.8	45.8	54.7	31.2	29.9
8	3.80	3.28	0.342	0.388	7.4	6.6	1.0	1.4	66.0	66.0	58.9	88.6
9	4.71	4.81	0.454	0.462	7.8	7.8	2.8	1.2	77.9	77.85	77.5	72.2
10	2.29	2.09	0.268	0.249	7.6	7.5	0.7	1.2	48.5	48.6	40.1	38.8

Table 3.4 Soil nutrient concentrations in top 15 cm of soil. Statistical significance can be seen in Appendix IV.

Treatment	Nitrate				Phosphorus			
	May		August		May		August	
	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured
Site	----------kg N ha^{-1}----------				----------kg P ha^{-1}----------			
1	35	40	36	92	67	79	53	118
2	86	65	29	21	210	78	136	125
3	34	77	27	26	101	54	73	63
4	113	145	31	34	85	403	65	407
5	75	66	27	30	57	196	41	98
6	103	92	25	48	32	134	25	148
7	71	65	15	23	91	141	64	103
8	39	223	12	27	38	170	37	164
9	104	47	17	38	146	653	101	653
10	53	81	21	15	33	478	62	253

Table 3.5 Soil nutrient concentrations from top 15 cm of soil con't. Statistical significance can be seen in Appendix IV.

	Potassium				Sulphate			
	May		August		May		August	
Treatment	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured	Non-manured	Manured
Site								
	----------kg K ha^{-1}----------				----------kg S ha^{-1}----------			
1	843	580	554	1056	16	21	7	20
2	699	638	827	888	47	6	4	7
3	812	581	710	682	397	22	10	41
4	1165	1435	964	1186	23	37	15	19
5	1075	1357	892	828	9	20	5	30
6	295	1000	215	991	247	151	25	48
7	946	882	711	719	121	10	11	15
8	518	909	477	625	59	41	173	29
9	519	1463	385	1547	323	9	63	9
10	263	599	246	514	5	15	4	4

3.5 Results

3.5.1 Soil microbial biomass carbon (MBC)

The August soil samples generally had higher mean MBC content than May sampled soils (Table 3.6 and 3.7). Not all the sites responded to manure addition in the same way (site x treatment, May $P < 0.0001$, August <0.001). Thus, the effect of manure

application was site dependent. Among the manure-amended fields, fields with longer manure management histories generally had higher levels of MBC than soils with no manure application for both sample dates. Finer textured soils also tend to have higher levels of MBC than coarser-textured soils for both sample dates. The type of livestock manure applied or the current crop grown in did not demonstrate a specific trend.

Table 3.6 Mean microbial biomass carbon of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture †	Manure History‡	Manure Type§
	----------μg C g^{-1} soil----------						
7	1491 *canola*	a	1019 *corn*	abcde	F	L	H
9	752 *barley*	defg	1394 *corn*	ab	F	L	C
4	989 *wheat*	bcde	1254 *wheat*	abc	F	L	H
5	866 *canola*	cdefg	1072 *corn*	abcd	F	L	C
3	960 *winter wheat*	bcde	754 *canola*	defg	F	L	C
1	619 *wheat*	defg	915 *barley*	bcdef	C	S	C
10	586 *barley*	efg	838 *barley*	cdefg	C	S	H
6	556 *canola*	efg	788 *corn*	cdefg	C	L	C
2	552 *barley*	efg	692 *canola*	defg	F	L	H
8	467 *barley*	fg	427 *wheat*	g	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.01
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 3.7 Mean microbial biomass carbon of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------μg C g^{-1} soil----------						
9	1651 *canola*	ab	1699 *canola*	a	F	L	C
4	1098 *barley*	cdefg	1581 *corn*	abc	F	L	H
7	1493 *wheat*	abcd	1196 *wheat*	abcdef	F	L	H
5	1187 *canola*	bcdef	1405 *corn*	abcde	F	L	C
3	1206 *winter wheat*	abcdef	1233 *canola*	abcdef	F	S	C
2	1113 *wheat*	cedfg	920 *barley*	efgh	F	L	H
1	754 *barley*	fgh	1077 *barley*	defg	C	S	C
10	1027 *canola*	defg	755 *corn*	fgh	C	S	H
6	457 *barley*	h	764 *canola*	fgh	C	L	C
8	634 *barely*	gh	759 *wheat*	fgh	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.2 Microbial Biomass Nitrogen (MBN)

The analysis of variance found that the sites were significantly different (sites, May and August, P ≤0.0001), but not all the sites reacted the same way for MBN (sites x treatment, May, P ≤0.001, August P<0.0001) (Table 3.8, and 3.9). Soils with longer manure application history and finer texture exhibited higher levels of MBN for both

dates. The type of manure applied and the type of crop grown did demonstrate minor influences on MBN levels. Fields applied with cattle manure and cropped to corn tended to have higher levels of MBN.

Table 3.8 Mean microbial biomass nitrogen of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------μg N g^{-1} soil----------						
6	281 *canola*	abc	497 *corn*	a	F	L	C
7	369 *canola*	ab	168 *corn*	bcde	F	L	H
9	208 *barley*	bcde	350 *corn*	ab	F	L	C
3	262 *winter wheat*	abcd	247 *canola*	abcd	F	S	C
5	183 *canola*	bcde	259 *corn*	abcd	F	L	C
4	179 *wheat*	bcde	248 *wheat*	abcd	F	L	H
1	43 *wheat*	cde	214 *barley*	bcde	C	S	C
2	128 *barley*	bcde	198 *canola*	bcde	F	L	H
10	-14 *barley*	e	131 *barley*	bcde	C	S	H
8	9.5 *barley*	de	-9.5 *wheat*	e	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.01
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 3.9 Mean microbial biomass nitrogen of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------μg N g^{-1} soil----------						
2	434 *barley*	a	316 *canola*	ab	F	L	C
4	221 *wheat*	bcdef	348 *wheat*	ab	F	L	H
3	333 *winter wheat*	ab	281 *canola*	bc	F	L	C
5	306 *canola*	ab	320 *corn*	ab	F	S	C
9	265 *barley*	bcd	144 *corn*	def	F	L	C
7	227 *canola*	bcd	126 *corn*	ef	F	L	H
10	166 *barley*	cdef	93 *barley*	f	C	S	C
8	159 *barley*	cdef	95 *wheat*	ef	F	L	H
6	109 *canola*	ef	152 *corn*	cedf	C	S	H
1	130 *wheat*	ef	132 *barley*	ef	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.01
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.3 Basal Respiration

The results for soil respiration in this project were highly variable (Table 3.10).

Observations were not normally distributed and therefore the data was log transformed.

There was a significant site x treatment effect ($P \leq 0.05$). Thus, each site responded

differently to manure application. Soil texture, type of livestock, and field manure history did not have a significant influence on soil respiration.

Table 3.10 Mean basal respiration of soil samples (0-15 cm) among various sites for May. Soil respiration levels as a measurement of labile carbon source and activity. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------mg CO_2 g^{-1} soil hr^{-1}----------						
1	363 *wheat*	abc	466 *barley*	a	C	S	C
4	436 *wheat*	ab	235 *wheat*	abc	F	L	H
7	74 *canola*	c	310 *corn*	abc	F	L	H
6	76 *canola*	c	270 *corn*	abc	C	L	C
8	238 *barley*	abc	167 *wheat*	abc	C	S	H
3	198 *winter wheat*	abc	177 *canola*	abc	F	S	C
5	198 *canola*	abc	153 *corn*	abc	F	L	C
2	181 *barley*	abc	125 *canola*	bc	F	L	H
10	169 *barley*	c	99 *barley*	bc	C	S	H
9	56 *barley*	c	91 *corn*	c	F	L	C

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.05

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.4 Dehydrogenase

Site effects greatly affected dehydrogenase responses to manure application in the May soil (site x treatment, $P \leq 0.0001$), but not in the soils sampled in August (site x treatment, ns) (Table 3.11 and 3. 12). The impact of manure addition was most significant in the August samples (treatment, $P \leq 0.05$), and not for the May samples. Sites receiving cattle manure tended to have higher dehydrogenase content than those receiving hog manure, particularly in the samples collected in May. In comparing long-term manure application histories to short-term in manure-applied fields, no differences were seen in the dehydrogenase enzyme responses. Coarse textured soils tended to have higher dehydrogenase responses than fine textured soils, especially in the August sampled soils. Current crop also did not appear to affect the dehydrogenase responses on either sampling dates.

Table 3.11 Mean dehydrogenase enzyme content of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- TPF ug g^{-1} soil hr^{-1} ----------						
9	11.37 *barley*	a	7.01 *corn*	abcd	F	L	C
3	3.37 *winter wheat*	cde	8.38 *canola*	ab	F	S	C
10	8.32 *barley*	ab	5.85 *barley*	bcde	C	S	H
6	5.98 *canola*	bcde	7.57 *corn*	abc	C	L	C
1	3.56 *wheat*	cde	7.19 *barley*	abc	C	S	C
8	5.66 *barley*	bcde	3.54 *wheat*	cde	C	S	H
2	4.16 *barley*	bcde	4.59 *canola*	bcde	F	L	H
5	3.07 *canola*	cde	4.46 *corn*	bcde	F	L	C
7	3.52 *canola*	cde	2.44 *corn*	de	F	L	H
4	1.80 *wheat*	*e*	2.00 *wheat*	e	F	L	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 3.12 Mean dehydrogenase enzyme content of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*	Manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	---------- TPF ug g^{-1} soil hr^{-1} ----------						
1	3.15 *wheat*	7.83 *barley*	5.49	ab	C	S	C
10	6.65 *barley*	7.58 *barley*	7.12	a	C	S	H
3	4.21 *winter wheat*	6.72 *canola*	5.47	ab	F	S	C
6	3.67 *canola*	6.40 *corn*	5.04	abcd	C	L	C
9	5.27 *barley*	5.19 *corn*	5.23	abc	F	L	C
2	3.65 *barley*	4.47 *canola*	4.06	bcde	F	L	H
5	2.51 *canola*	3.88 *corn*	3.20	bcde	F	L	C
4	2.39 *wheat*	3.64 *wheat*	3.02	cde	F	L	H
7	2.87 *canola*	2.51 *corn*	2.69	de	F	L	H
8	2.42 *barley*	2.73 *wheat*	2.58	e	C	S	H
Treatment	3.68 a	5.49 b					

ANOVA

	df	Prob>F
Site	9	<0.05
Treatment	1	<0.05
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.5 Glutaminase

The nitrogen mineralizing soil enzyme glutaminase was found to have significant differences between the different sites (May, $P \leq 0.0001$; August, $P \leq 0.01$) (Table 3. 13 and 3.14). Although the overall average for the manure-amended soils was higher than the non-manured soils, only the August samples exhibited a significant treatment effect ($P<0.01$). The values from the enzyme assay analysis were notably higher for the August treatment ($P \leq 0.0001$). Glutaminase enzyme potential for May ranged from 0.13 to 80 mg NH_4-N kg^{-1} soil hr^{-1} with an average of 22 mg NH_4-N kg^{-1} soil hr^{-1}. Whereas for August, the glutaminase values ranged from 4.8 to 360 mg NH_4-N kg^{-1} soil hr^{-1} with an average of 86 mg NH_4-N kg^{-1} soil hr^{-1}. Soils amended with cattle manure showed higher glutaminase content than hog manure-amended soils. Soils with long-term manure application exhibited higher levels of glutaminase enzyme contents in the May soil samples. The history of manure application did not appear to affect soil glutaminase enzyme contents in the August samples. The type of crop grown also influenced soil glutaminase contents in the May sampling. Soils cropped to corn had significantly higher glutaminase content for both sample dates relative to fields cropped to cereals or pulses, respectively.

Table 3.13 Mean glutaminase enzyme potential of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*	Manured *Crop*	Site Overall		Texture †	Manure History‡	Manure Type§
	----------mg NH_4-N kg^{-1} soil hr^{-1}----------						
5	35.3 *canola*	44.0 *corn*	39.7	a	F	L	C
6	30.0 *canola*	41.3 *corn*	35.7	ab	C	L	C
9	32.6 *barley*	40.4 *corn*	36.5	ab	F	L	C
2	30.9 *barley*	30.3 *canola*	30.6	abc	F	L	H
8	27.6 *barley*	25.2 *barley*	26.4	bc	C	S	H
10	18.5 *barley*	25.1 *barley*	21.8	cd	C	S	H
7	19.1 *canola*	8.3 *corn*	13.7	de	C	S	C
3	12.6 *winter wheat*	11.9 *canola*	12.3	de	F	S	C
7	4.8 *canola*	6.9 *corn*	5.9	e	F	L	H
4	1.2 *wheat*	3.7 *wheat*	2.5	e	F	L	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 3.14 Mean glutaminase enzyme potential of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*	Manured *Crop*	Site Overall		Texture †	Manure History‡	Manure Type§
	----------mg NH_4-N kg^{-1} soil hr^{-1}----------						
1	175.2 *wheat*	111.0 *barley*	143.1	a	C	S	C
9	91.2 *barley*	154.6 *corn*	122.9	ab	F	L	C
6	44.4 *canola*	143.8 *corn*	94.1	abc	C	L	C
5	83.3 *canola*	126.1 *corn*	104.7	abc	F	L	C
3	95.1 *winter wheat*	86.1 *canola*	90.6	abc	F	S	C
10	67.6 *barley*	74.9 *barley*	71.3	bc	C	S	H
8	70.8 *barley*	60.2 *wheat*	65.5	bc	C	S	H
4	58.6 *wheat*	69.3 *wheat*	64.0	bc	F	L	H
2	33.3 *barley*	62.5 *canola*	47.9	c	F	L	H
7	43.5 *canola*	58.9 *corn*	51.2	c	F	L	H
Treatment	76.3 a	94.7 b					

ANOVA

	df	Prob>F
Site	9	<0.01
Treatment	1	<0.01
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.6 Urease

Urease enzyme potential was found to differ greatly among sites (May, $P \leq 0.0001$; August, $P \leq 0.01$) (Table 3.15 and 3.16). Site effects also influenced the urease enzyme responses to manure in the May sampling (site x treatment, $P<0.0001$). Urease increased from seeding time (May 25.7 mg NH_4-N kg^{-1} soil h^{-1}) to harvest, August 57.5 mg NH_4-N kg^{-1} soil h^{-1}). The potential urease activity ranged from 3.7to 126 mg NH_4-N kg^{-1} soil hr^{-1}. For both sample dates, manure application tended to increase the soil's urease enzyme content in most cases. On average, the manure-amended soils had a higher urease response (63 mg NH_4-N kg^{-1} soil hr^{-1}) than the non-amended field (52 mg NH_4-N kg^{-1} soil hr^{-1}) but this difference was only statistically significant at two sites (Table 31.5). Soils amended with livestock manure of different types did not demonstrate significant differences in urease content. Long-term manure application influenced the soil's urease content in May, but this effect wasn't seen in the August samples. Fine-textured soils also showed higher urease enzyme potentials in the May samples. Texture alone did not show significant differences in urease content. Planted crop influences on urease enzyme potential were difficult to ascertain.

Table 3.15 Mean urease enzyme potential of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture †	Manure History‡	Manure Type§
	--------mg NH_4-N kg^{-1} soil hr^{-1}---------						
7	42.5 *canola*	a	30.0 *corn*	abc	F	L	H
5	22.4 *canola*	cd	40.8 *corn*	a	F	L	C
4	19.2 *wheat*	cd	40.7 *wheat*	a	F	L	H
2	37.9 *barley*	ab	30.0 *canola*	abc	F	L	H
6	17.9 *canola*	abc	30.5 *corn*	abc	C	L	C
8	24.5 *barley*	bcd	21.4 *wheat*	cd	C	S	H
10	24.1 *barley*	bcd	21.3 *barley*	cd	C	S	H
9	23.6 *barley*	bcd	22.4 *corn*	cd	F	L	C
3	13.7 *winter wheat*	d	19.0 *canola*	cd	F	S	C
1	12.7 *wheat*	d	17.5 *barley*	cd	C	S	C

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.01
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 3.16 Mean urease enzyme potential of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*	Manured *Crop*	Site Overall		Texture †	Manure History‡	Manure Type§
	----------mg NH_4-N kg^{-1} soil hr^{-1}----------						
4	60.2 *wheat*	89.6 *wheat*	74.9	a	F	L	H
5	59.3 *canola*	81.9 *corn*	70.6	a	F	L	C
1	46.7 *wheat*	72.4 *barley*	59.6	abc	C	S	C
3	52.0 *winter wheat*	70.2 *canola*	61.1	abc	F	S	C
2	67.2 *barley*	67.9 *canola*	67.6	ab	F	L	H
10	67.8 *barley*	56.1 *barley*	62.0	abc	C	S	H
6	32.8 *canola*	57.7 *corn*	45.3	cde	C	L	C
9	51.7 *barley*	49.9 *corn*	50.8	bcde	F	L	C
8	32.2 *barley*	50.8 *wheat*	41.5	de	C	S	H
7	46.7 *canola*	36.0 *corn*	41.4	e	F	L	H
Treatment	51.7 a	63.3 b					
ANOVA							
	df	Prob>F					
Site	9	<0.01					
Treatment	1	<0.01					
Sitex Treatment	9	ns					

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.7 Alkaline Phosphatase

Soils sampled in May showed differences among sites and their responses to manure application (site P $\leq$0.001; site x treatment P $\leq$0.001) (Table 3.17). Fine-textured soils from August tended to have higher phosphatase potentials (Table 3.18). These soils were also generally cropped to corn. No trends were observed among phosphatase enzyme content means for different current crop or the type of livestock manure applied. Although soils planted to barley had lower observable phosphatase potentials.

Table 3.17 Mean phosphatase enzyme content of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P=0.05).

Site	Non-manured *Crop*		Manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- PNP *u*g g soil $^{-1}$ h $^{-1}$----------						
4	243 *wheat*	bcde	384 *wheat*	a	F	L	H
7	313 *canola*	ab	306 *corn*	abc	F	L	H
3	228 *winter wheat*	bcde	264 *canola*	abcd	F	S	C
6	176 *canola*	cdef	238 *corn*	bcde	C	L	C
1	165 *wheat*	def	222 *barley*	bcde	C	S	C
8	220 *barley*	bcde	164 *wheat*	def	C	S	H
5	179 *canola*	cdef	216 *corn*	bcde	F	L	C
2	171 *barley*	def	189 *canola*	bcdef	F	L	H
10	169 *barley*	def	72 *barley*	f	C	S	H
9	157 *barley*	def	111 *corn*	ef	F	L	C

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 3.18 Mean phosphatase enzyme content of soil samples (0-15 cm) among various sites for August.

Site	Non-manured *Crop*	Manured *Crop*	Texture†	Manure History‡	Manure Type§
	---------- PNP *u*g g soil $^{-1}$ h $^{-1}$----------				
4	172 *wheat*	258 *wheat*	F	L	H
9	243 *barley*	200 *corn*	F	L	C
5	203 *canola*	228 *corn*	F	L	C
7	222 *canola*	166 *corn*	F	L	H
1	127 *wheat*	212 *barley*	C	S	C
10	199 *barley*	123 *barley*	C	S	H
2	132 *barley*	192 *canola*	F	L	H
3	176 *winter wheat*	184 *canola*	F	S	C
6	110 *canola*	181 *corn*	C	L	C
8	160 *barley*	161 *wheat*	C	S	H

ANOVA

	df	Prob>F
Site	9	ns
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

3.5.8 Correlations among parameters

At the beginning of the growing season (May), significant correlations were observed among the microbial parameters and between microbial and soil parameters more frequently than at harvest (August). The number of statistically significant correlations between microbial parameters was greater in the soil samples collected from the non-manured fields (Table 3.19) than in those collected from the manure-amended fields (Table 3.20) for May. However, soils sampled in May showed stronger correlations for manure-amended fields than those not receiving manure. Organic C was not correlated with any of the microbial parameters in the non-manured field. Total N was correlated with MBC, MBN and phosphatase in the non-manured fields (Table 3.21). In the manure-amended field, both organic C and total N were significantly correlated with MBC and urease (Table 3.22). Soil ammonium, sulfate, phosphate, pH and EC levels exhibited minimal and inconsistent relationships with soil microbial parameters in all sites. Total N, nitrate, OM and soil texture were correlated with microbial parameters to varying degrees. Microbial biomass C had stronger correlations with soil enzyme responses in the non-amended fields.

At harvest time, there were few correlations amongst the microbial parameters. MBC levels in the August non-manure amended samples showed greater correlations to MBN, urease and phosphatase (Table 3.23). Correlations among the microbial parameters in the manure-amended field samples showed greater range in the fall sampling (Table 3.24). Urease and MBN were strongly correlated in the soils collected from manure-amended fields in the August sampling. In contrast to the May sampling, the organic C was highly

correlated with MBC, MBN and phosphatase in the soils collected from the non-manure amended fields (Table 3.25). MBC, MBN and phosphatase had the most numerous strong correlations with our selected soil parameters. In soils collected from the manure-amended fields, only the MBC showed more significant correlations with the selected soil parameters (Table 3.26). Urease and dehydrogenase responses were not highly correlated with other microbial parameters in the August sampling. Glutaminase did not correlate with any soil microbial parameters in the August sampling.

