4R NUTRIENT STEWARDSHIP:

Magnitude and significance of the N2O priming effect associated with long-term applications of manure

CANADIAN FERTILIZER INSTITUTE
INSTITUT CANADIEN DES ENGRAIS

4R nutrient stewardship

1. ADF file number, Project title, and reporting period.

Project No. 200110209: Magnitude and significance of the N_2O priming effect associated with long-term applications of manure

Reporting Period: April 2013 – March 2014

2. Specify project activities undertaken during this reporting period .

a.) Methodology: *Include approaches, experimental design, tests, materials, sites, etc. Please note that any significant changes from the original work plan will require written approval from the Ministry.*

Research continues at the Dixon long-term research plots located in the Black sol zone (NW21-37-23-W2) near Humboldt, SK. Plots at the site received manure applications starting in the fall of 1996 and terminating in the fall of 2009. The site was divided into two treatment blocks: a liquid swine manure (LSM) block and a solid cattle manure (SCM) block. The two blocks are situated adjacent to each other in the same field; each block included a control with no fertilizer application (zero N), urea, LSM, and SCM amendments at 1×, 2×, and 4× the soil test recommendation (i.e., equivalent to 50-kg plant available N). The two blocks were set up in Randomized Complete Block Design (n=4). Since the spring of 2011 there has been an annual spring application of urea fertilizer over the entire field—including the former control (zero N) plots.

Nitrous oxide flux measurements have been made yearly since the spring of 2009—continuing throughout the 2013 growing season. As in the past, the plots were sampled more frequently (1–2 times per week) at the start of the season (i.e., during spring thaw and following seeding) and less frequently (once every 2–3 weeks) at the end of the season. In addition, gas fluxes were measured 24–48 hours after any significant precipitation event during the sampling season. Flux measurements were obtained using non-steady state, vented chambers installed at 69 locations (i.e., 18 treatments × 4 replicates[1]). Collars (15-cm × 20.3-cm i.d.) for the chambers were manually driven into the soil to a depth of 5-cm and anchored using three, 1-gauge nails inserted through pre-drilled holes in each collar. Once a chamber was sealed to the collar, gas samples were collected at 15-min intervals (three samples per chamber, collected at 15-, 30- and 45-min). In addition ambient air samples (n = 10) were collected on each date and the concentration of N_2O in the ambient air used as a check on the time-zero measurement. Gas samples were transported back to the lab in evacuated sealed tubes for N_2O analysis by gas chromatography in the Department of Soil Science.

Starting in 2011, denitrification enzyme activities (DEAs) were assessed using a modified version of the procedure described by Alef and Nannipieri (1995). Briefly, soil samples were extracted at three times over each growing season (before and after fertilization, and prior to harvest). Field moist subsamples were mixed in a slurry and incubated for 90 min in sealed bottles containing a 10% acetylene atmosphere to inhibit reduction of N_2O to N_2. A 10-mL headspace sample was collected every 30 minutes and injected into evacuated tubes. The headspace samples were analyzed for N_2O using standard gas chromatography.

Microbial community analysis has been initiated using phospholipid fatty acid analysis (PLFA), which was carried out using the method described by Helgason et al. 2010. Briefly, fatty acids were extracted from lyophilized, ground soil (4.0 g) using a single phase chloroform, methanol, phosphate buffer solution. Fatty acids were separated on a solid phase extraction column; phospholipids were methylated and the resulting fatty acid methyl esters were analyzed using a Hewlett Packard 5890 Series II gas chromatograph with a 25-m Ultra 2 column (J&W Scientific). Peaks were identified using fatty acid standards and MIDI identification software (MIDI Inc., Newark, DE) and quantified based on the addition of internal standard methyl nonadecanoate (19:0). Total biomass was calculated as the sum of all identified PLFA peaks. As well, samples for more targeted analysis of nitrifier and denitrifier abundance—measured using functional genes encoding for key enzymes in the pathways leading to N_2O production—have been collected and are just now being analyzed.

