# Assessing Potential Soil Microbial "Priming Effects" on N<sub>2</sub>O Emissions as a Result of a Fertilizer Change-Over from Long-Term Manure Applications to Urea.

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# Introduction

Nitrogen fertilizer use in agriculture has been shown to impact nitrous oxide (N<sub>2</sub>O) emissions, and when environmental conditions are favourable, lead to an increase of N<sub>2</sub>O (Whalen, 2000; Rochette *et al.*, 2008). Manure as fertilizer can add or maintain soil organic carbon (OC) and nitrogen (N). Increases in available C and N in the soil can lead to greater N<sub>2</sub>O emissions (Bergstrom *et al.*, 1994). However, depending on the type of manure applied different amounts of OC and N may be added. Solid manures such as solid cattle manures tend to have higher C:N ratios, and more of the added N is bound in organic form (Qian and Schoenau, 2002), and may not be readily available upon application (Helgason *et al.*, 2001), and can be lost as N<sub>2</sub>O under higher moisture environmental conditions (Rochette *et al.*, 2008). Inputs of manures, especially over the long-term, may create conditions in the soil that are favorable for increased microbial activity. This increased activity may carry over if more readily available N (urea) is applied to the system, creating a "priming effect" that may result in increased N<sub>2</sub>O emissions.

Nitrous oxide emissions are facilitated through microbial activity in the soil by both nitrification and denitrification processes. While nitrification primarily occurs under drier, aerobic conditions in the soil, denitrification occurs when the soil is anaerobic, typically with the water filled pore space greater than 60% (Linn and Doran, 1984). Higher N<sub>2</sub>O losses can be seen after increases to soil moisture and N additions, typically after a rainfall event following fertilization (Rochette *et al.*, 2008), or after spring snowmelt (Corre *et al.*, 1996).

Initial research at a site near Dixon, Saskatchewan, showed that denitrification enzyme activities (DEA) in plots with a history of manure applications had a higher activity than plots with a history of urea applications and the control (no manure or urea applied) plots (Farrell, 2011); suggesting that long-term manure applications can produce conditions that may "prime" the soil microbial communities for increased  $N_2O$  emissions when a more readily available fertilizer (urea) is applied to the agroecosytem. However, exactly how  $N_2O$  emissions will be impacted on an agricultural system in the semi-arid prairie of Saskatchewan as a result of a change-over from long-term manure application to a urea application is not well understood. This study examined how potential changes in the microbial activity for nitrogen transformation may have been impacted by long-term application of manure, and how this may lead to a

potential "priming effect" that could result in increased  $N_2O$  emission after a more readily available N source (urea) is applied.

#### **Material and Methods**

#### Site Description

The Dixon long-term research site is located near Dixon, SK (NW21-37-23-W2), on a Black Chernozemic soil with a loamy texture (Stumborg *et al.*, 2007). This long-term research site has had continuous manure applications beginning in 1996 that were subsequently terminated in the fall of 2009. The historically applied amendments are divided into two treatment blocks: a liquid swine manure block and a solid cattle manure block. The two blocks are situated adjacent to each other on the same agricultural landscape. Each block included a control with no fertilizer application, urea, liquid swine manure (LSM), and solid cattle manure (SCM) amendments at a  $1\times$ ,  $2\times$ , and  $4\times$  agronomic application rate. This rate equates to 50-kg plant available N. The two blocks are set up in Randomized Complete Block Design (n=4). Beginning in 2011 annual spring fertilizer applications of urea over the entire field, including all historically applied blocks and plots therein has been applied to the site.

## Gas Sampling

Field gas emissions were sampled using a modified standard gas sampling protocol (Yates *et al.*, 2006). An aluminum vented, non-steady state chamber was placed in each historically amended plot and a 20-ml headspace sample was taken every 15-min over a 45-min sampling event. The samples were transported back to the lab in evacuated sealed tubes, until analysis by gas chromatography in the Department of Soil Science for nitrous oxide emissions. Fluxes were calculated using R statistical software, version 2.15.3 (R-project, 2013), and the HMR flux calculation package (Pedersen, 2012). Gas emissions were monitored from snowfall to snowmelt over three growing seasons from 2011 to 2013.

## Enzyme Assay

Denitrification enzyme activities were assessed using a modified procedure from Methods in Applied Soil Microbiology and Biochemistry (Alef and Nannipieri (eds.), 1995). Soil samples were extracted at multiple times over the growing season. Field moist subsamples were incubated in a slurry solution with an added acetylene inhibitor over 90-min. A headspace sample of 10-ml was taken every 30 minutes and injected into evacuated tubes. The headspace samples were analyzed by gas chromatography for N<sub>2</sub>O. DEA assays were carried out during the 2011, 2012 and 2013 growing seasons.

## **Preliminary Results**

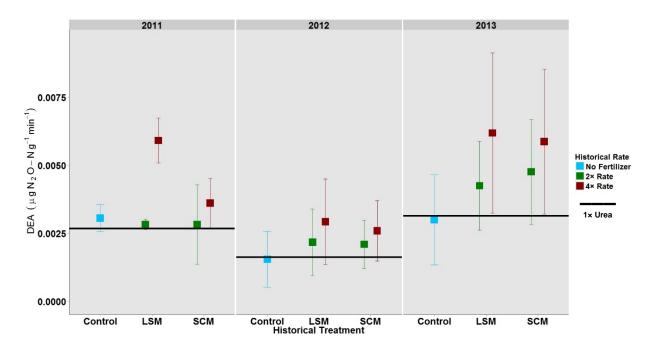


Figure 1. Cumulative denitrification enzyme activities (DEA) for historically applied amendments and application rate for the historically applied control, liquid swine manure (LSM), solid cattle manure (SCM), and urea amendments.