Overall, many of the parameters were positively correlated with clay content. MBC had the most positive correlations with all the parameters. Dehydrogenase and glutaminase also showed a greater tendency for negative correlation, when correlations were significant. Soil respiration measurements were not well correlated with dehydrogenase.

Table 3.19 Coefficients of pair-wise correlation (r) matrix for microbial parameters for 10 Manitoba soils May 2000 in non-manured field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase
MBN	0.68***				
Dehydrogenase	ns	ns			
Urease	0.38*	ns	ns		
Glutaminase	-0.43**	ns	0.40*	ns	
Phosphatase	0.59***	0.48**	ns	0.42**	ns

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 3.20 Coefficients of pair-wise correlation (r) matrix for microbial parameters for 10 Manitoba soils May 2000 in manure-amended field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase
MBN	0.42**				
Dehydrogenase	ns	0.36*			
Urease	0.33*	ns	-0.42**		
Glutaminase	ns	ns	ns	ns	
Phosphatase	ns	ns	-0.32*	0.47**	-0.45**

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 3.21 Coefficients of pair-wise correlation (r) matrix between microbial and soil parameters for 10 Manitoba soils May 2000 in non-manured field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase	Phosphatase
% Organic C	ns	ns	ns	ns	ns	ns
% Total N	0.43**	0.32*	ns	ns	ns	0.37*
Nitrate	0.50***	ns	-0.47**	ns	-0.42**	0.38*
Ammonia	ns	ns	ns	ns	ns	ns
Phosphate	ns	ns	ns	0.46**	ns	ns
Potassium	0.62***	0.38*	-0.55***	ns	ns	0.46**
Sulphate	ns	0.39*	0.37*	ns	ns	ns
%Clay	0.72***	0.47**	-0.36*	0.35*	ns	0.33*
%Sand	-0.61**	ns	ns	ns	ns	-0.37*
pH	ns	ns	0.64**	ns	ns	ns
EC	0.39*	0.59***	ns	ns	ns	ns

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 3.22 Coefficients of pair-wise correlation (r) matrix between microbial and soil parameters for 10 Manitoba soils May 2000 in manure-amended field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase	Phosphatase
% Total C	0.55***	ns	-0.37*	0.51***	ns	ns
% Total N	0.51***	ns	-0.60***	0.64***	ns	0.52***
Nitrate	0.38*	ns	ns	0.37*	-0.42**	0.47**
Ammonia	ns	ns	ns	ns	ns	ns
Phosphate	0.46**	ns	ns	ns	ns	ns
Potassium	0.57***	ns	ns	0.51***	ns	ns
Sulphate	ns	ns	-0.43**	ns	ns	ns
%OM	0.53***	ns	-0.46**	0.70***	ns	0.54***
%Clay	0.52***	ns	0.50**	0.63***	ns	0.42**
%Sand	-0.46**	ns	0.47**	-0.52***	ns	ns
pH	ns	0.35*	ns	ns	ns	ns
EC	ns	ns	ns	ns	ns	0.57***

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 3.23 Coefficients of pair-wise correlation (r) matrix for microbial parameters for 10 Manitoba soils August 2000 in non-manured field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase	Phosphatase
MBN	0.53***					
Dehydrogenase	ns	ns				
Urease	0.33*	ns	ns			
Glutaminase	ns	ns	ns	ns		
Phosphatase	0.66***	ns	ns	ns	ns	
Respiration	ns	ns	ns	ns	ns	ns

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 3.24 Coefficients of pair-wise correlation (r) matrix for microbial parameters for 10 Manitoba soils August 2000 in manure-amended field samples combined.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase	Phosphatase
MBN	0.32*					
Dehydrogenase	ns	ns				
Urease	ns	0.52***	0.36*			
Glutaminase	ns	ns	ns	ns		
Phosphatase	0.40*	0.38*	ns	0.37*	ns	
Respiration	ns	ns	ns	ns	ns	ns

ns = not significant.
*, **, *** Significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 3.25 Coefficients of pair-wise correlation (r) matrix between microbial and soil parameters for 10 Manitoba soils August 2000 in non-manured field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase	Phosphatase	Respiration
% Organic C	0.73***	0.50***	ns	ns	ns	0.61***	ns
% Total N	0.49**	0.59***	ns	ns	ns	0.45**	ns
Nitrate	0.32*	ns	ns	ns	ns	0.34*	ns
Ammonia	ns	ns	ns	ns	ns	ns	ns
Phosphate	0.33*	0.42**	0.31*	ns	ns	ns	ns
Potassium	ns	0.54***	-0.39*	ns	ns	ns	0.43**
Sulphate	0.34*	ns	ns	ns	ns	ns	ns
%Clay	0.57***	0.48**	ns	0.36*	ns	0.32*	ns
%Sand	-0.61***	-0.38**	ns	ns	ns	-0.42**	ns
pH	ns	ns	ns	ns	ns	ns	0.65***
EC	0.64***	0.46**	ns	ns	ns	0.34*	ns

ns = not significant.

*, **, *** Significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 3.26 Coefficients of pair-wise correlation (r) matrix between microbial and soil parameters for 10 Manitoban soils August 2000 in manure-amended field samples.

	MBC	MBN	Dehydrogenase	Urease	Glutaminase	Phosphatase	Respiration
% Organic C	0.68***	0.33*	ns	ns	ns	0.44**	ns
% Total N	0.65***	0.48**	ns	ns	ns	0.48**	ns
Nitrate	ns	-0.36*	ns	ns	ns	ns	0.39*
Ammonia	ns	ns	ns	ns	ns	ns	ns
Phosphate	0.58***	ns	ns	ns	ns	ns	ns
Potassium	0.48**	ns	ns	0.32*	ns	ns	ns
Sulphate	ns	ns	ns	ns	ns	ns	ns
%Clay	0.57***	0.54**	-0.39*	ns	ns	ns	ns
%Sand	-0.58***	-0.39*	0.45**	ns	ns	ns	ns
pH	ns	ns	ns	ns	ns	ns	0.40**
EC	ns	ns	ns	ns	ns	ns	ns

ns = not significant.

*, **, *** Significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

3.6 Discussion

The strongest, most evident trend among all the parameters observed in the study is the differences in responses among sites. The many site x treatment interactions emphasizes how many of the parameters react differently to manure application at various sites. There are many factors that influence the productivity and biochemical characteristics of a soil. These same factors also influence the biological composition of the soil. Climatic differences, soil physical and chemical properties and other organisms all affect the activity of the microbial community resulting in site-specific differences. It is not surprising that these site effects are evident in this study and that the responses to manure addition is also site specific as all of the factors influencing the microbial community also affect microbial biomass carbon and nitrogen, soil enzymatic response and many other biological parameters.

Manure application influenced microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), dehydrogenase, and urease contents for some sites on at least one of the sample dates. At most sites, soils sampled in May exhibited significantly greater MBC levels in the manure-amended soil than non-manure amended soils. Other researchers have found that MBC levels rise with the application of organic amendments (Fauci and Dick, 1994; Zaman et al., 1999a, 1999b), including manure (Lalande et al., 2000). If microbial biomass C were well correlated to gross N mineralization and it may be a useful indicator of soil fertility as shown by Zaman et al. (1999a).

Soil microbial biomass nitrogen (MBN) levels were strongly impacted by manure application. Although in general, manure-amended sites often possessed higher levels of MBN in the spring, the non-manure amended samples frequently had higher levels in the August samples. Fields with a longer history of manure application had higher levels of MBN. Zaman et al. (1999a) found that mineral N can increase MBN levels because ammonium can be readily assimilated by microorganisms depending on available C substrate levels. MBN levels in this study were not correlated to nitrate or ammonium levels, and both of which were higher, but varied among sites in the August sampled manure-amended soils. MBN did not prove to be useful in distinguishing the impact of manure amendment on soil nitrogen status.

Dehydrogenase content was significantly higher in the fall, and manure application effects were also visible at that time. The increased levels of microbial activity, as indicated by dehydrogenase, could be attributed to increased levels of soil organic C and inputs of available soil C from manure application (Kandeler and Eder, 1993; Lalande et al., 2000; Marinari et al., 2000). There was also some distinction between sites with a history of long-term vs. shorter-term manure applications, where short-term manure application histories had higher levels of dehydrogenase content. However, soil texture may have played a greater influence on this observable trend.

Measured soil urease content was significantly increased by manure application as indicated by a manure treatment effect in the August samples. Other research has also found this to be the case (Zaman et al., 1999b; Lalande et al., 2000). In addition, our

study observed that the influence of manure application on urease content was greater for sites with long-term manure-amended soils in the May. Urease enzyme content has proven to be a useful tool to differentiate between manure amendment histories. The positive correlation between nitrate and urease contents in the May samples could be attributed to nitrification. As urease breaks down urea (found in manure) to ammonium, the ammonium is nitrified further into nitrate (Hasan, 2000).

Soil respiration was not responsive to manure application. Other long-term manure application studies have found that basal respiration rates were correlated with SOM and available C (Svensson and Pell, 2001). We found soil respiration to have very few correlations to chemical and biological parameters. Since greater EOC levels were observed in the manure-amended fields, differences in basal respiration were anticipated. Most measures of soil respiration include longer soil incubations than that used in our study or longer periods of time. Other researchers have found longer incubations times provide better correlation with MBC, MBN, N mineralization (Franzluebebbers et al., 1995). However, extended periods of monitoring basal respiration would not provide a convenient method of quantifying soil microbial activity and would not be a useful fertility analysis.

Manure application did not influence soil phosphatase and glutaminase contents significantly in this study. Although glutaminase is an N cycling enzyme, very little research has been conducted on glutaminase's role as a soil quality parameter and its responses to organic amendments. Other researchers have found that phosphatase

responses were greater in manure-amended soils than in non-manure amended soils due to organic phosphorus contained within the manure (Oberson et al., 1996). Our study showed correlations between phosphastase, MBC, MBN, dehydrogenase, urease, and glutaminase. Thus, the active microbial community in this study may be influencing the phosphatase responses, and not all the phosphatase content is due to abiontic soil enzymes.

The type of manure applied to soil can also affect microbial parameters. Dehydrogenase, glutaminase, and MBN generally showed increased responses to cattle manure application. Cattle manure commonly has a higher C:N ratio than hog manure (Qian and Schoenau, 2002). With more available energy sources in cattle-amended soils, it is not surprising that certain microbial parameters would be higher in these soils. Organic amendments have been shown to positively influence dehydrogenase activity (Ritz et al., 1992; Hadas et al., 1996; Albiach et al., 2000; Lalande et al., 2000; Parham et al., 2002). As most dehydrogenase responses to organic-amendments tend to be short in duration (Hadas et al., 1996), it is not surprising that the impact of manure type is only seen in the spring sampling. Although a general effect of manure application on glutaminase responses was not apparent, the effect of different manure types did. Glutaminase soil enzyme levels were consistently higher in cattle manure-amended soils in the spring and at harvest. This could be related to the higher concentration of available carbon in cattle manure (Qian and Schoenau, 2002). Manure application has also been shown to significantly increased MBN levels relative to legume or inorganic amendments (Fauci and Dick, 1994). Fauci and Dick (1994) found long-term beef manure application had a greater influence on microbial biomass than pea vine amendments. Although

inorganic fertilizer can increase MBN, manure application can increase MBN to a greater extent and for a longer duration than inorganic N amendments (Zaman et al., 1999a). MBN responses in manure-amended soil reflect microbial assimilation of N in the presence of available carbon from the manure application (Zaman et al., 1999b).

Texture had a large impact on microbial parameters. There are many factors that influence microbial interaction with different types of soil particles. Greater amounts of available water, cation exchange capacity (CEC), organic matter, and microbial activity are typically seen in fine-textured soils than coarse textured soils. Fine textured soils had higher MBC levels for all sites. Thomsen and Olesen (2000) found that fine-textured soils increased the physical protection of their nutrient substrates. So clay particles may not only protect soil nutrients, it may also protect microbial populations; keeping them more nourished and hydrated (Chenu and Stotzky, 2002). In contrast, coarse-textured soils were found to have greater dehydrogenase responses. It may be that coarse textured soils have better aeration than clay because of the larger pore sizes (Chenu and Stotzky, 2002). Coarse-textured soils also tend to have increased C mineralization from manure possible due to greater aeration and less physical protection of substrates (Thomsen and Olesen (2000). This greater aeration may have enhanced differences in dehydrogenase content between the manure and non-amended fields. Better aeration increases mineralization potential as nutrients are metabolized more efficiently.

Texture imparted the greatest influence on phosphatase enzyme content after site effects. It is not surprising that soils with higher percentages of clay would exhibit significantly

higher phosphatase responses. It is well known that soil enzymes, such as phosphatase are stabilized by clay minerals and organic matter (Burns, 1986; Gianfreda and Bollag, 1994). Not only can fine textured soils contain more active phosphatase enzymes, manure application can enhance phosphatase enzyme responses. However, phosphatase enzyme activity is recognized as being controlled by a negative feedback loop (Clarholm, 1993; Sinsbaugh et al., 1993; Tadano et al., 1993; Dormaar and Chang, 1995; Olander and Vitousek, 2000). Deficient phosphate levels would stimulate enzyme production and activity if controlled by a negative feedback mechanism. Although all sites generally had high levels of phosphate, an abundance of phosphate in the environment should of suppressed enzyme production and activity. Other researchers also failed to observe distinct expression of a negative feedback mechanism (Harrison, 1982; Adams, 1992). Although the manure-amended fine- textured soils exhibited higher phosphatase enzyme responses, they also had higher phosphate levels, but they were not well correlated. Thus, some soils may not have reached the threshold level of phosphate required to trigger enzyme inhibition for that soil's texture. The soils' accumulated, stable, abiontic phosphatases were not influenced by current P levels (Marinari et al., 2000). And lastly, the high phosphate levels could be halting further production of the phosphatase enzymes, although both phosphate and phosphatise levels were high. Higher phosphatase responses seen in fine textured soils could be offering a protective environment against soil's proteolytic enzymes and allow the accumulation of phosphatase on soil colloids over time. Microbial biomass C and N were also influenced by texture, and could also be protected and nourished in the micropore environment of finer-textured soils.

The effect of crop was most evident with corn relative to the cereals or pulses. Not only did corn produce a large amount of plant biomass, but ut us considered a high nutrient demanding crop that is frequently planted in soils receiving high rates of manure application. At some of this study's sites, producers grew corn because of its high nutrient demands as a means to counter balance the effects of excessive nutrient loading. Whether it was due to crop effects or nutrient loading, soils collected from these cornfields had higher levels of MBC, glutaminase and urease content. Other studies have found continuous corn to have the highest bacterial biomass compared to other rotations (Jordan et al, 1995). Thus, some of the aforementioned parameters may be influenced by the standing crop. Dehydrogenase and respiration were not influence by the type of standing crop. Crop influences were not observed on alkaline phosphatase. This may reflect the microbial origin of alkaline phosphatase. Acid phosphatase analyses are typically used to access phosphatase production from both plant and microorganisms (Tabatabai, 1982). Alkaline phosphatase activities are associated with bacterial populations.

3.6 Conclusions

Texture, crop, manure type, seasonal climatic variations and soil manure history all influenced microbial parameters. MBC, glutaminase, urease and dehydrogenase contents were the most sensitive to long-term manure application and could be useful in predicting soil nutrient cycling. Overall, microbial parameters appear to be site dependent as all sites were significantly differentiated. The response to manure application was also seen

to be site dependent. Site conditions are what make site and the microbial responses to them unique. As a result, approaches to soil nutrient management of manure should be responsive to varying site characteristics.

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4. NITROGEN MINERALIZATION POTENTIAL IN MANURE-AMENDED SOIL AND ITS RELATIONSHIP TO VARIOUS SOIL BIOCHEMICAL AND CHEMICAL CHARACTERISTICS.

4.1 Abstract

The difficulties in predicting N mineralization in soil are compounded by the addition of manure. The relationship of various physical, biochemical and chemical indices to estimates of soil nitrogen mineralization were analyzed in ten long-term manure-amended sites and compared to adjacent fields with minimal to no manure history. Parameters included KCl extractable NH_4^+, mineral N production during a seven day laboratory incubation, the content of urease, glutaminase, phosphatase enzymes in soil, soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), NO_3^-, S, P, K, pH, electrical conductivity (EC), organic carbon content (OC), extractable organic carbon content (EOC), and total N. The influence of site, manure application history, crop, manure type, manure application duration, and texture differences was observed. Site was the most significant factor in this study, suggesting the need for a site-specific approach to mineralization modelling. A step-wise regression analyses was used to select the best predictors of plant available nitrogen, a field-based estimate of N mineralization. The significance of the components in the regression equations varied between manure and non-amended soils. The most significant variables influencing N mineralization in the manure-amended soils were MBN, urease, organic carbon, pH and sand content (R^2 =

0.76). In sites not receiving manure, the most significant variables influencing N mineralization were soil NH_4^+, NO_3^-, urease and glutaminase contents ($R^2 = 0.80$). Thus, not only are biological parameters influential in predicting field N mineralization, physical and chemical soil characteristics were also significant. This further enforces the need for a broad approach to predicting N mineralization that may require examination of individual site and manure characteristics.

4.2 Introduction

In Manitoba, there is a growing trend towards large intensive livestock operations (ILOs). These operations tend to produce large volumes of manure in a concentrated area. For example, in 2002 the rural municipality of Hanover had 430 ILOs in an area less than 746 km^2 (Gibson, 2002). In some cases, a small associated landbase has resulted in application of manure in excess of crop nutrient requirements either through the application of high rates of manure or repeated application to the same land, particularly land adjacent to the manure source. High rates of manure application affect the soil's chemical, physical and biological properties including increased N mineralization potential (Whalen et al., 2001). Understanding N mineralization in manure-amended soils is important to prevent the over-application of nutrients to soil systems and protect against environmental damage (Chang and Janzen, 1996). Conversely, properly utilized manure is a valuable resource improving soil quality and fertility.

The efficient use of nutrients in crop production requires that sufficient nutrient is available to support plant growth, while avoiding the accumulation of excess nutrients in forms that may have an adverse impact on the environment. One of the challenges in the efficient utilization of the nutrients contained in manure is accurately predicting the release of nutrients from organic forms. For decades researchers have been attempting to measure and predict N mineralization in soil. Many factors influence N mineralization *in situ*. In this paper, we will examine a variety of parameters relevant to nitrogen mineralization and assess their ability to predict plant available nitrogen, a field-based estimate of nitrogen mineralization. In addition the impact of long-term manure application on these relationships will be assessed.

4.3 Objective of the Study

The purpose of this study is to assess the sensitivity of various chemical indices to manure application and to assess the degree to which these indices may be used to in predicting plant available nitrogen, a field-based estimate of N mineralization.

4.4 Material and Methods

4.4.1 Experimental Setup and Site Description

The experimental design of this project was previously described in Chapter 3 (Section 3.4.1). All sites were located in southern Manitoba and the samples collected in late spring (May) and at harvest time (August) of 2000. The experimental design was a

randomized complete block with ten blocks and two treatments per block. Each block represented a site and each site contained two treatments (manure-amended field, and non-amended field). These two fields were separate, but adjacent. Climatic information from the nearest weather station and more detailed site histories can be found in the Appendix A. Each field was under different management, had different soil types (Table 3.1) and was planted to different crops (Table 3.2). This experimental set-up is not a classical randomized block design in that cropping practices were not consistent between blocks and minor soil and field management differences existed between fields at each site. While long-term controlled manure-amendment studies across soil type would have been preferred, sites of this nature do not exist in Manitoba. Our design attempted to capture the long-term influence of manure amendment in a wide range of soils relative to soils that did not have a history of intensive manure application. The use of side-by-side producer fields was seen as the most effective means of assessment.

Four soil samples, each being a composite of ten soil cores collected to a depth of 15 cm, were randomly selected from each field treatment. The 10 soil cores were collected from a 4 m^2 area. For row crops, five samples were taken in-row and five samples between-rows and combined for each composite sample. Samples were stored in labelled polyethylene bags at 4 °C until analysis. Gravimetric moisture content of each soil sample was measured for each sample period. Bulk density of each sample was measured in August and used to determine the soil's volumetric moisture content (Table 3.2). A portion of the refrigerated soil was air-dried and passed through a 2 mm sieve. It was used to measure chemical soil parameters. To remove the effect of temperature and moisture, soils were brought to 70% of field capacity and incubated for seven days at 25

°C prior to the measurement of microbial parameters. Methodologies used to analyze microbial biomass carbon and nitrogen, glutaminase, urease, phosphatase, dehydrogenase, basal respiration and various soil and manure chemical parameters can be found in Chapter 3, Appendix III and IV.