[1] Note: there were only three (3) replicates for three of the treatments.

b.) Research accomplishments in the reporting period. *(Describe progress towards meeting objectives. Please use revised objectives if Ministry-approved revisions have been made to original objectives.)*

Objectives	Progress
1) Installation of bases for soil flux chambers; gas sampling and analysis; soil sampling (YEAR 2).	Gas sampling and analysis for the 2013–14 season have been completed. Due to the late spring in 2014; the start of the spring thaw measurements and soil sampling were delayed until late April and mid May, respectively, of this year; consequently, data analysis for these samples is just now getting under way.
2) Process soil samples; extract DNA and PLFA; complete qPCR and data analysis.	Collection of soil samples for the PLFA and DNA analyses was completed last fall. Sample processing was delayed while the graduate student on the project (Ryan Pearce; M.Sc.) recovered from a severe knee injury that required surgery. Ryan is currently in the process of completing the microbial analyses and will start the data analyses once the lab work is finished.
add additional lines as required	

c.) Discussion: *Provide discussion necessary to the full understanding of progress made during this reporting period and the relevance of any findings. Detail any major concerns or project setbacks.*

Previous research at the Dixon site (ADF Project No. 20080135) indicated that denitrification enzyme activities (DEA) in the long-term manure-amended plots were greater than those in the urea-amended and control (zero N) plots (Farrell, 2011). This suggested that long-term manure applications could produce conditions that might "prime" the soil microbial communities for enhanced denitrification, thus exacerbating N_2O emissions when a more readily available fertilizer (e.g., urea) is applied to the soil. However, exactly how N_2O emissions might be impacted as a result of a change-over from long-term, fall manure applications to a spring urea application was unknown. The present study was established to examine how long-term applications of manure affected the soil microbial community and whether this would lead to a potential "priming effect" that could result in increased N_2O emission after a more readily available N source (urea) is applied. The specific objectives of the study were to (i) monitor N_2O emissions over the course of multiple growing seasons in order to quantify N_2O emissions from plots with a long-term history of manure application; (ii) examine how microbial community structure and abundance was impacted by long-term manure applications; and (iii) assess the link between soil microbial community abundance and/or activity and N_2O emissions.

Greenhouse gas sampling and N_2O flux measurements for the 2013 growing season have been completed and the annual growing season (i.e., net cumulative) flux estimates calculated. The seasonal flux estimates, together with those for the 2011 and 2012 growing seasons, are summarized in Figs. A1 and A2 for the liquid swine manure (LSM) and solid cattle manure (SCM) plots, respectively. The preliminary results indicate that present day N_2O emissions from the plots with a history of long-term manure applications were generally greater than those from the historical control plots (i.e., plots that received no fertilizer N during the long-term manure study, but which now receive the same amount of fertilizer N as the manured plots). This is especially true of the plots with a history of long-term LSM applications (see Fig. A1). Long-term applications of SCM had a similar, though much weaker, effect on present day N_2O emissions (see Fig. A2)—with cumulative seasonal N_2O-N losses that were generally less than those from the LSM plots. In any given year, however, the magnitude of the total seasonal N_2O-N losses depended upon the amount of fertilizer N applied, as well as on the rate and duration of the spring thaw and the amount and timing of the growing season precipitation.

Denitrification enzyme activities in the plots with a history of long-term manure applications remain high (see Fig. A3)—even though it has now been three years since the last manure application. This effect was generally stronger in the plots with a history of LSM applications, and suggests that long-term applications of manure-N, especially at high application rates, produces a 'priming' effect that can—under the right environmental and management conditions—exacerbate fertilizer-induced N_2O emissions.

The total PLFA content of the soils is used as an indicator of total microbial biomass. At the Dixon site, total

PLFAs were strongly correlated with the soil water content—increasing as the soil moisture increased. Consequently, high soil moisture leads to a larger, more active microbial community which, if it coincides with a period when there is a ready supply of available N can result in enhanced denitrification activity and increased N_2O emissions. Moreover, preliminary indications are that the highest PLFA values were generally associated with the long-term manure plots that had received the highest (4×) application rate of either LSM or SCM.