The DEA activities across all three years of sampling post fertilizer change-over show that the historically applied manure amendments produce a higher DEA activity than the historically applied  $1\times$  urea amendment. The manure amended plots also show that higher historically applied rates of manure fertilizer result in higher potential N<sub>2</sub>O production after the change-over to a more readily available urea fertilizer. The historically applied control only showed a higher DEA activity then the  $1\times$  urea amendment in 2011, which was the first year of the fertilizer change-over (Figure 1).

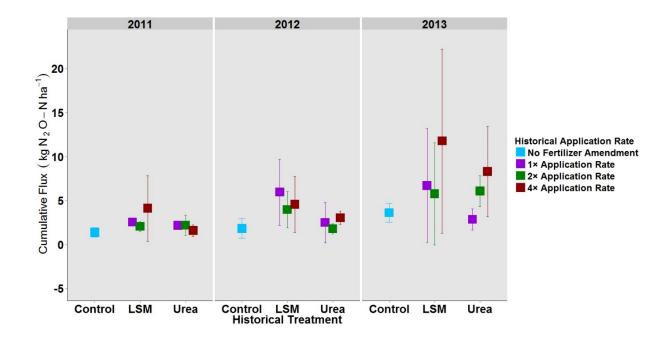


Figure 2. Cumulative flux of  $N_2O$  for the yearly historically applied liquid swine manure block for the 2011, 2012 and 2013 Growing season.

The preliminary results for the cumulative field  $N_2O$  emissions show that the historically applied manure amended plots with yearly applications resulted in a higher loss of  $N_2O$  than the historically applied control plots (Figure 2 and Figure 3). Figure 2 illustrates how the historically applied LSM plots are producing a greater loss of  $N_2O$  over the growing season than the historically applied urea treated plots at equivalent historically applied application rates. The magnitude of the  $N_2O$  losses also increased over the three years of monitoring. In 2011, the first year of fertilizer change-over resulted in the smallest cumulative loss of  $N_2O$ . While, it was during the 2013 growing season that the largest  $N_2O$  cumulative losses were observed. It was also interesting to note that the cumulative loss of  $CO_2$ , estimated from the coinciding  $CO_2$ measurements, showed the largest  $CO_2$  loss in 2011, the first year of annual urea application after the termination of the manure fertilizer application, opposite to what the  $N_2O$  results show.

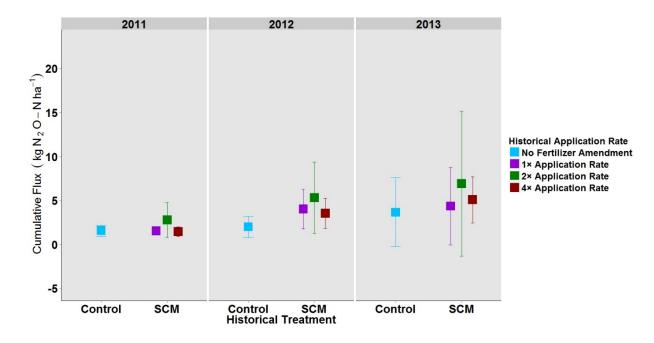


Figure 3. Cumulative flux of  $N_2O$  for the yearly historically applied solid cattle manure block for the 2011, 2012 and 2013 Growing season.

The impact of long-term solid cattle manure application is not as prevalent as was seen with the historically applied liquid swine manure having losses of smaller magnitude especially at the highest application rates then the LSM plots. However, there is still a similar trend of larger losses of  $N_2O$  from the manure amended plots over the historical control.

# Discussion

The higher DEA activities measured in the historically applied manure amended plots, especially with higher application rates, suggest a "priming effect" potentially creating a greater loss of  $N_2O$  under the appropriate environmental conditions. This effect appears to still be influencing the loss of N<sub>2</sub>O three years after the change-over from long-term manure applications to annual urea applications. The increasing size of the error bars may be suggesting that this effect is beginning to level off as time increases from the change-over from manure fertilizer application. This is supported by the preliminary field emissions data, which show a higher loss of N<sub>2</sub>O from the higher rates of historically applied manure plots compared to the historically applied control plots (plots receiving no fertilizer application over the course of the long-term study, but receiving the annual urea applications starting in the spring of 2011). The highest total losses of N<sub>2</sub>O were measured on the historically applied LSM plots (Figure 2). The higher loss of N<sub>2</sub>O from LSM plots over SCM plots may be the result of the greater availability of N from the LSM amended plots to the microbial communities throughout the duration of the long-term manure research study. In summary, the magnitude of the potential N<sub>2</sub>O loss from an agroecosystem in a semi-arid prairie does appear to be impacted by long-term manure applications when subjected to a fertilizer change-over to urea. When given the right moisture conditions, N<sub>2</sub>O losses can be greater.

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