4.4.2 KCl extractable NH_4^+

The procedure used for determining ammonium extracted by 2M KCl followed the general methods of Gianello and Bremner (1986a, b). Out of 12 chemical methods, Gianello and Bremner (1986a) found the NH_4^+ released during a 4 hr extraction in 100 °C 2M KCl and subsequent distillation (Equation 4.1) to be one of the best correlated to biological laboratory measures of N mineralization. Therefore, this method was selected for use in this study. This method involves measuring total-KCl extractable NH_4^+ and cold-KCl extractable NH_4^+ and determining the difference. These three values were used in this study (total-, hot-, and cold-KCl extractable NH_4^+). Duplicates of 3.0 g of soil were placed in 250 mL Tecator (Ensinger, Inc., Washington, PA) digestion tubes. Twenty mL of 2M KCl was added to each set of tubes. The tubes were mixed using a vortex mixer. One set was heated at 100°C for 4 h in a Tecator digestion block and was analyzed for ammonia following distillation of the KCl extraction (total-KCl extractable NH_4^+). The remaining set was left for 2 hr at room temperature and was used for the cold-KCl extraction. Normally, the cold-KCl extraction method uses 4 hr of standing at room temperature. This was shortened to allow for better lab time efficiency. The NH_4^+ content of each digest was determined using steam distillation for 5.5 minutes with 0.2 g of MgO (heavy-dried at 700 °C for two hours). Steam was passed through the distillation

unit for 2 minutes between samples. The distillate was collected in 5 mL of boric indicator solution (containing bromocreasol green and methyl red) buffered to pH of 5.2. This acidic buffer converts ammonia gas released in the distillation process to ammonium. The distillate is then back-titrated with 0.0025M H_2SO_4 using a Mettler DL 21 titrator (Mettler Instruments AG 1989, Greigensee, Switzerland). A KCl blank, distilled water blank and ammonium solution blanks (3.5 ppm, 7 ppm and 35 ppm ammonium-N) were used as quality controls.

$$[\text{Hot-KCl extractable } NH_4^+] = [\text{Total-KCl extractable } NH_4^+] - [\text{Cold-KCl extractable } NH_4^-] \quad (4.1)$$

4.4.3 Laboratory N mineralization

After a portion of each soil sample had its moisture content adjusted to 70% field capacity (FC) and was allowed to stabilize in a humidified chamber, a sub sample was taken for determining nitrate and ammonium using 0.5M K_2SO_4 as an extractant. Whalen et al. (2001) found the highest mineralization rates with 75% FC at 20°C. The humidified chamber was composed of a flat-bottom Tupperware bin with 2.5 cm of water on the bottom. The soil samples were stored in plastic drinking cups standing in the water. The soil samples were exposed to room temperature and indoor lighting for approximately 8 hr a day, 5-days a week. After 10 days, this soil was sampled and NH_4^+ and NO_3^- determined. Some researchers use a non-linear regression equations following first-order kinetics (Jalil et al., 1996: Zaman et al., 1999a, b; Deng and Tabatabai, 2000; Whalen et al., 2001). However, long incubations require constant rewetting that affects

the slopes of regression equations (Whalen et al., 2001). Therefore, in this study, a simple mass balance approach was used (4.2).

$$\text{Net N mineralized } (\mu g\ N\ g^{-1}\ \text{soil}\ d^{-1}) = \frac{[NH_4^+\text{-N} + NO_3^-\text{N}]_{final} - [NH_4^+\text{-N} + NO_3^-\text{-N}]_{initial}}{\text{Incubation period}} \quad (4.2)$$

4.4.4 Field N mineralization

Soil samples taken to 15 cm depth, shortly after seeding and prior to harvest were analyzed for NH_4^+ and NO_3^- content using 0.5M K_2SO_4 as an extracting solution. Plant biomass per square meter was sampled prior to harvest and the straw and grain sub-samples were analyzed separately for total nitrogen content (dry combustion, LECO Corporation, St. Joseph, MI). Field N mineralization was estimated using a general mass balance calculation (Equation 4.3) where:

$$\frac{[[NH_4^+\text{-N} + NO_3^-\text{N}]_{final} + \text{Plant Biomass-N}] - [NH_4^+\text{-N} + NO_3^-\text{N}]_{inital}}{\text{days of growth}}$$

$$= \text{Net field N mineralization (kg N/ha/day)} \quad (4.3)$$

4.4.6 Statistical analyses

A two-way analysis of variance was conducted between site and manure application, then site and dates of sampling using JMP IN 5 software (© 2005 SAS Institute, Inc.). Where significant site x treatment interactions were detected the 20 sites were examined using one-way analysis. A least Significant Difference (LSD) with a Tukey-Kramer Honestly Significant Difference (HSD) test was used during the one-way analyses of variance for site x treatment where the results are displayed according to numerical value. Beside each site, the manure application history, current crop grown, manure type and soil

texture are displayed. Sites 2, 4, 5, 6, 7, and 9 were considered to have longer manure application histories (> 25 years of manure application). The current owner of site 6 suspects the manure-amended field as having more than 25 years of manure application. When this farming operation was purchased in 1965, it was already a well-established operation. Texture classes were also simplified to fine and coarse textured soils. Sites 1, 6, 8, and 10 were considered more coarse textured soils (Clay < 30%). Sites 2, 3, 4, 5, 7, and 9 were regarded as having fine textured (Clay > 30%). Sites 1, 3, 5, 6, and 9 received cattle manure; the remaining sites received hog manure. Pair-wise correlations were conducted for the manure and non-amended fields separately for all the soil parameters analyzed.

4.5 Results

4.5.1 KCl extractable NH_4^+

Two KCl extraction methods were evaluated for their ability to predict mineralizable nitrogen (Gianello and Bremner,1986a, b). In this study, hot-KCl extractable NH_4^+-N refers to the difference between total-KCl extractable NH_4^+-N (NH_4^+-N released from heat digestion and distillation) and cold-KCl extractable NH_4^+-N (NH_4^+-N released from distillation). The calculated values for hot-KCl extractable NH_4^+ ranged from –18 to 48 mg NH_4-N kg^{-1} soil for all samples. Total- and cold-KCl extractable NH_4^+-N were found to be significantly higher for the May sampling than the August sampling. Hot-KCl extractable NH_4^+-N was not affected by date. Hot-KCl extractable NH_4^+-N was the only parameter that responded to the present of manure application on both sample dates (Table 4.1 and 4.2). When examining total-KCl extractable NH_4^+-N results separated by

date, site effects were more significant in the May sampling ($P \leq 0.001$) (Table 4.3) than at the August sampling ($P \leq 0.05$) (Table 4.4). A significant effect of manure addition was seen in the total-KCl extractable NH_4^+-N (May, $P \leq 0.05$) and hot-KCl extractable NH_4^+-N (both dates, $P \leq 0.05$). Soils with finer texture, longer manure application histories and soils receiving hog manure tended to have higher levels of total-KCl extractable NH_4^+-N for both sample dates. Cold-KCl extractable NH_4^+-N analyses showed significant site effects in the May samples (Table 4.5), but not in the August samples (Table 4.6). However, the August samples had a tendency for higher levels of cold-KCl extractable NH_4^+-N in fine textured soils, amended with cattle manure over along period of time. Hot-KCl extractable NH_4^+-N showed no significant site effects, but hot-KCl extractable NH_4^+-N was higher in the manure amended soils for both samples dates. Some samples had higher values for cold-KCl extractable NH_4^+-N than total-KCl extractable NH_4^+-N resulting in negative values for hot-KCl extractable NH_4^+-N. Fine textured soils with long histories of hog manure appeared to have higher levels of hot-KCl extractable NH_4^+-N.

In comparing hot-KCl extractable NH_4^+-N and cold-KCl extractable NH_4^+-N to other soil parameters, soil samples collected in the spring (May) had fewer significant correlations to various soil chemical, physical and biochemical indices (Tables 4.7 – 4.10). Significant correlations occurred more frequently among parameters in non-manured soils. Hot-KCl extractable NH_4^+-N had the fewest correlations with strongest correlations occurring in the non-amended August samples (phosphatase, 0.53***; % organic C, 0.52**; % total N, 0.49**). In addition, more microbial parameters, such as

glutaminase and phosphatase were negatively correlated to cold- and total-KCl extractable NH_4^+-N in the August non-manured samples. These included MBC, MBN, glutaminase, and phosphatase. Cold- and total-KCl extractable NH_4^+-N were positively correlated with organic C %, total N %, and clay % for most sample dates and treatments.

Table 4.1 Mean hot-KCl extractable NH_4^+-N of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA.

Site	Manured *Crop*	Non-manured *Crop*	Texture†	Manure History ‡	Manure Type§
	--------- mg NH_4-N kg^{-1} soil ---------				
4	23.0 *wheat*	17.5 *wheat*	F	L	H
5	20.7 *corn*	-0.4 *canola*	F	L	C
10	18.9 *barley*	0.97 *barley*	C	S	H
8	15.9 *wheat*	7.6 *barley*	C	S	H
3	14.4 *canola*	12.1 *winter wheat*	F	S	H
9	12.5 *corn*	8.0 *barley*	F	L	C
6	11.1 *corn*	6.3 *canola*	C	L	C
7	10.2 *corn*	6.7 *canola*	F	L	H
2	5.2 *canola*	3.4 *barley*	F	L	H
1	2.4 *barley*	3.9 *wheat*	C	S	C
Treatment	13.4 a	6.6 b			
ANOVA	df	Prob>F			
Site	9	ns			
Treatment	1	<0.05			
Site x Treatment	9	ns			

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.2 Mean hot-KCl extractable NH_4^+-N of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA.

Site	Manured *Crop*	Non-manured *Crop*	Texture†	Manure History‡	Manure Type§
	--------- mg NH_4-N kg^{-1} soil ---------				
7	18.8 *corn*	10.6 *canola*	F	L	H
4	16.9 *wheat*	17.6 *wheat*	F	L	H
1	15.5 *barley*	9.0 *wheat*	C	S	C
8	14.0 *wheat*	13.3 *barley*	C	S	H
6	13.7 *corn*	0.72 *canola*	C	L	C
5	13.6 *corn*	12.7 *canola*	F	L	C
9	12.5 *corn*	7.9 *barley*	F	L	C
2	12.1 *canola*	6.8 *barley*	F	L	H
10	11.1 *barley*	11.6 *barley*	C	S	H
3	11.5 *canola*	10.2 *winter wheat*	F	S	C
Treatment	14.0 a	10.0 b			
ANOVA	df	Prob>F			
Site	9	ns			
Treatment	1	<0.05			
Site x Treatment	9	ns			

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.3 Mean total-KCl extractable NH_4^+-N of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA.

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture †	Manure History‡	Manure Type§
	--------- mg NH_4-N kg^{-1} soil ---------						
4	86.0 *wheat*	119.2 *wheat*	102.6	a	F	L	H
7	105.5 *canola*	52.6 *canola*	79.1	a	F	L	H
5	80.1 *corn*	47.8 *canola*	63.4	bcd	F	L	C
1	76.3 *barley*	55.4 *wheat*	65.9	bc	C	S	C
3	63.0 *canola*	44.0 *winter wheat*	53.5	cd	F	S	C
8	57.7 *wheat*	46.0 *barley*	51.9	cd	C	S	H
2	50.2 *canola*	44.1 *barley*	47.2	cd	F	L	H
9	48.3 *corn*	43.4 *barley*	45.9	cd	F	L	C
10	47.2 *barley*	32.7 *barley*	40.0	d	C	S	H
6	45.6 *corn*	37.2 *canola*	41.4	cd	C	L	C
Treatment	66.0 a	52.2 b					

ANOVA	df	Prob>F
Site	9	<0.001
Treatment	1	<0.05
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.4 Mean total-KCl extractable NH_4^+-N of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA.

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture †	Manure History ‡	Manure Type§
	--------- mg NH_4-N kg^{-1} soil ---------						
3	39.6 *canola*	59.7 *winter wheat*	49.7	ab	F	S	H
7	54.1 *corn*	50.2 *canola*	52.2	ab	F	L	H
5	52.3 *corn*	53.9 *canola*	53.1	a	F	L	C
4	50.3 *wheat*	53.4 *wheat*	51.9	ab	F	L	H
9	52.2 *corn*	33.9 *barley*	43.1	abcd	F	L	C
2	47.1 *canola*	43.7 *barley*	45.4	abc	F	L	H
8	43.26 *wheat*	46.7 *barley*	45.0	abc	C	S	H
1	44.6 *barley*	32.6 *wheat*	38.6	bcd	C	S	C
6	39.9 *corn*	19.8 *canola*	29.9	d	C	L	C
10	32.4 *barley*	38.3 *barley*	35.4	cd	C	S	H

ANOVA	df	Prob>F
Site	9	<0.05
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.5 Mean cold-KCl extractable NH_4^+-N of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA.

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture †	Manure History‡	Manure Type§
	--------- mg NH_4-N kg^{-1} soil ---------						
4	63.0 *wheat*	112.0 *wheat*	87.5	a	F	L	H
7	95.3 *corn*	53.6 *canola*	74.5	ab	F	L	H
1	74.0 *barley*	42.5 *wheat*	58.3	bc	C	S	C
5	59.4 *corn*	56.1 *canola*	57.8	bc	F	L	C
3	48.6 *canola*	39.4 *winter wheat*	44.0	cd	F	S	C
2	45.1 *canola*	43.9 *barley*	44.5	cd	F	L	H
8	41.8 *wheat*	35.7 *barley*	38.8	cd	C	S	H
9	35.8 *corn*	33.4 *barley*	34.6	cd	F	L	C
6	34.4 *corn*	33.3 *canola*	33.9	cd	C	L	C
10	28.2 *barley*	26.2 *barley*	27.2	d	C	S	H

ANOVA	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.6 Mean cold-KCl extractable NH_4^+-N of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Texture†	Manure History‡	Manure Type§
	--------- mg NH_4-N kg^{-1} soil ---------				
3	28.2 *canola*	49.5 *winter wheat*	F	S	C
9	21.4 *corn*	44.3 *barley*	F	L	C
5	38.7 *corn*	41.2 *canola*	F	L	C
7	35.4 *corn*	39.5 *canola*	F	L	H
2	35.0 *canola*	36.9 *barley*	F	L	H
4	33.5 *wheat*	35.8 *wheat*	F	L	H
8	29.3 *wheat*	33.5 *barley*	C	S	H
1	29.2 *barley*	23.7 *wheat*	C	S	C
10	21.3 *barley*	26.7 *barley*	C	S	H
6	26.3 *corn*	19.0 *canola*	C	L	C

ANOVA	df	Prob>F
Site	9	ns
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.7 Coefficients of pair-wise correlations (r) matrix for KCl extractable NH_4^+-N and various soil parameters for 10 sites collected in May in non-amended fields. Only significant correlations are shown (*, **, * significant at P ≤0.05, P ≤0.01, and P ≤0.001).**

	Hot-KCl extractable NH_4^+	Cold-KCl extractable NH_4^+	Total-KCl extractable NH_4^+
Cold-KCl extractable NH_4^+	ns		
Total-KCl extractable NH_4^+	0.36*	0.91***	
Nitrate	ns	0.56***	0.51***
Ammonium	ns	ns	ns
Phosphorus	ns	ns	ns
Potassium	ns	0.63***	0.50**
Sulphate	ns	ns	ns
Organic C %	ns	0.60***	0.53***
Total N %	ns	0.43**	0.40*
Clay %	ns	0.59***	0.43**
Sand %	ns	-0.53***	-0.43**
pH	ns	-0.63***	-0.60***
EC	ns	ns	ns
EOC	ns	ns	ns
MBC	ns	ns	ns
MBN	ns	ns	ns
Dehydrogenase	ns	ns	ns
Urease	ns	ns	ns
Glutaminase	ns	ns	ns
Phosphatase	ns	-0.35*	-0.33*

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 4.8 Coefficients of pair-wise correlations (r) matrix for KCl extractable NH_4^+-N and various soil parameters for 10 sites collected in May in manure-amended fields. Only significant correlations are shown (*, **, * significant at P ≤0.05, P ≤0.01, and P ≤0.001).**

	Hot-KCl extractable NH_4^+	Cold-KCl extractable NH_4^+	Total-KCl extractable NH_4^+
Cold-KCl extractable NH_4^+	ns		
Total-KCl extractable NH_4^+	-0.38*	0.90***	
Nitrate	ns	ns	ns
Ammonium	ns	ns	ns
Phosphorus	ns	ns	ns
Potassium	ns	ns	ns
Sulphate	ns	ns	ns
Organic C %	ns	0.35*	-0.44**
Total N %	ns	0.34*	0.44**
Clay %	ns	ns	0.39*
Sand %	ns	ns	-0.36*
pH	ns	ns	ns
EC	0.37*	ns	0.33*
EOC	ns	ns	ns
MBC	ns	ns	ns
MBN	ns	ns	ns
Dehydrogenase	ns	ns	ns
Urease	ns	ns	ns
Glutaminase	ns	-0.38*	-0.41**
Phosphatase	ns	0.40*	0.45**

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 4.9 Coefficients of pair-wise correlations (r) matrix for KCl extractable NH_4^+-N and various soil parameters for 10 sites collected in August in non-amended fields. Only significant correlations are shown (*, **, * significant at P ≤0.05, P ≤0.01, and P ≤0.001).**

	Hot-KCl extractable NH_4^+	Cold-KCl extractable NH_4^+	Total-KCl extractable NH_4^+
Cold-KCl extractable NH_4^+	-		
Total-KCl extractable NH_4^+	0.44**	0.89***	
Nitrate	ns	ns	ns
Ammonium	ns	ns	ns
Phosphorus	ns	ns	ns
Potassium	ns	ns	0.38*
Sulphate	ns	ns	ns
Organic C %	0.52**	0.35*	0.56***
Total N %	0.49**	0.49**	0.66***
Clay %	ns	0.33*	0.40*
Sand %	-0.37*	-0.43**	-0.56***
pH	ns	ns	ns
EC	ns	0.33*	ns
EOC	ns	ns	ns
MBC	ns	0.51***	0.58***
MBN	ns	0.50**	0.48**
Dehydrogenase	ns	ns	ns
Urease	ns	ns	ns
Glutaminase	ns	-0.33*	ns
Phosphatase	0.53***	ns	-0.38*
Respiration	ns	ns	ns

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Table 4.10 Coefficients of pair-wise correlations (r) matrix for KCl extractable NH_4^+-N and various soil parameters for 10 sites collected in August in manure-amended fields. Only significant correlations are shown (*, **, * significant at P ≤0.05, P ≤0.01, and P ≤0.001).**

	Hot-KCl extractable NH_4^+	Cold-KCl extractable NH_4^+	Total-KCl extractable NH_4^+
Cold-KCl extractable NH_4^+	ns		
Total-KCl extractable NH_4^+	0.44**	0.81***	
Nitrate	ns	ns	ns
Ammonium	0.39*	ns	ns
Phosphorus	ns	ns	ns
Potassium	ns	ns	ns
Sulphate	ns	ns	ns
Organic C %	ns	0.39*	0.50**
Total N %	ns	0.32*	0.45**
Clay %	ns	0.34*	0.39*
Sand %	ns	ns	-0.35*
pH	-0.34*	-0.35*	-0.52***
EC	ns	ns	ns
EOC	ns	ns	ns
MBC	ns	ns	ns
MBN	ns	ns	ns
Dehydrogenase	ns	ns	ns
Urease	ns	ns	ns
Glutaminase	ns	ns	-0.31*
Phosphatase	ns	-0.32*	ns
Respiration	ns	0.33*	0.37*

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

4.5.2 Laboratory N mineralization

The impact by manure application on laboratory measures of N mineralization was site dependent (site x treatment, $p< 0.0001$; Table 4.11). Manure-amended fields tended to have greater levels of laboratory N mineralization as did soils whose current crop was corn and soils with a long history of manure application (Table 4.11). Soils amended with cattle manure tended to have greater levels of N mineralization in the laboratory incubation relative to those receiving hog manure. Texture and manure history did not appear to influence laboratory N mineralization rates. Laboratory N mineralization rates from August manure-amended soils had the highest degree of correlation to soil nutrient levels and microbial biomass C and N (Table 4.12). Only glutaminase was correlated to laboratory N mineralization in the non-manured soils in the August sampled soil. The laboratory N mineralization was poorly correlated to parameters measured in the May sampled soil. Only ammonium and MBC in the manure-amended sample had significant correlations to laboratory N mineralization.

