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.