Table 4.11 Mean laboratory incubated N mineralization (mg N kg soil $^{-1}$ day $^{-1}$) of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- mg NH_4-N kg^{-1} soil ----------						
9	5.007 *corn*	a	3.156 *barley*	bcde	F	L	C
1	4.978 *barley*	a	4.330 *wheat*	ab	C	S	C
6	3.756 *corn*	abc	1.986 *canola*	e	C	L	C
7	3.741 *corn*	abc	3.542 *canola*	bcd	F	L	H
5	3.269 *corn*	bcde	2.378 *canola*	cde	F	L	C
10	3.046 *barley*	bcde	3.239 *barley*	bcde	C	S	H
3	3.034 *canola*	bcde	3.141 *winter wheat*	bcde	F	S	C
4	3.079 *wheat*	bcde	2.289 *wheat*	de	F	L	H
8	2.988 *wheat*	bcde	2.465 *barley*	cde	C	S	H
2	2.124 *canola*	e	2.357 *barley*	cde	F	L	H

ANOVA	df	Prob>F
Site	9	<0.001
Treatment	1	<0.001
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.12 Coefficients of pair-wise correlations (r) matrix between laboratory N mineralization and various soil parameters for May and August sampled soils. Only significant correlations are shown (*, **, * significant at P ≤0.05, P ≤0.01, and P ≤0.001).**

	May		August	
	Non-manured	Manured	Non-manured	Manured
Hot-KCl extractable NH_4^+-N	ns	ns	ns	ns
Cold-KCl extractable NH_4^+-N	ns	ns	ns	ns
Total-KCl extractable NH_4^+-N	ns	ns	ns	ns
Nitrate	ns	ns	ns	0.53***
Ammonium	ns	0.35*	ns	ns
Phosphorus	ns	ns	ns	0.32*
Potassium	ns	ns	ns	0.54***
Sulphate	ns	ns	ns	ns
Organic C %	ns	ns	ns	ns
Total N %	ns	ns	ns	ns
Clay %	ns	ns	ns	ns
Sand %	ns	ns	ns	ns
pH	ns	ns	ns	ns
EC	ns	ns	ns	ns
EOC	ns	ns	ns	ns
MBC	ns	0.42**	ns	0.38*
MBN	ns	ns	ns	-0.47**
Dehydrogenase	ns	ns	ns	ns
Urease	ns	ns	ns	ns
Glutaminase	ns	ns	0.50**	ns
Phosphatase	ns	ns	ns	ns

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

4.5.3 Estimated Field N mineralization

The response of estimated field N mineralization rates to manure treatment varied among sites (site x treatment P<0.001; Table 4.13). Fine-textured soils with long manure application histories tended to exhibit net N losses. Soils to which cattle manure had been applied tended to have higher levels of estimated net field N mineralization, but not always. The field-based estimate of N mineralization generally had higher levels for manure-amended soil than non-manure amended soils (manure-amended 0.60 ± 2.08 kg N/ha/day, non-manured 0.12 kg ± 1.19 N/ha/day) but this difference was not consistent

across sites. Although all soils sampled with different manure treatments and sample dates had various correlations with soil parameters, manure-amended soils had a higher frequency of significant correlations with soil microbial parameters, such as MBC, MBN, dehydrogenase, and urease (Table 4.14). Cold- and total-KCl extractable NH_4^+-N were negatively correlated to estimated field N mineralization for May. In non-manure amended soils, nitrate was also negatively correlated to estimated field N mineralization, but positively correlated in August.

Table 4.13 Mean field N mineralization (kg N ha $^{-1}$ day $^{-1}$) of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing LSmeans, all pairs, Tukey HSD.

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- kg N ha $^{-1}$ day $^{-1}$----------						
6	5.840 *corn*	a	0.515 *canola*	bcde	C	L	C
1	0.478 *barley*	bcde	1.073 *wheat*	b	C	S	C
3	0.535 *canola*	bcde	1.065 *winter wheat*	b	F	S	C
7	0.925 *corn*	bc	-0.530 *canola*	ef	F	L	H
8	0.300 *wheat*	bcdef	0.883 *barley*	bc	C	S	H
9	0.478 *corn*	bcde	0.671 *barley*	bcd	F	L	C
10	0.73 *barley*	bcdef	0.383 *barley*	bcdef	C	S	H
2	0.148 *canola*	bcdef	-0.040 *barley*	cdef	F	L	H
5	-0.658 *corn*	f	-0.298 *canola*	def	F	L	C
4	-2.50 *wheat*	g	-2.08 *wheat*	g	F	L	H

ANOVA	df	Prob>F
Site	9	<0.001
Treatment	1	<0.01
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table 4.14 Coefficients of pair-wise correlations (r) matrix between estimated field N mineralization and various soil parameters for May and August sampled soils. Only significant correlations are shown (*, **, * significant at P ≤0.05, P ≤0.01, and P ≤0.001).**

	May		August	
	Non-manured	Manured	Non-manured	Manured
Laboratory N mineralization	0.33*	ns	0.33*	ns
Hot-KCl extractable NH_4^+-N	ns	ns	ns	ns
Cold-KCl extractable NH_4^+-N	-0.76***	ns	ns	ns
Total-KCl extractable NH_4^+-N	-0.68***	-0.36*	ns	ns
Nitrate	-0.70***	ns	0.34*	ns
Ammonium	ns	ns	ns	ns
Phosphorus	ns	ns	ns	ns
Potassium	-0.53***	ns	-0.43**	ns
Sulphate	ns	ns	ns	ns
Organic C %	-0.53***	-0.42**	-0.41***	ns
Total N %	ns	-0.44**	ns	ns
Clay %	ns	-0.50**	-0.63***	-0.50**
Sand %	ns	0.62***	0.48***	0.62***
pH	ns	ns	0.41**ns	ns
EC	ns	ns	ns	ns
EOC	ns	-0.58***	0.54***	ns
MBC	ns	ns	ns	-0.42**
MBN	ns	0.47**	ns	0.33*
Dehydrogenase	ns	0.40**	ns	ns
Urease	ns	ns	ns	-0.35*
Glutaminase	ns	ns	0.35*	ns
Phosphatase	ns	ns	ns	ns
Respiration	0.33*	ns	ns	ns

ns = not significant.
*, **, *** Significant at P ≤0.05, P ≤0.01, and P ≤0.001, respectively.

Stepwise regression analysis was used to estimate potential field N mineralization with selected physical, chemical and biological parameters (Table 4.11). Variables selected in the predicted regression models varied, depending on whether if the analysis was run with the entire data set combined or separated according to manure application treatments. The percentage of sand in samples had the greatest impact on field N mineralization models for the manure-amended and combined data set. The percentage

of total C, and laboratory N mineralization were the most significant variables in regression models for soils not amended with manure. The R^2 values were higher when modeling was done separately on the manure-amended treatments opposed to the combined data set.

Table 4.15 Step-wise regression analyses of field N mineralization with various physical, chemical and biological parameters.

	Field N mineralization regression model equations	R^2 (RMSE)	P (model)
All combined	$y = 1.685 - 0.0094\ Urease_{AUG}$** - 0.552 EC_{MAY} *** + 0.638 Laboratory Nmin *** -0.0036 MBC_{MAY} *** + 0.0008 MBN_{MAY} ***	0.62 (1.08)	***
Manured	$y = 4.292 - 1.02\ pH_{MAY}$ ** + 0.007 MBN_{MAY}*** + 0.019 $Urease_{AUG}$ *** + 0.084 Sand% *** + 0.214 $OC\%_{AUG}$*	0.76 (1.07)	***
Non-manured	$Y = 1.777 + 0.0028\ Glutaminase_{AUG}$** + 0.013 cold KCl_{AUG}* -0.015 $Nitrate_{MAY}$** - 0.025 cold KCL_{MAY}*** - 0.0057 $Urease_{AUG}$*	0.80 (0.57)	***

*, **, and *** significant at P ≤0.05, P ≤0.01, and P ≤0.001.

4.6 Discussion

Cold-KCl extractions were sensitive to site location differences, but not sensitive to manure application. Hot-KCl extractable NH_4^+-N was influenced by manure treatment. The use of cold-KCl extractable NH_4^+-N as the base value for calculating net hot-KCl extractable NH_4^+-N may have removed the influence of site. Some sites had very little differences between the cold- and total-KCl extractable NH_4^+-N and this resulting in a net negative value for hot-KCl extractable NH_4^+-N (e.g., site 5 of the May sampling). Manure-amended effects were more prominent in the hot-KCl extractable NH_4^+-N

suggesting its selective ability in detecting the effects of manure addition on N availability. However, cold- and total-KCl extractable NH_4^+-N were better correlated to soil and chemical parameters (e.g., total N%, organic C % and phosphatase) than was hot-KCl extractable NH_4^+-N in the non-manured soils. Hot-KCl extractable NH_4^+-N appears to be sensitive to different soil textures. Thus may be related to the cation exchange capacity (CEC), and resulting higher nutrient holding capacity of the soil, or alternatively the role of clays in protection organic matter. In this study, fine textured soils generally had high levels of total-, cold- and hot-KCl extractable NH_4^+-N. Future research should examine the significance of soil texture, manure application history and type of manure applied as well as crop effects to allow more conclusive statements to be made.

Laboratory N mineralization differed among sites in its response to manure application. Furthermore, the type and history of manure application may have influenced the amount of N released during laboratory N mineralization. Soils receiving cattle manure had increased laboratory N mineralization rates, this effect was not correlated to OC% and EOC levels of corresponding sites. Cattle manure, which tends to be straw-laden (higher C:N ratio), influences the quality of organic carbon in the long term amended soils, potentially increasing nutrient availability (Qian and Schoenau, 2002). The duration of repeated manure application can increase total N. Other studies have found that manure-amended soils with higher levels of total N and P have increased mineralization potentials (Whalen et al., 2001). However, we found no correlation between total N and mineralization.

The effect of manure application on estimates of field N mineralization varied for different sites. In general, greater mineralization occurred in manure-amended fields and was observed in both the estimated field N mineralization and laboratory N mineralization at many sites. It was anticipated that the longer the manure application history, the greater the N mineralization rate. Negative values found in the estimated field N mineralization could be attributed to nitrogen losses via leaching, volatilization, runoff, and denitrification (Paul and Clark, 1996). The above –normal precipitation levels during the growth season of the study year (Appendix I) and the positive correlations between field N mineralization and sand content suggest better mineralization in the larger aerated macropores of coarse textured soils. The negative correlation with clay content and spring NO_3^- content suggests denitrification as a significant pathway of N loss. This underscores one of the potential shortcomings of the field-based approach for estimating N mineralization.

The majority of the variables examined suggest differences in nutrient dynamics between manure-amended and non-amended soils. Therefore, manure-amended soils should be analyzed separately from non-manured soils in predicting N mineralization. In non-amended soils, the variation in N mineralization was best described by nitrate and various measurements of ammonia released from cold-KCl extractable NH_4^+-N, glutaminase and urease. The use of more biologically-based parameters (MBC, MBN, dehydrogenase, phosphatase and respiration) did not improve the prediction of mineraliztion for non-manured soils. Although the inclusion of KCl-extractable NH_4^+-N, NO_3^- and organic

matter have improved the estimates of potentially mineralizable nitrogen in other studies (McTaggart and Smith, 1993), they were not selected in the step-wise regression models in the manure-amended soils. While many researchers found KCl extractable NH_4^+-N to be strongly correlated to plant uptake of N and N mineralization potential, most of these studies used laboratory-based incubations (Gianello and Bremner, 1986a,b; Ha'ava and Waring, 1992; Smith and Li, 1993; Campbell et al., 1995) and not subjected to field conditions. Although field N mineralization was correlated to estimated values for field N mineralization, laboratory N mineralization rates were poorly correlated to soil parameters,

For predicting N mineralization from manure-amended fields, physical (pH, sand%, OC %) and biological (urease, MBN) factors were selected in the step-wise regression model. However, nitrate was not selected suggesting that the nitrate test was not good predictor of potential N mineralization in the manure-amended soils. The combined step-wise regression selected laboratory N mineralization, emphasizing the potential for short-term incubations to aid in predicting N mineralization. The use of short-term incubations of a week is more practical and economical for producers and commercial soil testing laboratories than the 24-week incubation often used in research studies. Our model did not include the detailed characteristics of the manure applied, just the type of manure. As continuous heavy annual manure applications are not recommended and manure characteristics can be very variable (Davis et al., 2002), our approach was to study the intrinsic N mineralization capability of long-term manure-amended soils. A more accurate determination of N mineralization would include local environmental

conditions, manure properties, subsurface soil sampling (especially in coarse textured soils), and soil atmospheric sampling (to account for denitrification and NH_3 volatilization losses). Others have found that the sampling time can affect N mineralization (Deng and Tabatabai, 2000). Further studies into the influence of sampling time on the ability to best predict field N mineralization in manure-amended soils may be advisable.

4.7 Conclusions

A majority of indices were sensitive to site characteristics, indicating the need to consider site-specific variables in the modelling N mineralization. The response to manure addition also appeared to be site-specific for many of the measured parameters. Hot-KCl extractable NH_4^+-N, laboratory N mineralization and field N mineralization were all found to increase from manure application. Although hot-KCl extractable NH_4^+-N resulted in few differences among sites, it was not selected in the step-wise regression. Biological indices, such as MBN, and urease were more effective in predicting field N mineralization in the manure-amended soils than other studied soil parameters. Site-specific variables, such as pH, percentage of sand and organic carbon also had a strong influence in the manure-amended step-wise model. In contrast, predicting field N mineralization in non-amended soils relied on nitrate, nitrogen cycling enzymes and extractable ammonium. Texture effects were seen in the total, cold-KCl extractable NH_4^+-N, laboratory N mineralization. The type of manure and duration of manure application was not always as significant as soil texture and crop system among our

parameters. Evidence of nitrogen losses was the greatest in fine textured soils perhaps due to denitrification. Therefore, future prediction models of N mineralization should account for measures of N movement through leaching, denitrification, climatic conditions, and manure characteristics.

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5. SOIL MICROBIAL COMMUNITY'S SUBSTRATE UTILIZATIONS PATTERNS FOLLOWING LONG-TERM MANURE APPLICATION IN DIFFERENT AGRICULTURAL SOILS ACROSS SOUTHERN MANITOBA

5.1 Abstract

Soil microbial community structure was studied using Biolog substrate utilization patterns in combination with various chemical, physical and physiological parameters to assess the impact of manure application on soil microbial diversity at 10 sites across southern Manitoba. Sites varied in soil texture, crops planted, manure type and duration of manure application. The effects from manure application were examined using various diversity indices (Shannon, Shannon evenness, and Simpson) and redundancy analysis (RDA). Recent manure applications did not influence Biolog substrate utilization patterns in the year of application, but long-term manure application did. Soil microbial communities were also significantly impacted by crop type. Correlations between various nutrient, physical and physiological soil parameters on community diversity as indicated by substrate utilization patterns underscore the implications of microbial community structure on nutrient mineralization.

5.2 Introduction

Carbon substrate utilization potentials as seen on Gram-negative (GN) Biolog plates have been used as a means to assess soil microbial diversity (Zak et al., 1994). Although more research in needed in standardizing and interpreting this methodology, processing and analysis of community substrate utilization pattern is far more convenient than genetic or phospholipid fatty acids analyses (Garland, 1996). Patterns of substrate utilization are an example of functional diversity, which is a product of genotypic and phenotypic expression in relation to environmental, ecological (Zak et al., 1994) and plant factors (Grayston et al., 1998).

Many researchers have found the addition of amendments not only change the activity of soil microbial communities, but also its community structure (Grayston et al., 1998, Peacock et al., 2001). This is seen with manure amendments (Peacock, et al., 2001), heavy metals (Knight et al, 1997), crop residue incorporation (Bending et al., 2002) and alternative farm management practices (Schutter et al., 2001). It is believed that soil management practices that increase soil organic matter, such as the application of manure will increase the size of the soil's microbial biomass and lead to the changes in the composition of the soil community (Peacock et al., 2001). Consequently, changes in community structure may influence how that community reacts to future amendments.

Microbial community structure is also seasonally dynamic (Schutter et al., 2001; Schutter et al., 2002). Greater soil microbial functional and structural diversity occurs in the spring and this has been attributed to the availability of more numerous and complex

nutrient compounds (Maire et al., 1999; Grayston et al., 2001). It is anticipated that the addition of manure will change the structure of the soil's microbial community and may ultimately lead to the changes in the nutrient cycling dynamics. In this chapter, soil metabolic function as expressed as substrate utilization patterns were used to assess the impact of manure application on the soil microbial community diversity.

5.3 Objectives of Study

The objectives of this study were to use substrate utilization patterns to identify the effects of long-term manure application on soil microbial community structure. To achieve this objective a comparison of microbial substrate utilization patterns in manure-amended fields relative to adjacent non-manure amended fields was undertaken.

5.4 Materials and Methods

5.4.1. Site description, soil sampling and environmental variables

The experimental design of this project was previously described in Chapter 3 (Section 3.4.1). All sites were located in southern Manitoba and the samples collected in late spring (May) and at harvest time (August) of 2000. The experimental design was a randomized complete block with ten blocks and two treatments per block. Each block represented a site and each site contains two treatments (manure-amended field, and non-amended field). This experimental set-up is not a classical random block design in that crops with a block were not consistent and minor soil and field management differences may differ between fields at one site. While long-term controlled manure-amendment studies across soil type would have preferred, sites of this nature do not exist in Manitoba. The design here attempts to capture the long-term influence of manure amendment in a wide range of soils relative to soils that have not had a history of intensive manure application. The use of side-by-side producer fields was seen as the most effective means of assessment. All soil biochemical and chemical methods are listed in Chapters 3 and 4.

5.4.2. Substrate utilization pattern analyses

Biolog GN microplates (Biolog Inc., 3938 Trust Way, Hayward, CA were used to assess the soil's microbial metabolic diversity. Each plate contains 95 wells with various carbon substrates and one well with no carbon substrate. In addition to the substrate of interest, each well contains compounds essential for microbial growth such as dried mixture of peptone, nutrients, and salts. A redox tetrazolium violet dye is also added to all wells as an indicator of metabolic activity. Metabolic activity reduces the clear-coloured tetrazolium violet dye to a purple coloured compound, formazan. Many researchers have used Biolog plates as a fast, effective method of assessing the functional diversity of a microbial community (Zak et al., 1994).

Two types of solutions were prepared and dispensed into milk dilution bottles. One set of bottles contained 99 mL of physiological saline solution (0.85%, 8.5 g NaCl in 1 L distilled water); the other set bottles contained 90 mL of water agar solution (0.2%, 2 g of purified grade agar in 1 L of physiological saline solution (0.85%). The second set of bottles also contained ten glass beads (5 mm diameter). All milk dilution bottles, pipette tips, and reagent reservoirs were autoclaved and cooled prior to being used.

Ten grams of soil on oven dry basis from each soil sample was weighed out and added aseptically to the milk dilution bottles containing 90 mL 0.2% agar-water solution. Plate replicates were not done for each soil subsamples as variability was determined by soil sample replicates, not plate replicates (Balser et al., 2002). These bottles were laterally shaken for half an hour and then 1 mL of the soil suspension was used to perform a 10^{-2}

dilution in the 99 mL physiological saline solution bottles, hand shaken between each transfer. The combined effect of the 10-fold dilution of the soil into 90 mL of solution and a 100-fold dilution of that suspension was a 10^{-3} g soil mL^{-1} dilution.

Each final dilution was poured into a sterile reagent reservoir (Eppendorf, Inc.) in a sterile hood (Labconco® Purifer™ Clean Bench). From this dispensed final dilution, 100 μL was added to each of the 96 wells on the microplate using a multichannel pipette (Eppendorf 8-channel Repeater™ Pipette) and incubated for 96 hours at 25°C. The microplate samples were run on an automated Biolog Microstation Reader (Hayward, CA) at a wavelength of 590 nm and the data recorded with Biolog's MicroLog™ System, Release 4.0 program (Hayward, CA) after incubations of 24, 48, 72 and 96 hours. Data was collected in the dual wavelength data format. A dual wavelength reading is the at-peak optical density (590 nm O.D.) values minus the off-peak O.D. (790 nm) (Gadzinski, 2001). This is done to prevent bubble-causing turbidity from producing a false positive. This dual wavelength (DW) data takes the respective well DW O.D. minus the water blank (cell A1) and multiplies it by the dilution factor (10^{-3}). This causes the water blank to be come zero and it gives the DW data in the same format as the 590 nm% change format data commonly used in similar studies (Garland and Mills, 1991; Garland, 1996; Ibekwe and Kennedy, 1998).

5.4.3. Statistical analysis

Two-way analysis of variance of the soil chemical and microbiological variables was performed using JMP-IN version 4 from SAS Institute Inc. (Cary, NC, USA). Where site x treatment effects were significant, an one-way analysis of variance was done. The

substrate utilization patterns were investigated using CANOCO version 4.5 software from Microcomputer Power (Ithaca, NY, USA). The substrate utilization pattern was analyzed first by calculating Shannon Diversity Index, Simpson Diversity Index (Staddon et al., 1997), and AWCD (Garland and Mills, 1991). A two-way analysis of variance was conducted between site and manure application, then site and dates of sampling using JMP IN 5 software (© 2005 SAS Institute, Inc.). A Least Significant Difference (LSD) with the Tukey-Kramer Honestly Significant Difference (HSD) test was used to differentiate effects. Beside each site, the manure application history, current crop grown, manure type and soil texture are displayed.

All biolog data was corrected to avoid zero and negative values and log transferred prior to multivariate analysis (Staddon et al., 1997). Detrended canonical analysis was run to test for unimodality, which there was none. Hence, the data is responding in linear gradients (linear response), not around some environmental optima (unimodal response) (ter Braak and Šmilauer, 2002). The transformed data was analyzed using linear methods: PCA (principal component analysis) and RDA (redundancy analysis). During the RDA, Monte Carlo permutation tests were done under the full model with 199 permutations. Co-variables such as: sites, manure application, manure history, manure type, crop, and soil texture were used against each other to determine the variance each co-variable.

5.5 Results

Average well colour development (AWCD) had significantly different responses to manure application at various sites (site x treatment $P<0.001$) (Table 5.1). Soils amended with cattle manure tended to have higher levels of AWCD than soils amended with hog manure. Texture and history of manure application did not affect AWCD in this study. Crop effects were evident, especially in the manure-amended soils. There was a tendency for higher AWCD values in sites cropped to wheat or corn.

Table 5.1 Mean average well colour development (AWCD) for manure-amended and non-manure-amended soils using 72 hr incubation on Biolog GN substrate utilization plates for soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing LSmeans , all pairs, Tukey HSD.

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
4	0.664 *wheat*	a	0.512 *wheat*	abc	F	L	H
1	0.642 *barley*	a	0.609 *wheat*	a	C	S	C
6	0.611 *corn*	a	0.351 *canola*	c	C	L	C
9	0.601 *corn*	a	0.537 *barley*	abc	F	L	C
3	0.565 *canola*	abc	0.594 *winter wheat*	ab	F	S	C
8	0.566 *wheat*	abc	0.533 *barley*	abc	C	S	H
5	0.466 *corn*	abc	0.559 *canola*	abc	F	L	C
2	0.349 *canola*	bc	0.524 *barley*	abc	F	L	H
7	0.506 *corn*	abc	0.460 *canola*	abc	F	L	H
10	0.471 *barley*	abc	0.464 *barley*	abc	C	S	H

ANOVA	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Shannon Diversity Index measures both the species richness (species number) and species eveness (the abundance of species) (Hill, 1973). Site effects had the strongest influence on the Shannon Diversity Index (Table 5.2). The application of manure did not influence microbial diversity according to the Shannon Diversity Index. However, fine textured soils tended to have greater species richness than coarse textured soils according to the Shannon Diversity Index. A history of long-term manure application did not appear to influence species richness. Soils cropped to corn appeared to be clustered together in the manure-amended soils and thus possess similar species richness. In the non-manure amended, soils cropped to wheat had the highest species richness. The type of manure did not appear to influence the values of Shannon Diversity Index.

Table 5.2 Mean Shannon Diversity Index for manure-amended and non-manure-amended soils using 72 hr incubation on Biolog GN substrate utilization plates for soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing LSmeans , all pairs, Tukey HSD.

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture †	Manure History‡	Manure Type§
3	4.25 *canola*	4.14 *winter wheat*	4.20	ab	F	S	C
4	4.24 *wheat*	4.20 *wheat*	4.22	s	F	L	H
1	4.23 *barley*	4.21 *wheat*	4.22	s	C	S	C
2	4.10 *canola*	4.18 *barley*	4.14	bc	F	L	H
5	4.14 *corn*	4.18 *canola*	4.16	abc	F	L	C
9	4.17 *corn*	4.16 *barley*	4.17	ab	F	L	C
7	4.14 *corn*	4.14 *canola*	4.14	bc	F	L	H
8	4.10 *wheat*	4.09 *barley*	4.10	cd	C	S	H
6	4.09 *corn*	4.02 *canola*	4.06	d	C	L	C
10	4.05 *barley*	4.03 *barley*	4.04	d	C	S	H

ANOVA	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Shannon Evenness measures how equally distributed the number of species are in a community (Begon et al., 1990; Staddon et al., 1997). All Shannon Evenness values were very high (close to one or over) indicating an even distribution of species within the communities. In the ANOVA of Shannon evenness index, sites reacted significantly differently to manure application (site x treatment, $P<0.0001$) (Table 5.3). Thus, there was a difference in the number or evenness of substrate used by the soil communities at different sites as they response to manure application. Long-term manure application history appears to be increasing Shannon Evenness values. Soils cropped to canola possessed higher species evenness on average than other crops. Soil texture and the type of manure applied did not influence the Shannon Evenness indices.

Table 5.3 Mean Shannon Evenness Index for manure-amended and non-manure-amended soils using 72 hr incubation on Biolog GN substrate utilization plates for soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing LSmeans, all pairs, Tukey HSD.

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
6	0.98 *corn*	c	1.04 *canola*	a	C	L	C
2	1.04 *canola*	ab	1.01 *barley*	abc	F	L	H
7	1.02 *corn*	abc	1.01 *canola*	abc	F	L	H
5	1.01 *corn*	abc	1.00 *canola*	abc	F	L	C
4	0.97 *wheat*	c	1.01 *wheat*	abc	F	L	H
10	1.00 *barley*	abc	0.99 *barley*	bc	C	S	H
9	0.99 *corn*	bc	0.99 *barley*	bc	F	L	C
8	0.98 *wheat*	c	0.99 *barley*	bc	C	S	H
1	0.99 *barley*	c	0.98 *wheat*	c	C	S	C
3	0.99 *canola*	c	0.98 *winter wheat*	c	F	S	C

ANOVA	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Simpson Dominance index compares the probability of observing two species belonging to different species in a given sample (Begon et al., 1990; Staddon et al., 1997). As the Simpson Dominance index increases, the greater the diversity as species richness is higher. This index is expressed in reciprocal terms (1/D), the lower the dominance (D), the higher the value, the greater diversity. The sites in this study reacted differently to manure application according Simpson dominance indices ($P<0.001$) (Table 5.4). Soils cropped to corn in the manure-amended fields and wheat in the non-manure amended fields appears to offer similar Simpson Dominance index values. Fine textured soils appeared to increase the level of Simpson dominance indices. Coarse-textured soils tended to have lower levels of species dominance than fine-textured soils. Manure application history and the livestock manure type did not affect the microbial community in terms of increased species dominance within a community.

Table 5.4 Mean Simpson Dominance Index for manure-amended and non-manure-amended soils using 72 hr incubation on Biolog GN substrate utilization plates for soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing LSmeans, all pairs, Tukey HSD.

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- mg NH4-N kg-1 soil ----------						
3	60.3 *canola*	a	52.8 *winter wheat*	ab	F	S	C
4	60.0 *wheat*	a	55.3 *wheat*	ab	F	L	H
1	59.8 *barley*	a	58.3 *wheat*	ab	C	S	C
9	56.0 *barley*	ab	52.5 *barley*	ab	F	L	C
5	48.5 *corn*	abc	53.8 *canola*	ab	F	L	C
2	39.7 *canola*	bc	53.5 *barley*	ab	F	L	H
7	53.0 *corn*	ab	52.0 *canola*	ab	F	L	H
6	50.5 *corn*	ab	31.8 *canola*	c	C	L	C
8	50.3 *wheat*	ab	50.0 *barley*	ab	C	S	H
10	45.5 *barley*	abc	46.5 *barley*	abc	C	S	H

ANOVA	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Principal component analysis (PCA) of the average well colour development in the Biolog substrate utilization patterns for all sites resulted in 35.3 % of the variance being described by principal component 1 and 7.2 % of the variance by principal component 2. In Figures 5.1 to 5.6, present PCA for Biolog substrate utilization patterns where grouped according to site, manure treatment, manure history, current crop, soil texture and manure type. Although there is evidence of some clustering, no clear separation was apparent. Principle component analysis plots of manure treatment, manure history, current crop, soil texture and manure type showed no separation between similar sample points (Figure 5.2 to 5. 6). Principal component analysis using only the manure-amended data was able to describe 39.0% of the variance on PCA1 and 8.2% of the variance on PCA 2 (Figure 5.3 and5.6).

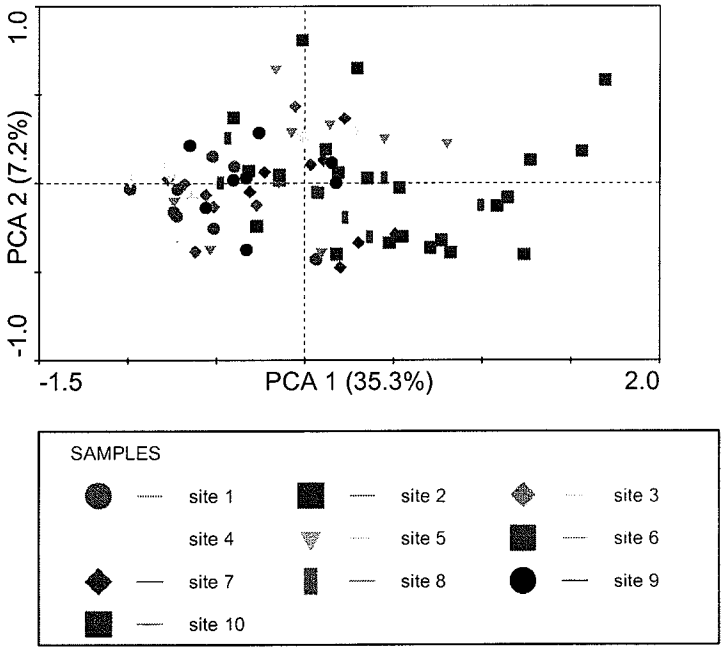

Figure 5.1 Principal component analysis of average well colour development in Biolog GN substrate utilization patterns after 72 hr incubation, categorized by sites. Percent of variance explained by each axis is in parenthesis.

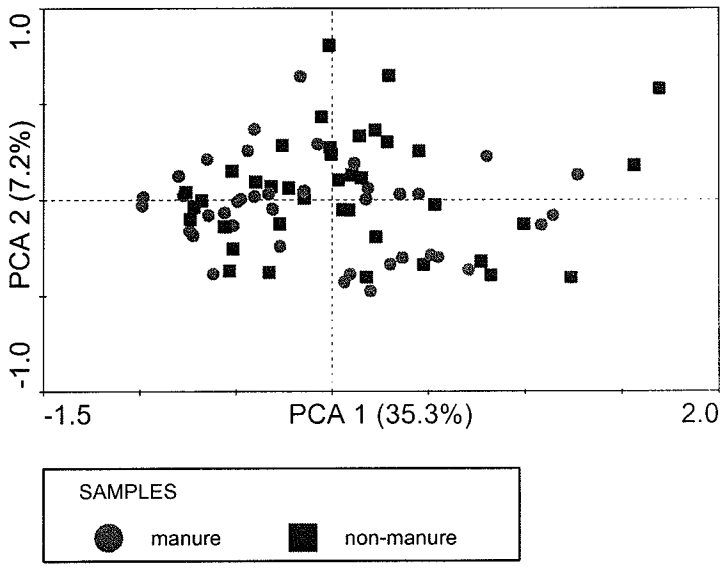

Figure 5.2 Principal component analysis of Biolog GN substrate utilization patterns after 72 hr incubation, categorized by manure treatment. Percent of variance explained by each axis is in parenthesis.

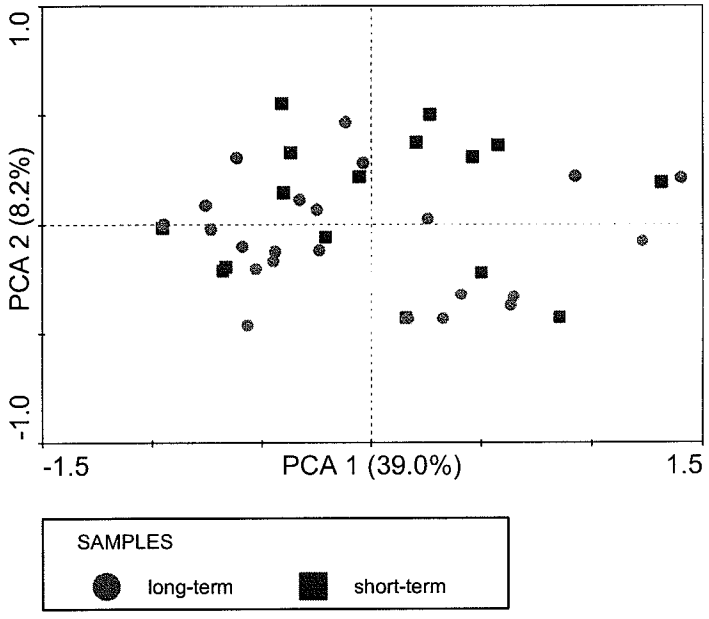

Figure 5.3 Principal component analysis of Biolog GN substrate utilization patterns after 72 hr incubation, categorized by manure history for manure-amended soils only. Percent of variance explained by each axis is in parenthesis.

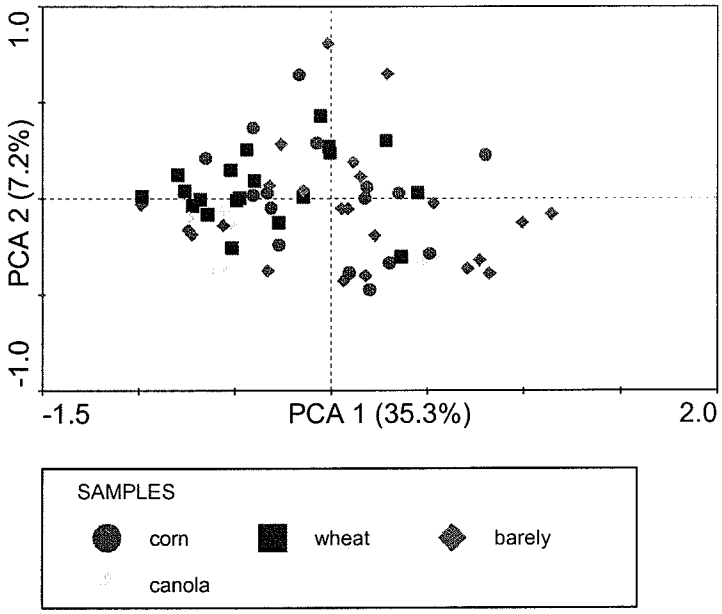

Figure 5.4 Principal component analysis of Biolog GN substrate utilization patterns after 72 hr incubation, categorized by current crop. Percent of variance explained by each axis is in parenthesis.

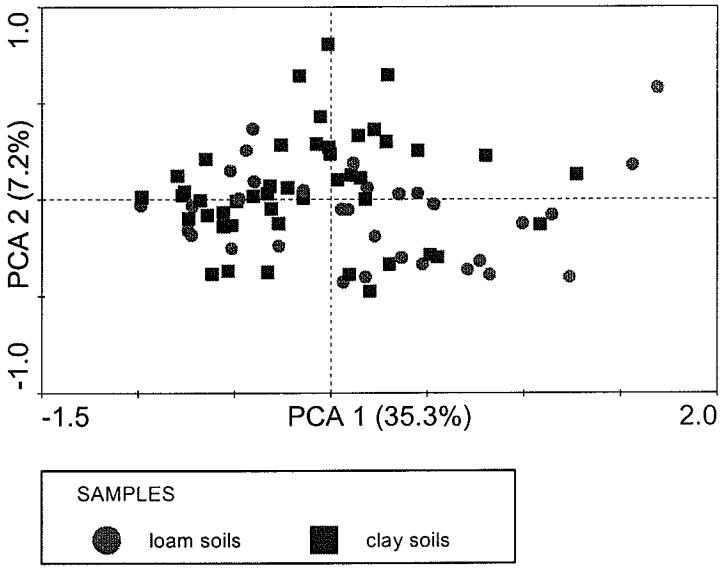

Figure 5.5 Principal component analysis of Biolog GN substrate utilization patterns after 72 hr incubation, categorized by soil texture. Percent of variance explained by each axis is in parenthesis.

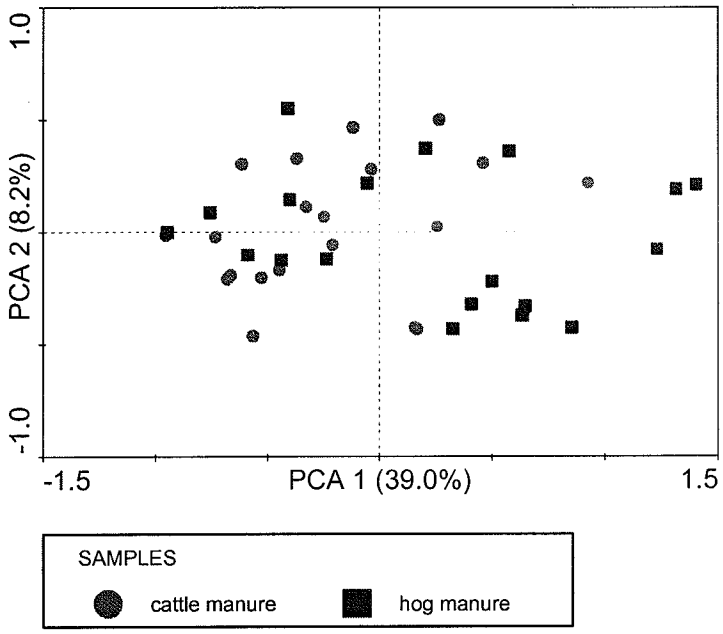

Figure 5.6 Principal component analysis of Biolog GN substrate utilization patterns after 72 hr incubation, categorized by manure type for manure-amended soils only. Percent of variance explained by each axis is in parenthesis.

Redundancy analysis (RDA) is a technique that constrains principal component analysis with measured environmental variables in an attempt to identify the degree to which these environmental variables account for the variation captured in one or more principle components. By including or excluding specific environmental variables, the impact on the overall percentage of variance explained, relative to an unconstrained RDA (no co-variables) can be determined. Redundancy analysis (RDA) analysis using no co-variables showed that 27.2% of the variation in AWCD was accounted for by RDA axis 1 (Table 5.5, Figure 5.7). By including various co-variables, the partial RDAs showed that site, followed by manure history and crop type accounted for more variation when the remaining variation was already accounted for by the selected co-variables. Soil texture,

manure application and manure type did not contribute to covariance between environmental and Biolog substrate utilization patterns.

According to the forward selection process from the Monte Carlo permutations, environmental variables that best explained the Biolog substrate utilization patterns include various sites (1, 2, 3, 6, and 7), canola, field N mineralization, % sand, non manure-amended, cold-KCl extractable NH_4^+ and hot-KCl extractable NH_4^+. When Biolog substrate utilization patterns for manure amended and non-manure amended soils without co-variables were analyzed with RDA manure-amended soils (RDA 1 34.2%, RDA 2 7.2%) (Figure 5.8) and non-manure-amended soils (RDA 1 34.7%, RDA 2 9.0%) (Figure 5.9) accounted for similar levels of described variance.

Table 5.5 Redundancy analysis (RDA) of average well colour development in GN Biolog substrate utilization pattern after 72 hr incubation represented as covariance values. Co-variables used in partial RDA analyses include site, crop, texture, manure application, manure application history, and manure type applied. The exclusion of a co-variable during partial RDA allows for determining the variation by that variable.

Analysis	RDA AXIS 1 (%)	RDA AXIS 2 (%)	RDA AXIS 3 (%)	RDA AXIS 4 (%)	Σ all eigenvalues variance	Σ all canonical eigenvalue variance
RDA no co-variables	27.2	4.1	3.4	2.7	1.00	0.602
Partial RDA adjusted for various co-variables						
RDA with all co-variables	7.9	2.1	1.8	1.4	0.632	0.234
RDA without sites as a co-variable	13.7	3.3	2.6	2.0	0.767	0.369
RDA without manure-amendment as a co-variable	7.9	2.1	1.8	1.4	0.632	0.234
RDA without manure type as a co-variable	7.9	2.1	1.8	1.4	0.632	0.234
RDA without manure history as a co-variable	9.6	2.2	2.1	1.6	0.671	0.272
RDA without texture as a co-variable	7.9	2.1	1.8	1.4	0.632	0.234
RDA without crop as a co-variable	8.2	3.0	2.1	1.6	0.671	0.273

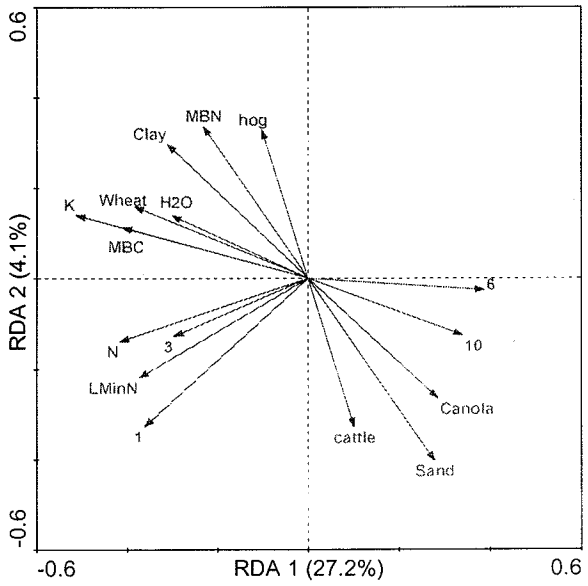

Figure 5.7 Redundancy analysis showing environmental variables that have greater than 25% correlations to the model using all combined data. (*Numbers* refer to site numbers, *long* and *short* refer to the duration of manure application, *Resp* refers soil respiration measurement, *LMinN* refers to laboratory N mineralization, *MBC* refers to microbial biomass carbon, and *MBN* refers to microbial biomass nitrogen,

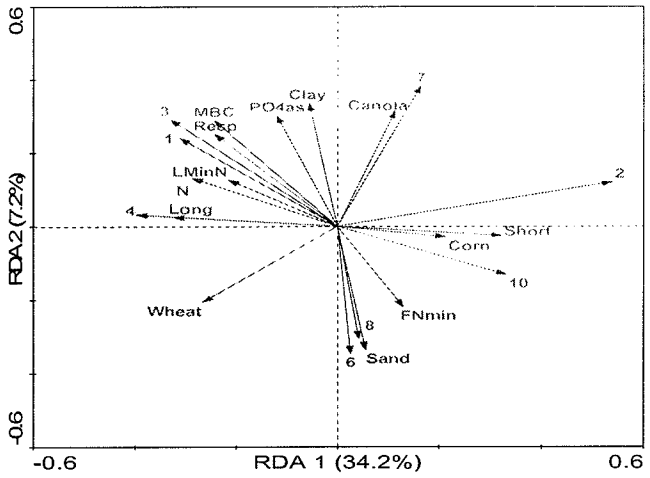

Figure 5.8 Redundancy analysis showing environmental variables (r^3>25%) for manure-amended data only. (*Numbers* refer to site numbers, *long* and *short* refer to the duration of manure application, *Resp* refers soil respiration measurement, *LMinN* refers to laboratory N mineralization, *FNmin* refers to estimated field N mineralization, *MBC* refers to microbial biomass carbon, and *PO4as* refers to phosphatase enzyme responses).

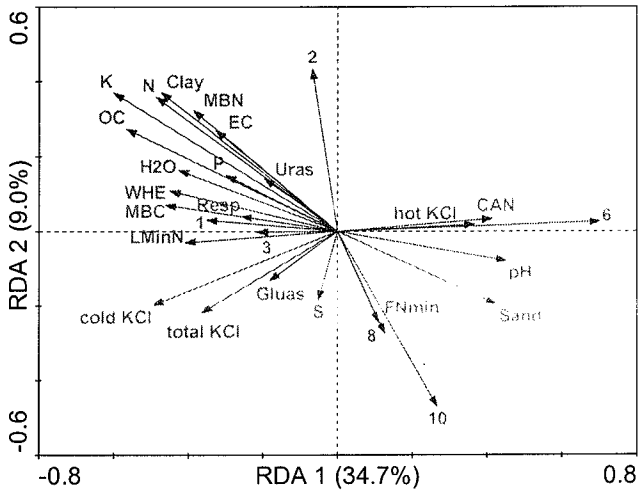

Figure 5.9 Redundancy analysis showing environmental variables (r^2>25%) for non-manure-amended data only. (*Numbers* refer to site numbers, *long* and *short* refer to the duration of manure application, *Resp* refers soil respiration measurement, *LMinN* refers to laboratory N mineralization, *FNmin* refers to estimated field N mineralization, *MBC* refers to microbial biomass carbon, *MBN* refers to microbial biomass nitrogen, *WHE*, and *CAN* refers to current crop wheat and canola, *OC* refers to organic carbon, *Gluas* erfers to glutaminase, *Uras* refers to urease and *PO4as* refers to phosphatase enzyme responses).

Separate partial RDA for manure amended and non-manure-amended soils proved that the selected co-variables did not improve the covariance explanation between the environmental variables and the Biolog substrate utilization pattern as all covariance levels remained the same for both treatments (non-manure-amended RDA 1 11.0%, RDA 2 4.2%; manure-amended RDA 1 10.2%, RDA 2 4.7%). Using forward selection, corn, some sites (2, 6, 8, and 10) and most significantly nitrate were the best environmental variables in explaining Biolog substrate utilization patterns in the manure-amended soils in the partial RDA analysis (Figure 5.8). Barley, selected sites (6, 8 and 10), hot-KCl extractable NH_4^+ and most notably cold-KCl extractable NH_4^+ were the best environmental variables in the non-manure-amended soils using forward selection in the partial RDA analysis (Figure 5.9). Most of these environmental variables also appear in the RDA scatter plots in Figures 5.7 – 5.9 along with other variables that contributed to the variance in the relevant models. Separating the data according to manure treatments helped to explain more variance in the RDA models. The RDA manure-amended scatter plot (Figure 5.8), manure management history, current crop- corn and sites 2, 4, and 10 had the greatest impact on RDA axis 1 which accounts for the largest variability in this model. Soil texture, phosphatase (PO_4ase) and sites 6 and 8 contributed to explaining the variance on RDA 2 axis. More variables were used to account for the variation in the RDA for the non-manure amended soil, especially along the primary axis (Figure 5.9). RDA axis 1 was strongly influenced by sites 1, 3 and 6, canola, hot-KCl extractable NH_4^+, laboratory N mineralization (LminN) and microbial biomass C (MBC). Sites 2, 8 and 10 and sulphate appeared to explain 8.0% of the variance in the non-manured soils on RDA axis 2.

5.6 Discussion

Sites were the dominant factor influencing microbial diversity as represented by Biolog substrate utilization patterns. This was further demonstrated with selected diversity indices and the partial RDA where sites were selected as a co-variable. Thus, site-dependent factors have a strong impact on the soil's functional diversity. Although the soil's metabolic diversity was not impacted by recent manure applications, thecurrent soil microbial diversity is considered high (Bending et al., 2002). As short incubations are more reflective of field conditions and not those imposed by laboratory incubations conditions (Maire et al., 1999), the high values of the Shannon diversity indices suggest that the current microbial populations are not nutrient deprived (Bending et al., 2002). None of the selected diversity indices appear to be influence by manure type. This was also apparent in the PCA ordination plots as there was no distinguishable clustering of the carbon rich cattle and carbon poor hog manures (Figure 5.6). Cattle manure with its higher C:N ratio, should contribute higher concentrations of organic carbon in the long term and potentially increase nutrient availability (Qian and Schoenau, 2002). The microbial community structure might be anticipated to respond to this carbon rich manure resource through increase species richness. Although species richness and evenness were not influenced by manure type, the size of microbial community, as indicated by an increase in AWCD, did respond to the type of manure applied. This may be attributed to an increase in readily available carbon sources (Grayston et al., 2001). Our soils had

higher levels of MBC, organic carbon (%), and various measure of nitrogen overall, without salinity problems and other studies (Pankurst et al., 2001), and therefore be expected to support a large microbial community and therefore a high value for AWCD. Although some researchers prefer to study substrate utilization potentials with acclimatized microbial soils (Maire et al., 1999; Bending et al., 2002), it is still useful to consider the function a diversity of more recently collected soil, reflecting the influence of soil environment on community composition.

Many other researchers have found that crop influences can greatly influence microbial diversity. Different crops are also found to emit different types and levels of root exudates (Grayston et al., 1998; Grayston et al., 2001; Schutter et al., 2001). Furthermore, as a crop matures, diversity increases (Grayston et al., 2001). Upon seed set, few plant exudates are released, and their energy is redirected to seed development. At this stage, soil microbial diversity is shown to decline and greater convergence between samples in PCA ordination plot can be seen at harvest (Shutter et al., 2001). In this study. partial RDA analyses detected differences in Biolog substrate utilizations in response to current crop, but the PCA ordination plots had convergent data points. Future studies should be careful in crop selection and the timing of soil sampling in the experimental design.

The negative correlations of many environmental variables along the primary RDA axis could be associated to the position of ordination plots of the substrate utilization patterns (not shown in the RDA plots, but similar to the PCA ordination plots). As many of the

environmental variables are correlated to each other (see Chapters 3 and 4), and certain variables are nominal, such as crop, texture, manure application, history and type, resulted in a split in variable array placement on the RDA plots. It is interesting that microbial biomass, phosphatase and various nutrient mineralization measures (such as respiration, laboratory N mineralization and various KCl extractable NH_4^+ measurements) tend to have a significant negative influence on the primary axis which explains 27 to 34% of the data variation. Nitrate and potassium levels also appear to decrease the soil's community substrate usage.

5.7 Conclusions

Manure application did not have a single uniform effect on the diversity of the soil microbial community. However, examination of long-term manure applications in contrast to short term applications did indicate an influence on the soil microbial community as seen in the RDA analyses. No significant manure treatment effects were seen in any of the diversity indices or AWCD. Overall, the microbial diversity as interpreted by the Biolog substrate utilization potentials is very site-dependent with all the indices and the RDA analyses. Crop effects were significantly different in the overall model. Manure type and history appeared to influence microbial community diversity. Soil types are not as influential, and their effects on microbial community's structure and function are less defined.

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6. GENERAL DISCUSSION

Site was the most influential factor affecting soil microbial properties and nutrient dynamics in our study. The strong impact of site characteristics underscores the need for site-specific assessment of a soil's nutrient supplying capacity. Responses to manure application and other aspects of nitrogen cycling were also different among sites, often resulting in significant site x treatment interactions. Thus, intensive studies that consider only a single or limited number of sites may provide useful insight, but their extrapolation to a broader range of soils is limited by these site-specific effects.

At most sites, the application of manure in the year prior to or the current year of sampling increased microbial biomass N $(MBN)_{MAY\ and\ AUGUST}$, microbial biomass C $(MBC)_{MAY}$, urease $_{MAY\ and\ AUGUST}$, dehydrogenase, extractable organic carbon $(EOC)_{MAY\ and\ AUGUST}$, $nitrate_{AUGUST}$, phosphorus $_{MAY\ and\ AUGUST}$, potassium $_{MAY\ and\ AUGUST}$, and total $\%N_{MAY\ and\ AUGUST}$. Laboratory and estimated field N mineralization tended to be higher in most manure-amended fields, although these effects were not consistent. Hence, manure application may have increased microbial activity and soil nutrient cycling. In the soils considered in this research, metabolic diversity indices were unaffected by manure application to soil. Parameters whose values change more slowly such as total N

and C, EC and pH were not also significantly affected by manure application when comparing all manure-amended fields to non-manured fields.

Predicting mineralization in long-term manure amended soils is challenging. Longer-term manure application has been shown to increase soil organic matter content (Jenkinson et al., 1994), and the rate of N and P mineralization in soil (Whalen et al., 2001). It is intuitive that this increased availability of carbon should support a larger and more active microbial community. Indeed, increases in microbial biomass and enzyme have been shown to correlate with increased soil C content (Hadas et al., 1996). Increased biological activity has been also shown to increase soil mineralization (Parham et al., 2002). In sites with longer histories (greater than 35 years) of manure application, nitrate, phosphorus, potassium, total C, total N, pH and EC levels were higher. Organic carbon, total N, K, MBC, MBN, urease, glutaminase, phosphatase, total KCl extractable NH_4^+N, hot KCl extractable NH_4^+N, field N mineralization, and the Shannon evenness index for bio-diversity tended also to be higher with longer histories of manure application. With so many soil characteristics affected manure application and its application history, it would be anticipated that the microbial community diversity would be affected more by manure application history. In short-term incubations, the microbial community uses the manure's readily available organic substrate to exhaustion (Zaman et al., 1999a). As not all the organic compounds contained in manure are used in the first year, the remaining compounds could enter a more recalcitrant nutrient pool in the soil, and provide a small, but persistent influence on nutrient cycling and the microbial community. As crops utilize plant available nitrogen (PAN) during the growing season,

soils subjected to long-term manure application may be able to sustain microbial activity into the fall. Estimates of community diversity were performed on samples collected in August and therefore the microbial community diversity may have been influenced by the more recalcitrant organic compounds that could be present in manure-amended soils as the Shannon evenness index indicated that the microbial population was unevenly distributed. Conversly, Shannon diversity, Simpson Dominance indices and AWCD indicated that species richness did not appear to be affected. Other studies have found soil microbial biomass and extracellular enzymes activities such as urease increase with manure application for a short period, then return to background levels over time (Zaman et al., 1999a). The long-term effects of extended manure application history may also be increased may soil microbial parameters.

Manure quality and mineralization depend not only on the animal species producing the manure, but also the age of the animals, how the animals and manure are handled and the climate (Davis et al., 2002). As not all of the nutrients in manure are released at the same rate and often manure is applied in successive years, the prediction of future nutrient available in manure-amended soils is difficult. Not all organic N applied in the manure is mineralized in the first year, and manure quality influences the rate and extent of nutrient mineralization following land application. For example, in a Nebraska study, 40% of total N in cattle feedlot manure was plant available in the first year, with 15% remaining useable in the second year (Eghball et al., 2002). Hog manure may have 90% of its total N to be plant available in the year of application and 2% available in the second year. The application of cattle manure produced higher levels of pH, EOC, $nitrate_{AUG}$, K, S,

MBN, dehydrogenase, laboratory N mineralization and Simpson diversity index than hog manure. Cattle manure generally had a higher C:N ratio and thus should increase carbon substrate availability and result in greater microbial activity (Qian and Schoenau, 2002). Although Eghball et al. (2002) cited average C:N ratios of beef cattle, dairy, and swine as 19, 16, and 14, respectively, they noted that the composition of the C and N constituents and inorganic/organic fraction ratios in the manure is more significant than a C:N ratio. The microbial community's size (MBN), activity (dehydrogenase) and metabolic diversity (AWCD for Biolog substrate utilization pattern) demonstrated that the microbial community responded to the quality of manure amendment and the nutrients it supplied. This was shown in the soil's nutrient cycling capabilities. Glutaminase, laboratory and field N mineralization all tended to have higher levels of activity in the cattle manure-amended soils than in the liquid hog manure amended soils. Thus, carbon rich cattle manure increased the soil's microbial activity and, over the longer term, increased N mineralization and release during the growing season. This contradicts the findings of Qian and Schoenau (2002) who found N availability decreases if the manure organic C:N ratio is over 15, as typically found in cattle manure. However, Qian and Schoenau dealt only with short-term nutrient availability and did not address the implications of long-term manure application.

The physical environment of the soil is an important factor in determining the nature of the microbial environment. Soil texture has an important influence of soil aeration and hydrology. Coarse textured soils tend to provide a more aerobic environment conducive to microbial activity. The sand content of the soil was positively correlated with

estimated field N mineralization. Sørensen and Jensen (1995) also observed that the addition of animal manure resulted in greater plant availability N in sandy soils during the initial months after application. However, the loss of nitrate from the root zone can be increased in coarse-textured soil due to rapid turn over of soil biomass N (Sørensen and Jensen, 1995) and the increased potential for leaching of NO_3^-. Thus, more nutrients are unaccounted for in the surface, as they turnover and are translocated to the subsurface in sandy soils. Up to 30% of the soil's profile N mineralization can accumulate in the 60 to 160 cm depth layer (Hadas et al., 1986). However, the microbial community activity and populations can vary with depth. Lalande et al., (2000) found greater ammonifier and nitrifier populations in the 15-to 30-cm soil layer in their study after hog manure application. Microbial biomass carbon and MBN levels were highest in their surface soil samples. Under this study's wetter than normal sampling conditions, greater loss of nitrate due to denitrification may have occurred, especially in clay soils. Chang and Janzen (1996) found significant gaseous N losses due to denitrification while modeling field N mineralization. Many of the clay soils in our study also appeared to have negative net N field mineralization. This is likely the result of greater rates of denitrification in heavy-textured soils in this relatively wet growing season. The combined effects of improved aeration in coarse-textured soils and denitrification in fine-textured soils are likely to have caused a shift to higher apparent N mineralization in sandy soils as measured under field conditions and may have affected the step-wise regression modeling for field N mineralization. Climatic conditions further affected soil texture's influence on the microbial community. Compared to coarse-textured soils, fine-textured soils have greater porosity with more micropores that can hold more water

(Brady, 1990). This further compounds problems of excess precipitation. Fine textured soils generally have a net negative charge and have a greater cation exchange capacity (CEC). This is demonstrated in the higher soil potassium concentrations apparent in fine-textured soils in this study. Micropores can provide more protection to the microbial community, abiontic enzymes and soil organic matter. Consequently, MBC, MBN, urease and phosphatase were all higher with fine-textured soils. Simpson dominance index of the metabolic capacity (Biolog) of the microbial community indicated higher values for fine-textured soils. However, Shannon diversity and evenness index and AWCD were not affected by texture. This implies that the level of diversity may be the same between different textured soils. The species dominance and evenness in relation to the substrate utilization patterns were higher in fine-textured soils. Under field conditions, the combined effect of soil texture on the behaviour of nitrates (leaching and denitrification) and the indirect effects of soil aeration on soil microbial activity and N mineralization resulted in a texture x manure addition interaction not evident in the laboratory-based measures.

The microbial community from soils cropped to corn, cereals and pulses appeared to influence soil microbial parameters, such as MBN, glutaminase, laboratory N mineralization, AWCD, Simpson dominance and Shannon diversity and evenness indices. The greatest differences in these parameters were most commonly seen between corn and the other crops studied. Corn was not only grown for silage, but also selected by the producer as a high N demanding crop to prevent groundwater contamination on heavily manure-amended fields close to the farm site. However, it should be noted that

corn was only grown on the manure-amended sites. This can make it difficult to distinguish between crop and other effects. Other studies have found fields cropped continuously to corn to exhibit higher MBC values than mixed rotations (Jordan et al., 1995). Although not examined here, the cropping history of a site can have long-term impacts on soil microbial parameters (Jordan et al., 1995) and indicate the need for further investigation. Since different crops can emit different qualities and quantities of exudates (Grayston et al., 1998; Grayston et al., 2001; Schutter et al., 2001), partial RDA analysis indicated that the current crop was able to account for some variation in microbial community diversity.

Traditionally, the nitrate soil test has been used to quantify available nitrogen and this estimate used to predict the supplemental nitrogen that is needed for a specific crop. Our results show that the nitrate test was not always the best predictor of N mineralization in manure-amended soils. However, nitrate was selected in the step-wise regressional analysis for N mineralization in non-manure amended sites. One of the difficulties in using a spring nitrate soil test is that loss of nitrate is not explicitly measured, but could influence the amount of plant available nitrogen (PAN). In the current study, the May soil samples were taken after organic and inorganic fertilizers were added. Thus, this measure integrates the carry over of nitrate from the previous year as well as the effect of field management and amendments on soil nitrate and ammonium content.
Although nitrate is a key by-product in N mineralization, it was not a significant parameter in modeling PAN in the manure-amended soils. Many parameters had significant manure treatment effects and the step-wise regression selected different

parameters for the non-amended and the manure-amended soils. Hence, soils with manure application need to be treated and assessed separately from non-manure amended soils.

The use of hot-KCl extractable NH_4^+ appeared to be valuable in estimating PAN only in non-amended soils and not the manure-amended or combined soil models. Soil NH_4^+ content (cold KCl-extractable NH_4^+) for both sample dates was also significantly related to PAN in the non-amended model and was selected in the stepwise repression. However, the relationship between NH_4^+ and PAN in non-manure amended soil differed for the two sample dates. For soils sampled in August, soil NH_4^+ content was positively related to PAN: whereas for samples collected in May there was a negative relationship between PAN and soil NH_4^+ content. This confounding result may be due to the mineralizaed levels of PAN in soil uncomplicated by manure application.

For most parameters, greater variability was observed in manure-amended soils, than in non-amended soils emphasizing the need to assess these two groups of soils separately. Manure application, plus the duration and type of manure applied also influenced soil mineralizing enzymes and the microbial populations. MBN, organic carbon %, pH, sand and urease all influenced the modeling of field N mineralization in manure-amended soils. Zaman et al. (1999a) found that microbial biomass C and N were the best parameters in describing gross N mineralization. They found gross N mineralization was significantly correlated to soil microbial biomass and urease. The enhancement of the soil microbial population by manure application not only provides a labile nutrient

source, but also promotes greater mineralization through enzyme activity. Future modeling should include greater evaluation of the impact of manure application on biological potential to accurately predict mineralization and minimize environmental impact.

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7. CONCLUSIONS

The influence of site was the dominant factor influencing N mineralization rates in this study. Site effects reflect a wide range of soil chemical and biochemical characteristics responding to soil pedogensis, climate and management and combine to influence the nature of the response to manure application. The dominant influence of site characteristics underscores the need include site-specific measures in approaches to describe the influence of manure application on N mineralization.

Manure application stimulated the soil microbial population as indicated by increases in parameters such as MBC, MBN, urease and dehydrogenase content. This effect was most strongly expressed in soil collected in the spring (May) where higher levels of MBC and greater differences between manure amended and non-amended soils were apparent. These effects were correlated with EOC levels, which were higher in manure-amended soils. Furthermore, soil nutrient (NH_4^+, NO_3^-, PO_4^{2-}, K^+) levels were higher in the spring due to availability of recently applied inorganic (fertilizers) and organic (manure) nutrients. This higher nutrient status may have contributed to greater microbial activity with the greatest responses seen in the manure-amended samples. The stimulatory effect of manure was expressed most dramatically in soils receiving more than 35 years of manure applications; nitrate, phosphorus, and potassium were higher in these soils.

Microbial diversity was not as significantly affected by manure application as estimated at the end of the growing season. But, soils with longer manure application histories had a higher Shannon evenness index of microbial diversity and were distinguishable using partial RDA analyses. With increased microbial activity and higher levels of nutrients in the manure-amended soils, it is not surprising that net field and laboratory N mineralization were significantly higher in manure-amended soils. Thus, manure–amended fields had a higher nitrogen mineralization potential and, provide greater levels of plant available nitrogen (PAN) than non-manure-amended soils. The inclusion of microbial parameters in regressions equation attempting to predict net field N mineralization provided an improved the prediction of field N mineralization.

In conclusion, of the parameters measured, none could be used as a single indicator for N mineralization in manure-amended soils. Although total N, EOC, nitrate, ammonium, P, K, MBC, MBN, dehydrogenase, glutaminase, urease, total KCl extractable NH_4^+N, hot KCl extractable NH_4^+N, and laboratory N mineralization were shown to significantly influenced by manure amendment, only MBN, organic carbon and urease were selected in stepwise regression as being able to predict field N mineralization. Furthermore, pH and the percentage of sand were also selected into the regression model for manure-amended soils. This suggests the importance of environmental site variables on the N mineralization process. Laboratory studies do not account for environmental factors and often remove their influence as much as possible, hence, ignoring the conditions that influence PAN in the field. Future prediction models should include site-specific

characteristics such as soil quality characteristics (chemical, and biological), and manure quality and history of application.

8. CONTRIBUTION TO KNOWLEDGE

The impact of manure application was found to vary among different sites. The response of the microbial community to manure-amendments is shown to have greater mineralization potential through nutrient cycling enzymes, laboratory and field N mineralization measurements. Soil with longer manure application histories also appeared to increase the soil's nutrient cycling abilities as demonstrated by soil microbial parameters, such MBC, MBN, glutaminase, urease and phosphatase. Thus soil biological properties and site-specific effects on these properties may be important considerations when managing soil fertility. The inclusion of biological indices improved the prediction of field N mineralization, but further benefit resulted from the inclusion of site-specific characteristics such as soil texture and chemistry. Overall, no one single variable was able to predict N mineralization very well. Even with the inclusion of biological and chemical parameters, a high degree of variability was still present. More research is needed to account for mineralization differences and to understand how different soil qualities influence mineralization. Although an established long-term experiment containing manure-amended soils would be beneficial in modeling a peculiar site, mineralization is site-dependent and the results from one site may not applied universally to all soils in a region such as southern Manitoba. Mineralization models also need to

include estimates for potential pathways of nutrient movement or loss such as leaching or denitrification.

9. APPENDICES

Appendix I – Site meteorological data

Table I-1 Meteorological data for Brandon for average normals (between 1971 – 2000) and year 2000 that are close to site 1.

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000	Normal
January	-16.9	-18.04	24.0	17.95
February	-9.9	-13.8	12.8	14.1
March	0.4	-6.4	34.4	22.2
April	4.1	3.5	7.2	31.0
May	10.8	11.4	51.8	52.7
June	13.2	16.1	66.0	74.4
July	18.5	18.4	133.0	75.8
August	17.5	17.5	46.0	69.2
September	11.5	11.4	66.4	50.1
October	5.5	4.4	33.4	27.7
November	-8.1	-6.1	70.6	17.7
December	-22.6	-14.9	39.8	19.2
Yearly	1.9	1.9	585.4	472

Table I-2 Meteorological **data for Selkirk for average normals (between 1971 – 2000) and year 2000 that are close to site 2 and 5.**

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000	Normal
January	-16.3	-17.46	21.0	16.03
February	-8.2	-13.3	33.0	11.3
March	-0.3	-5.9	30.2	21.8
April	4.6	4.1	5.4	26.0
May	11.6	12.4	55.2	56.6
June	15.0	17.3	176.2	93.0
July	20.0	19.8	129.5	79.6
August	19.2	18.7	97.0	74.5
September	12.2	12.5	62.5	57.5
October	7.1	5.5	27.6	35.6
November	-4.3	-4.9	94.4	23.7
December	-21.3	-14.1	34.0	14.7
Yearly	3.3	2.9	766.0	510.4

Table I-3 Meteorological data for Baldur for average normals (between 1971 – 2000) and year 2000 that are close to site 3.

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000	Normal
January	-14.3	-16.41	19.0	22.9
February	-7.8	-12.7	37.8	23.4
March	0.1	-5.9	22.2	26.4
April	3.7	3.6	5.2	32.2
May	11.0	11.6	58.6	62.6
June	13.7	16.4	113.7	91.7
July	19.0	18.8	77.1	69.2
August	18.2	18.0	123.3	73.8
September	11.6	11.9	58.3	49.8
October	6.1	4.9	18.7	39.3
November	-6.9	-4.9	93.8	26.8
December	-20.9	-13.5	40.0	24.5
Yearly	2.8	2.6	667.7	542.5

Table I-4 Meteorological data for Steinbach for average normals (between 1971 – 2000) and year 2000 that are close to site 4 and 7.

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000	Normal
January	-15.8	-17.37	20.2	21.8
February	-8.3	-13.0	30.3	14.4
March	0.2	-5.5	22.1	19.4
April	4.5	4.1	7.9	28.7
May	12.0	11.9	58.4	58.9
June	14.7	16.6	208.7	95.2
July	19.4	19.1	154.8	80.3
August	18.7	18.1	123.4	68.5
September	12.0	12.1	35.1	59.7
October	7.3	5.4	22.2	44.6
November	-4.2	-5.0	106.9	26.9
December	-20.7	-14.1	53.9	21.1
Yearly	3.4	2.7	834.9	539.4

Table I-5 Meteorological data for Morden for average normals (between 1971 – 2000) and year 2000 that are close to site 6.

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000	Normal
January	-14.4	-15.6	14.7	19.18
February	-5.5	-11.7	22.4	19.2
March	1.3	-4.9	12.0	25.0
April	5.2	4.7	5.4	35.5
May	12.4	12.9	47.2	63.3
June	15.5	17.7	90.8	84.4
July	19.9	20.1	39.0	71.2
August	19.9	19.1	166.4	69.9
September	12.9	13.3	40.2	52.7
October	7.7	6.2	19.6	44.8
November	-4.5	-4.3	75.5	27.4
December	-19.0	-12.5	30.2	20.8
Yearly	4.3	3.8	563.4	533.3

Table I-6 Meteorological data for Pilot Mound for climate normals (between 1971 – 2000) and year 2000 that are close to site 8.

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000*	Normal
January	-15.2	-17.6	-	21.01
February	-7.8	-12.8	28.4	16.6
March	-0.2	-6.5	18.1	22.7
April	3.3	3.5	9.0	35.9
May	10.7	11.3	50.0	58.2
June	13.2	15.8	124.2	82.1
July	18.1	18.7	44.2	63.6
August	17.7	17.9	85.8	69.6
September	11.1	11.6	48.0	56.0
October	5.6	5.1	20.8	37.7
November	-7.0	-5.4	30.8	20.2
December	-21.3	-14.4	-	20.3
Yearly	2.4	2.3	incomplete	503.9

* incomplete data whose sum is 459.3 mm of moisture.

Table I-7 Meteorological data for Stony Mountain for climate normals (between 1971 – 2000) and year 2000 that are close to site 9 and 10.

	Mean Temperature (°C)		Total Precipitation (mm water equiv.)	
Month	2000	Normal	2000	Normal
January	-16.7	-17.98	36.1	21.96
February	-8.8	-13.8	25.5	15.8
March	0.0	-6.3	26.1	20.7
April	4.5	3.8	6.6	26.5
May	12.2	12.1	66.8	54.8
June	15.3	16.9	197.4	88.9
July	20.2	19.5	112.2	71.5
August	19.8	18.4	63.9	68.6
September	12.4	12.3	61.1	53.1
October	7.1	5.1	29.8	39.0
November	-4.6	-5.4	90.4	27.1
December	-20.8	-14.7	56.5	22.6
Yearly	3.4	2.5	772.4	510.4

Appendix II

Methods of Selected Parameters

II.1 Microbial Parameters

II.1 2 Microbial Biomass C and N (modified from Voroney et al., 1993)

Two 25 g portions of moist incubated soil for each sample were placed into 100-mL square glass bottles. One set of samples was fumigated for 24 hours then extracted. This reflected the soil's microbial biomass C and N. This other unfumigated set was to be extracted immediately to determine the background levels of soluble C and N. The bottles that were to be fumigated were placed in a desiccator lined with freshly moistened paper towels. A 100-mL beaker containing 50 mL $CHCl_3$ and a few boiling chips was placed in the middle of the desiccator. The desiccator was sealed and evacuated until the $CHCl_3$ boils vigorously for 1 minute. After 24 hours, the vaccum was released and the samples were vacuumed 3 times (approx. 30 seconds each time) to remove excess $CHCl_3$. To both sets of fumigated and unfumigated samples, 50 mL of 0.5 M K_2SO_4 was added. The bottles were stoppered and shaken on a lateral shaker for 1 hour at 150 rpm. The samples were filtered through pre-washed Whatman #5 filter paper (12.5 cm) into 20 ml polyethylene scintillation vials. The filterate was analyzed on an autoanalyzer for dissolved organic carbon, total nitrogen, nitrate and ammonia.

II.2 Soil Enzymes

II. 2.1 Urease (modified from Frankenberger and Tabatabai 1991)

Five grams of moist incubated soil in a 50 mL Falcon centrifuge tubes. Tubes were also included for soil blanks (5 g of soil, randomly chosen) and solution blanks (no soil). Nine millilitres of THAM buffer (0.05M pH 9) to all samples and blanks. The assay was began by adding 1 ml of substrate (0.2M urea) to each sample and solution blank tubes. The soil blanks received 1ml of deionized water. The tubes were capped, vortexed (approx. 15 sec), and then placed into a water bath (37°C) for two hours. After 2 hours, the enzyme assay was stopped by adding 20 mL KCl-Ag_2SO_4 solution (3 M KCl-100 ppm Ag_2SO_4) with the bottle pipette disperser to all tubes (samples and all blanks). The tubes are then recapped, vortexed, and the soil assay mixture filtered through pre-washed Whatman #2 filter paper (12.5 cm) into 20 mL polyethylene scintillation vials. When possible, the filtrate samples were analysed the sample day as the assay was done. The ammonia concentrations in the collected filtrate were determined used an Orion Ammonia Electrode probe. A standard curve using 1.4 ppm, 14 ppm, and 140 ppm NH_4Cl-N was determined on the probe reader and repeated on regular intervals. Quality control was also maintained using 10 ppm $(NH_4)_2SO_4$-N.

II.2.2 Phosphomonoesterase (modified from Tabatabai, 1982)

Into 50 mL Falcon centrifuge tubes, weight out 0.5 g of moist incubated soil. Tubes were also included for soil blanks (5 g of soil, randomly chosen) and solution blanks (no soil). Add 8 mL of THAM buffer (0.1 M pH 11) and 2 ml of substrate, *p*-nitrophenyl phosphate tetrahydrate (15 mM) to each sample and solution blank tube. The soil blanks get 8 mL of

THAM buffer (0.1 M pH 10) and 2 mL of deionized water. The tubes were capped, vortexed (approximately. 15 sec), and then placed into a water bath (37°C) for half an hour. During the reaction, *p*-nitrophenyl phosphate tetrahydrate is converted into a bright yellow end product, *p*-nitrophenol. A standard curve of 0, 1, 2, 3, 4, and 5 ppm *p*-nitrophenol is used to determine the concentration of *p*-nitrophenol in the filtrate. Five millilitres of standard was added into the 50 mL Falcon centrifuge tubes and placed in to the water bath at the same time as the sample tubes. The assay is stopped by adding 2mL of 0.5 M of $CaCl_2$ and 8 mL of 0.04 M NaOH to all sample, blanks, and standard curve tubes. After adding the stopping solutions, the tubes are vortexed again and filtered through Whatman #2 filter paper (12.5 cm). The filtrate was collected into glass vials and the absorbance read at 400 nm. Dilutions with deionized water were done when the sample absorbance was no within range of the standard curve. Most dilutions required an one to ten dilution range.

II.2.3 Glutaminase (modified from Frankenberger and Tabatabai 1995)

Five grams of moist incubated soil in a 50 mL Falcon centrifuge tubes. Tubes were also included for soil blanks (5 g of soil, randomly chosen) and solution blanks (no soil). The assay was begun by adding 10 mL of glutamine substrate + THAM buffer (0.1 M THAM and 0.05 M L-Glutamine, pH 10) to each sample and solution blank tubes. The soil blanks get 9 mL of THAM buffer (0.1 M pH 10) and 1 mL of deionized water. . The tubes were capped, vortexed (approx. 15 sec), and then placed into a water bath (37°C) for two hours. After 2 hours, the enzyme assay was stopped by adding 20 mL KCl-Ag_2SO_4 solution (3 M KCl-100 ppm Ag_2SO_4) with the bottle pipette disperser to all

tubes (samples and all blanks). The tubes are then recapped, vortexed, and the soil assay mixture filtered through pre-washed Whatman #2 filter paper (12.5 cm) into 20 mL polyethylene scintillation vials. When possible, the filtrate samples were analyzed the sample day as the assay was done. The ammonia concentrations in the collected filtrate were determined used an Orion Ammonia Electrode probe. A standard curve using 1.4 ppm, 14 ppm, and 140 ppm NH_4Cl-N was determined on the probe reader and repeated on regular intervals. Quality control was also maintained using 10 ppm $(NH_4)_2SO_4$-N.

II.2.4 Dehydrogenase (modified from Tabatabai, 1982)

1.5 g moist incubated soil was placed in a 10 mL glass Kimax tube. Two sets of solution reagent and random soil blanks were include for quality control. Then, approximately 0.02 g $CaCO_3$ was added to each tube. This was followed by 1.5 mL of deionised water. The assay began by adding 0.6 mL substrate (3% 2,3,5-triphenyl tetrazolium chloride in water) to the test tube containing soil sample or solution reagent blanks. In the soil blanks, 0.6 ml deionized water was added instead of 3% triphenyl tetrazolium. All test tubes were vortexed (approx. 15 sec per tube), placed in water bath at 37°C and shaken for 24 hours. A standard curve consisting of 5, 10, 15, and 20 ppm of triphenyl formazan (TPF) in methanol was also incubated at this time. A sample of ASC Methanol was also included for the lowest portion of the standard curve. After 24 hours, the tubes were removed from the water bath. Adding 10 mL ACS methanol stopped the assay reaction. The tubes were then vortexed (approx. 15 sec per tube), then centrifuge for 15 minutes at 3000 rpm. The absorbance of the sample and blank supernatants, and the standard curve was measured on a spectrophotometer at 485 nm. The concentration of end product, TPF

is an indication of potential dehydrogenase activity and is determined using the calibration curve.

Appendix III – Manure and site characteristics

Table III.1 The average and standard deviation of selected manure characteristics on a dry weight basis (105°C).

Site	Livestock Manure Type	Moisture	EC	pH	Ammonium-N	Organic nitrogen	Total Nitrogen	Nitrite and nitrate -N	Phosphorus
		%	dS/m		g/kg				
BRA	Feed Cattle	74±6	4.0+0.5	9.4±0.1	0.13±0.07	17.3±5.5	17.5±5.6	134.6±60.1	4.8±0.9
ESK	Hog	97+0.3	16.8±0.0	7.4±0.0	70.33±7.0	29.8±1.9	100.6±7.6	306.8±27.9	45.4±4.0
KIL	Feed Cattle	59±4.0	2.1±0.7	6.8±0.6	0.27±0.27	20.2±4.3	23.1±5.8	2608.5±1704.8	7.1±1.6
KSB	Hog	98±0.7	18.9±0.1	6.7±0.1	120.25±43.66	34.6±15.4	170.0±48.3	217.5±121.6	27.4±7.2
LKP	Dairy Cattle	80±1.3	7.1±2.9	9.2±0.7	0.26±0.13	24.9±7.4	26.1±8.5	893.0±1186.8	22.6±9.7
MOR	Dairy Cattle	76±20.9	18.4±1.1	6.9±0.1	31.40±5.09	16.9±1.3	52.0±4.0	97±24.11	6.3±4.7
MSB	Hog	96±4.1	18.6±1.2	7.6±0.1	98.75±50.90	56.3±28.2	155.6±78.8	470.2±306.3	43.0±5.2
SLK	Hog	97±2.1	16.4±10.1	7.5±0.6	131.08±116.13	25.4±10.3	129.2±75.6	901.5±1139.7	26.2±11.8
STM	Dairy	56±4.9	19.5±4.8	8.1±0.3	0.45±0.16	18.7±4.9	20.1±4.6	903.3±648.1	6.9±1.3
SWL	Hog	98±1.0	14.0±0.9	6.9±1.0	165.15±99.16	48.0±23.9	215.3±114.2	1860.5±1115.4	48.4±6.3

Table III.2 Field management practices.

Site	2000 Crop		Crops in Field History[1]		Manure Method	Manure Application Rate	Inorganic Fertilizers		Tillage
	Non-manure	Manure	Non-Manure	Manure			Non-Manure	Manure	
							---N – P – K – S kg/ha--		
BRA	Wheat	Barley	Wh, B, Ca	B, Wh, Ca	Broadcast incorporated	4 tonnes/ha	82-37-11-11	87-37-11-11	Zero-tillage*
ESK	Barley	Canola	B, Wh, Ca	Ca, B, Wh	Injected	7654 l/ha	90-22-0-0	112-56-0-22	Minimum
KIL	Winter Wheat	Canola	Ca, Wh, P, O	Ca, Wh, Wi	Broadcast incorporated	4 tonnes/ha	112-34-17-0	112-34-0-13	Zero-tillage*
KSB	Wheat	Wheat	Wh, B, Ca, O	Wh, B, Ca, O	Broadcast incorporated	1530 l/ha	134-17-11-0	none	Conventional
LKP	Canola	Corn	Ca, Wh, B, F	Co, Ca, Wh, F, B	Broadcast incorporated	4 tonnes/ha	140-45-0-22	109-39-0-0	Conventional
MOR	Canola	Corn	Ca, Wh, Su,	Ca, Co	Broadcast incorporated	9185 l/ha	90-34-0-0	none	Conventional
MSB	Canola	Corn	Ca, Wh, B, Co	Co, Wh, Ca, B	Injected	10716 l/ha	0-39-11-11	34-11-0-11	Conventional
SLK	Barely	Wheat	B, Ca, F, Wh	Wh, Ca, B, Co	Injected	12247 l/ha	84-39-11-6	none	Conventional
STM	Barley	Corn	Ba, Co, Veg. (75yrs)	Co, B, O-P-B, A	Broadcast incorporated	4-8 tonnes/ha	101-34-0-11	39-22-0-6	Conventional
SWL	Barely	Barley	Untamed hay field	B, O, Fallow	Broadcast incorporated	17850 l/ha previous year on fallow	45-28-22-6	none	Conventional

[1]Crops in Field History, crop abbreviations: A – alfalfa, Ba – barley, Ca- canola, Co- corn, F – flax; O – oats, P- peas, Su – sunflower, Veg – various garden vegetables, Wh-red spring wheat, Wi – winter wheat.

Table III.3 Livestock and manure handling and storage.

Site	Livestock Manure Type	Manure Phase	Number of A.U.	Type of Manure Storage Facility	Storage covered	Use of bedding	Manure collection	Additional Comments
BRA	Feed Cattle	Solid	113	Piled	No	Wheat	Bobcat	-
ESK	Hog	Liquid	300	Above ground steel tank	No	No	Flush	Farrow to finish
KIL	Feed Cattle	Solid	140	Piled	No	Wheat straw	Bobcat	-
KSB	Hog	Liquid	250	Concrete under ground tank – one cell	Yes	No	Flush	Farrow to finish
LKP	Dairy Cattle	Solid	300	Piled	No	Wheat straw	Bobcat	Contains animal waste and straw only
MOR	Dairy Cattle	Liquid	100	Earthen Storage - one cell	No	Barley straw	Sweep + Pump out	Manure also contains waste water from cleaning dairy
MSB	Hog	Liquid	300	Concrete under ground tank – one cell	Yes	No	Flush	Farrow to finish
SLK	Hog	Liquid	850	Concrete under ground tank – one cell	No	Wheat Straw	Sweep + Pump out	Farrow to finish, straw only used for brooding area
STM	Dairy Cattle	Solid	1000 – cattle 50 - hogs	Piled	No	Wheat/ Barley Straw	Bobcat	Hogs make up small % of operation, not included in study
SWL	Hog	Liquid	126	Concrete under ground tank – one cell	Liquid-Yes	Barley Straw	Sweep + Pump out	Farrow to finish, straw only used for brooding area

Appendix IV

Analysis of variance for various soil parameters

Table IV.1 Mean volumetric moisture content of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------%----------						
4	53.2 *wheat*	a	49.5 *wheat*	ab	F	L	H
7	41.6 *corn*	bcd	50.3 *canola*	ab	F	L	H
5	45.6 *corn*	abc	45.9 *canola*	abc	F	L	C
2	38.6 *canola*	cde	34.2 *barley*	def	F	L	H
1	32.5 *barley*	defg	27.1 *wheat*	fgh	C	S	C
3	31.7 *canola*	efg	31.0 *winter wheat*	efg	F	S	C
8	30.2 *wheat*	efg	31.4 *barley*	efg	C	S	H
9	30.6 *corn*	efg	29.4 *barley*	efg	F	L	C
6	25.2 *corn*	fgh	18.3 *canola*	h	C	L	C
10	24.2 *barley*	gh	17.7 *barley*	h	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.001
Treatment	1	<0.05
Site x Treatment	9	<0.01

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.2 Mean volumetric moisture content ofsoil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture †	Manure History‡	Manure Type§
	----------%----------						
4	38.2 *wheat*	abc	47.0 *wheat*	a	F	L	H
5	40.7 *corn*	ab	40.4 *canola*	ab	F	L	C
2	29.4 *canola*	bcdef	35.5 *barley*	abcd	F	L	H
7	34.7 *corn*	abcd	35.4 *canola*	abcd	F	L	H
1	34.8 *barley*	abcd	28.9 *wheat*	bcdef	C	S	C
9	24.3 *corn*	defg	28.8 *barley*	bcdef	F	L	C
3	26.2 *canola*	cdefg	25.3 *winter wheat*	defg	F	S	C
6	22.3 *corn*	efg	14.6 *canola*	g	C	L	C
8	17.8 *wheat*	fg	21.8 *barley*	fg	C	S	H
10	17.3 *barley*	fg	14.6 *barley*	g	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.05

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.3 Mean 70% field capacity of soil samples (0-15 cm) among various sites. Only one sample per treatment block was used to determine field capacity. No statistics were done for field capacity.

Site	Manured *Crop*	Non-manured *Crop*	Texture†	Manure History‡	Manure Type§
	----------%----------				
4	39.8 *wheat*	35.5 *wheat*	F	L	H
7	28.9 *corn*	38.3 *canola*	F	L	H
5	35.8 *corn*	33.2 *canola*	F	L	C
8	20.8 *wheat*	27.5 *barley*	C	S	H
9	26.7 *corn*	27.3 *barley*	F	L	C
2	24.1 *canola*	23.1 *barley*	F	L	H
3	22.0 *canola*	23.1 *barley*	F	S	C
1	22.4 *barley*	18.7 *wheat*	C	S	C
6	20.3 *corn*	17.9 *canola*	C	L	C
10	16.4 *barley*	20.1 *barley*	C	S	H

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.4 Mean organic carbon percentage of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two- way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	----------%----------						
4	6.44 *wheat*	5.66 *wheat*	6.05	a	F	L	H
5	6.16 *corn*	5.89 *canola*	6.02	a	F	L	C
7	5.10 *corn*	5.34 *canola*	5.22	b	F	L	H
9	4.81 *corn*	4.71 *barley*	4.78	b	F	L	C
2	4.35 *canola*	3.94 *barley*	4.15	c	F	L	H
8	3.28 *wheat*	3.80 *barley*	3.54	d	C	S	H
3	3.67 *canola*	3.76 *winter wheat*	3.71	cd	F	S	C
1	3.70 *barley*	3.28 *wheat*	3.49	d	C	S	C
6	3.36 *corn*	2.28 *canola*	2.81	e	C	L	C
10	2.09 *barley*	2.29 *barley*	2.19	f	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.5 Mean organic carbon percentage of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	----------%----------						
5	5.93 *corn*	5.79 *canola*	5.86	a	F	L	C
4	4.33 *wheat*	5.67 *wheat*	5.00	ab	F	L	H
7	4.96 *corn*	4.97 *canola*	4.97	ab	F	L	H
9	4.90 *corn*	3.96 *barley*	4.43	bc	F	L	C
2	4.58 *canola*	4.25 *barley*	4.25.	bc	F	L	H
8	3.80 *wheat*	3.60 *barley*	3.70	c	C	S	H
3	3.28 *canola*	3.76 *winter wheat*	3.52	c	F	S	C
1	3.70 *barley*	3.65 *wheat*	3.68	c	C	S	C
6	3.25 *corn*	1.83 *canola*	2.34	d	C	L	C
10	2.25 *barley*	2.74 *barley*	2.94	d	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.6 Mean total nitrogen percentage of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	----------%----------						
5	0.54 *corn*	0.48 *canola*	0.51	a	F	L	C
4	0.54 *wheat*	0.44 *wheat*	0.49	ab	F	L	H
9	0.45 *corn*	0.46 *barley*	0.46	bc	F	L	C
7	0.44 *corn*	0.42 *canola*	0.43	cd	F	L	H
2	0.40 *canola*	0.38 *barley*	0.39	de	F	L	H
3	0.40 *canola*	0.37 *winter wheat*	0.38	e	F	S	C
8	0.34 *wheat*	0.38 *barley*	0.36	e	C	S	H
1	0.38 *barley*	0.35 *wheat*	0.36	e	C	S	C
6	0.27 *corn*	0.34 *canola*	0.31	f	C	L	C
10	0.25 *barley*	0.27 *barley*	0.26	f	C	S	H
Treatment	0.41 a	0.38 b					

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.05
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.7 Mean total nitrogen percentage of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	----------%----------						
5	0.50 *corn*	0.47 *canola*	0.80	a	F	L	C
4	0.47 *wheat*	0.42 *wheat*	0.44	ab	F	L	H
9	0.44 *corn*	0.36 *barley*	0.40	bc	F	L	C
2	0.41 *canola*	0.39 *barley*	0.40	bc	F	L	H
7	0.41 *corn*	0.40 *canola*	0.40	bc	F	L	H
1	0.37 *barley*	0.35 *wheat*	0.36	c	C	S	C
3	0.33 *canola*	0.37 *winter wheat*	0.35	c	F	S	C
8	0.33 *wheat*	0.37 *barley*	0.35	c	C	S	H
6	0.21 *corn*	0.33 *canola*	0.27	d	C	L	C
10	0.23 *barley*	0.27 *barley*	0.25	d	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.8 Mean pH of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- -log[H^+] ----------						
9	7.78 *corn*	ab	7.83 *barley*	a	F	L	C
10	7.53 *barley*	abc	7.63 *barley*	abc	C	S	H
3	7.65 *canola*	abc	7.53 *winter wheat*	abc	F	S	C
2	7.60 *canola*	abc	7.35 *barley*	abcdef	F	L	H
6	7.58 *corn*	abc	7.23 *canola*	abcdef	C	L	C
7	7.13 *corn*	abcdef	7.48 *canola*	abcd	F	L	H
5	7.43 *corn*	abcde	6.90 *canola*	bcdef	F	L	C
8	6.58 *wheat*	def	7.35 *barley*	bcdef	C	S	H
1	7.35 *barley*	abcdef	6.85 *wheat*	cdef	C	S	C
4	6.55 *wheat*	ef	6.48 *wheat*	f	F	L	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.01

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.9 Mean pH of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- -log[H^+] ----------						
9	7.78	abcd	8.18	a	F	L	C
6	7.58	abcd	8.03	ab	C	L	C
2	7.90	abc	7.63	abcd	F	L	H
8	7.45	abcde	7.83	abcd	C	S	H
3	7.83	abcd	7.50	abcd	F	S	C
10	7.70	abcd	7.78	abcd	C	S	H
7	7.20	abcde	7.73	abcd	F	L	H
1	7.45	abcde	6.93	de	C	S	C
5	7.40	abcde	7.05	cde	F	L	H
4	7.15	bcde	6.55	e	F	L	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	<0.01

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.10 Mean EC of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- dS m^{-1} ----------						
9	1.20 *corn*	ab	2.80 *barley*	a	F	L	C
3	2.65 *canola*	ab	0.93 *winter wheat*	ab	F	S	C
7	1.52 *corn*	ab	2.61 *canola*	ab	F	L	H
4	1.57 *wheat*	ab	2.00 *wheat*	ab	F	L	H
6	1.58 *corn*	ab	1.72 *canola*	ab	C	L	C
5	1.50 *corn*	ab	1.32 *canola*	ab	F	L	C
8	1.44 *wheat*	ab	1.02 *barley*	ab	C	S	H
2	1.27 *canola*	ab	1.22 *barley*	ab	F	L	H
10	1.15 *barley*	ab	0.74 *barley*	ab	C	S	H
1	0.96 *barley*	ab	0.64 *wheat*	b	C	S	C

ANOVA

	df	Prob>F
Site	9	<0.01
Treatment	1	ns
Site x Treatment	9	<0.05

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.11 Mean EC of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	---------- dS m^{-1} ----------						
8	1.39 *wheat*	0.63 *barley*	1.01	ab	C	S	H
9	1.07 *corn*	1.19 *barley*	1.12	a	F	L	C
7	0.80 *corn*	0.17 *canola*	0.98	abc	F	L	H
1	1.13 *barley*	0.56 *wheat*	0.84	abcd	C	S	C
4	1.08 *wheat*	0.87 *wheat*	0.97	abc	F	L	H
3	0.99 *canola*	0.92 *winter wheat*	0.96	abc	F	S	C
5	0.89 *corn*	0.68 *canola*	0.78	bcd	F	L	C
2	0.68 *canola*	0.87 *barley*	0.77	bcd	F	L	H
6	0.78 *corn*	0.55 *canola*	0.66	cd	C	L	C
10	0.58 *barley*	0.50 *barley*	0.54	d	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.05
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.12 Mean extractable organic carbon of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- µg C g^{-1} soil ----------						
5	111.7 *corn*	a	*canola*		F	L	C
9	77.9 *corn*	bc	90.0 *barley*	ab	F	L	C
2	69.2 *canola*	bcd	55.8 *barley*	cde	F	L	C
3	66.5 *canola*	bcd	52.7 *winter wheat*	cde	F	S	C
8	66.0 *corn*	bcd	61.7 *barley*	bcd	C	S	H
4	63.5 *wheat*	bcd	29.6 *wheat*	ef	F	L	H
1	62.4 *barley*	bcd	40.0 *wheat*	def	C	S	C
7	54.7 *corn*	cde	45.8 *canola*	def	F	L	H
10	48.6 *barley*	cdef	48.4 *barley*	cdef	C	S	H
6	25.7 *corn*	ef	19.8 *canola*	f	C	L	C

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.13 Mean extractable organic carbon of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	---------- μg C g^{-1} soil ----------						
8	88.6 *wheat*	58.9 *barley*	73.8	a	C	S	H
9	72.2 *corn*	77.5 *barley*	74.8	a	F	L	C
3	75.4 *canola*	43.4 *winter wheat*	59.4	b	F	S	H
1	64.1 *barley*	41.8 *wheat*	55.4	bc	C	S	C
6	63.3 *corn*	35.7 *canola*	49.6	bc	C	L	C
2	60.1 *canola*	42.5 *barley*	51.6	bc	F	L	H
5	54.8 *corn*	29.9 *canola*	42.3	cd	F	L	C
10	38.8 *barley*	40.0 *barley*	39.4	cd	C	S	H
4	35.6 *wheat*	12.8 *wheat*	24.2	e	F	L	H
7	29.9 *corn*	31.2 *canola*	30.5	de	F	L	H
Treatment	58.3 a	41.4 b					

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.14 Mean nitrate of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*	Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	---------- kg N ha^{-1} ----------						
8	164.7 *wheat*	21.5 *barley*	98.1	cd	C	S	H
4	122.5 *wheat*	86.6 *wheat*	104.5	b	F	L	H
9	33.7 *corn*	71.3 *barley*	52.5	bcd	F	L	C
7	48.9 *corn*	63.6 *canola*	56.2	cd	F	L	H
6	59.4 *corn*	55.0 *canola*	57.2	bc	C	L	C
2	52.2 *canola*	55.3 *barley*	53.7	bc	F	L	H
5	51.1 *corn*	54.0 *canola*	52.5	bc	F	L	C
3	50.0 *canola*	22.3 *winter wheat*	36.1	bcd	F	S	C
10	49.0 *barley*	30.0 *barley*	39.9	d	C	S	H
1	24.6 *barley*	20.0 *wheat*	22.3	a	C	S	C

ANOVA

	df	Prob>F
Site	9	<0.05
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.15 Mean nitrate of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	---------- kg N ha^{-1} ----------						
1	58.3 *barley*	a	20.1 *wheat*	bcd	C	S	C
6	32.3 *corn*	b	13.5 *canola*	cd	C	L	C
4	29.1 *wheat*	bc	23.4 *wheat*	bcd	F	L	H
9	26.5 *corn*	bcd	12.4 *barley*	cd	F	L	C
3	23.5 *canola*	bcd	17.5 *winter wheat*	bcd	F	S	C
5	23.4 *corn*	bcd	19.2 *canola*	bcd	F	L	C
8	21.7 *wheat*	bcd	10.0 *barley*	d	C	S	H
2	17.4 *canola*	bcd	19.8 *barley*	bcd	F	L	H
7	17.3 *corn*	bcd	13.5 *canola*	cd	F	L	H
10	9.3 *barley*	d	12.0 *barley*	cd	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.16 Mean phosphorus of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------kg P ha^{-1} ----------						
9	400.8 *corn*	a	89.8 *barley*	cd	F	L	C
10	293.5 *barley*	ab	20.5 *barley*	d	C	S	H
4	247.3 *wheat*	abc	52.3 *wheat*	d	F	L	H
2	48.8 *canola*	d	129.0 *barley*	bcd	F	L	H
5	120.5 *corn*	cd	34.8 *canola*	d	F	L	C
8	104.0 *wheat*	cd	23.3 *barley*	d	C	S	H
7	86.8 *corn*	cd	55.9 *canola*	d	F	L	H
6	82.0 *corn*	cd	19.5 *canola*	d	C	L	C
3	61.7 *canola*	d	33.0 *winter wheat*	d	F	S	C
1	48.8 *barley*	d	41.0 *wheat*	d	C	S	C

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.17 Mean phosphorus of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------kg P ha^{-1} ----------						
9	401.3 *corn*	a	61.8 *barley*	cd	F	L	C
4	250.0 *wheat*	b	39.8 *wheat*	d	F	L	H
10	155.0 *barley*	bc	38.0 *barley*	d	C	S	H
8	100.8 *wheat*	cd	22.8 *barley*	d	C	S	H
6	90.8 *corn*	cd	15.3 *canola*	d	C	L	C
2	76.8 *canola*	cd	83.5 *barley*	cd	F	L	H
1	72.3 *barley*	cd	32.3 *wheat*	d	C	S	C
7	63.4 *corn*	cd	39.5 *canola*	d	F	L	H
5	60.3 *corn*	cd	25.3 *canola*	d	F	L	C
3	44.8 *canola*	d	38.5 *winter wheat*	d	F	S	C

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.18 Mean sulphate of soil samples (0-15 cm) among various sites for May

Site	Manured *Crop*	Non-manured *Crop*	Texture†	Manure History‡	Manure Type§
	----------kg S ha^{-1} ----------				
3	243.5 canola	13.3 winter wheat	F	S	C
9	5.25 corn	198.3 barley	F	L	C
6	92.6 corn	152.0 canola	C	L	C
7	56.2 corn	74.3 canola	F	L	H
8	25.5 wheat	36.4 barley	C	S	H
2	3.53 canola	28.0 barley	F	L	H
4	22.4 wheat	14.3 wheat	F	L	H
1	12.9 barley	10.0 wheat	C	S	C
5	12.1 corn	5.5 canola	F	L	H
10	9.00 barley	3.25 barley	C	S	H

ANOVA

	df	Prob>F
Site	9	ns
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.19 Mean sulphate of soil samples (0-15 cm) among various sites for August

Site	Manured *Crop*	Non-manured *Crop*	Texture†	Manure History‡	Manure Type§
	----------kg S ha^{-1} ----------				
8	106.1 wheat	17.8 barley	C	S	H
9	5.3 corn	38.7 barley	F	L	C
3	25.3 canola	6.3 winter wheat	F	S	C
5	18.4 corn	3.2 canola	F	L	C
1	12.6 barley	4.3 wheat	C	S	C
4	11.9 wheat	9.0 wheat	F	L	H
6	9.4 corn	6.8 canola	C	L	C
7	4.4 corn	3.5 canola	F	L	H
2	4.0 canola	2.8 barley	F	L	H
10	2.5 barley	2.5 barley	C	S	H

ANOVA

	df	Prob>F
Site	9	ns
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.20 Mean potassium of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------kg K ha^{-1} ----------						
9	898 *corn*	a	318 *barley*	fg	C	S	H
4	881 *wheat*	a	715 *wheat*	abc	F	L	H
5	833 *corn*	ab	660 *canola*	abcd	F	L	C
6	614 *corn*	abcde	181 *canola*	bcdef	C	L	C
7	542 *corn*	bcdef	580 *corn*	bcdef	F	L	H
8	558 *wheat*	bcdef	318 *wheat*	fg	C	S	H
1	518 *barley*	cdef	356 *barley*	efg	C	S	C
3	357 *canola*	efg	498 *canola*	cdef	F	S	C
2	429 *canola*	cdefg	392 *canola*	defg	F	L	H
10	368 *barley*	defg	162 *barley*	g	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.21 Mean potassium of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------kg K ha^{-1} ----------						
9	949 *corn*	a	236 *corn*	de	F	L	C
4	758 *wheat*	ab	592 *wheat*	abcd	F	L	H
1	648 *barley*	abc	340 *barley*	bcde	C	S	C
6	608 *corn*	abcd	132 *corn*	e	C	L	C
5	508 *corn*	bcde	547 *corn*	bcd	F	L	C
2	508 *canola*	bcde	545 *canola*	bcd	F	L	H
7	441 *corn*	bcde	436 *corn*	bcde	F	L	H
3	436 *canola*	bcde	419 *canola*	bcde	F	S	C
8	384 *wheat*	bcde	293 *wheat*	cde	C	S	H
10	315 *barley*	cde	151 *barley*	e	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.0001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.22 Mean ammonium of soil samples (0-15 cm) among various sites for May. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from two-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*	Site Overall		Texture†	Manure History‡	Manure Type§
	----------μg NH_4^+-N g^{-1}soil ----------							
8	1.06 *wheat*		1.65 *wheat*	1.35	a	C	S	H
7	1.21 *corn*		1.63 *canola*	1.42	a	F	L	H
9	1.26 *corn*		1.52 *barley*	1.39	a	F	L	C
3	1.21 *canola*		0.77 *winter wheat*	0.99	ab	F	S	C
4	0.96. *wheat*		1.20 *wheat*	1.08	a	F	L	H
1	0.92 *barley*		0.82 *wheat*	0.87	abc	C	S	C
5	0.61 *corn*		0.36 *canola*	0.48	bcd	F	L	C
2	0.44 *canola*		0.32 *barley*	0.38	cd	F	L	H
6	0.36 *corn*		0.31 *canola*	0.34	cd	C	L	C
10	0.07 *barley*	f	0.23 *barley*	0.15	d	C	S	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	ns
Site x Treatment	9	ns

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied

Table IV.23 Mean ammonium of soil samples (0-15 cm) among various sites for August. Letters indicate degree of similarity by comparing all means using Tukey HSD (P<0.05) from one-way ANOVA (site x treatment).

Site	Manured *Crop*		Non-manured *Crop*		Texture†	Manure History‡	Manure Type§
	----------μg NH_4^+-N g^{-1}soil ----------						
7	2.74 *corn*	a	0.94 *canola*	c	F	L	H
5	2.43 *corn*	ab	0.34 *canola*	c	F	L	C
8	1.29 *wheat*	bc	0.73 *barley*	c	C	S	H
1	0.98 *barley*	c	0.23 *wheat*	c	C	S	C
3	0.46 *canola*	c	0.83 *winter wheat*	c	F	S	C
10	0.40 *barley*	c	0.73 *barley*	c	C	S	H
6	0.60 *corn*	c	0.48 *canola*	c	C	L	C
9	0.36 *corn*	c	0.37 *barley*	c	F	L	C
4	0.14 *wheat*	c	0.35 *wheat*	c	F	L	H
2	0.28 *canola*	c	0.20 *barley*	c	F	L	H

ANOVA

	df	Prob>F
Site	9	<0.0001
Treatment	1	<0.001
Site x Treatment	9	<0.0001

† Texture: F - fine-textured soil, C- coarse textured soil
‡ Manure history: L - long term manure application, S- short term manure application
§ Manure type: H- hog manure applied. C- cattle manure applied