More targeted analysis of nitrifier and denitrifier abundance—using functional genes encoding for key enzymes in the pathways leading to N_2O production—is currently underway and is expected to be completed over the next 3–4 months. Quantitative PCR (qPCR) will be used to assess the abundance of: (1) amoA genes, responsible for the rate determining step in nitrification; (2) nirK genes, involved in the conversion of NO_2^- to NO; and (3) nosZ genes, responsible for conversion of N_2O to N_2. Assessing the genetic potential of nitrifiers and denitrifiers following fertilization in these soils will help to clarify the changes in microbial N cycling that have occurred as a function of manure type as well as application rate and frequency. This information will be related to the cumulative (yearly) N_2O production and DEA to elucidate the nature of N_2O emissions from fertilizer N in soils with a history of manure application.

d.) List and briefly discuss any interim conclusions.

Data from the current (2013/14) reporting period, together with those obtained during 2011 and 2012, demonstrate that soil environmental conditions and soil management practice can have a profound impact on the total N_2O emissions associated with crop production. Long-term applications of LSM and, to a lesser extent, SCM can (at high application rates) impact the soil microbial community and cause a "priming" effect that can exacerbate fertilizer-induced N_2O emissions from agricultural soils. The data also indicate that DEA is a good predictor of the priming effect and the potential for enhanced N_2O emissions.

3. List any technology transfer activities undertaken in relation to this project: *Include conference presentations, talks, papers published etc.*

Pearce, R., B. Helgason, R. Lemke and R. Farrell. 2013. Do long-term manure applications "prime" the soil for increased N_2O emissions? ASA, CSSA, & SSSA International Annual Meetings, 3–6 Nov., Tampa, FL (Oral presentation).

Pearce, R., B. Helgason, R. Lemke, and R. Farrell. 2013. Magnitude and significance of the N_2O priming effect associated with long-term applications of manure. 2013 Annual Meeting of the Canadian Society of Soil Science, 23–25 July, Winnipeg, MB (Oral presentation).

Pearce, R., B. Helgason, R. Lemke, and R. Farrell. 2014. Assessing Potential Soil Microbial 'Priming Effects' on N_2O Emissions As a Result of a Fertilizer Change-over from Long-Term Manure Applications to Urea. Soils and Crops Workshop, March 11-12, Saskatoon, SK (Oral presentation).

4. Identify any changes expected to industry contributions, in-kind support, collaborations or other resources.

Changes to industry contributions and other resources have not been made, nor are they anticipated.

5. Appendices: *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited, acknowledgments.*

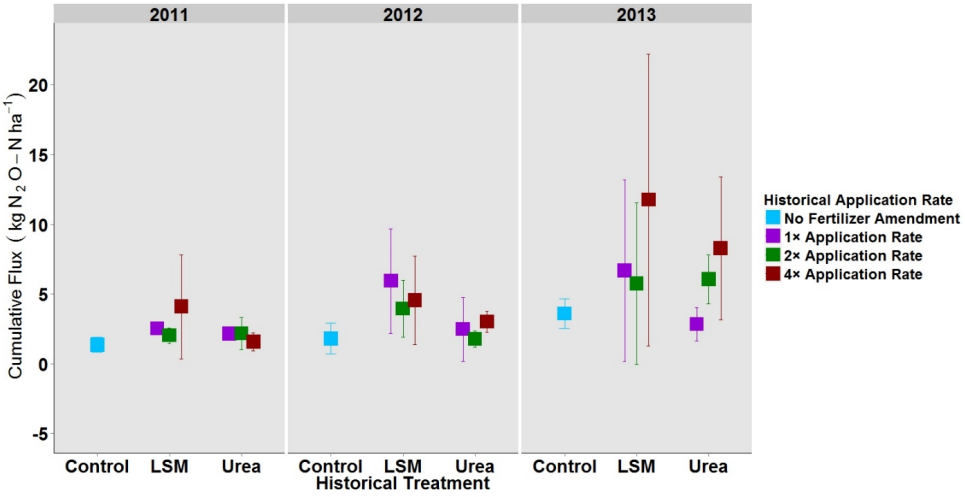

Figure A1. Cumulative N_2O flux of for the yearly historically applied liquid swine manure (LSM) plots for the 2011, 2012 and 2013 growing seasons.

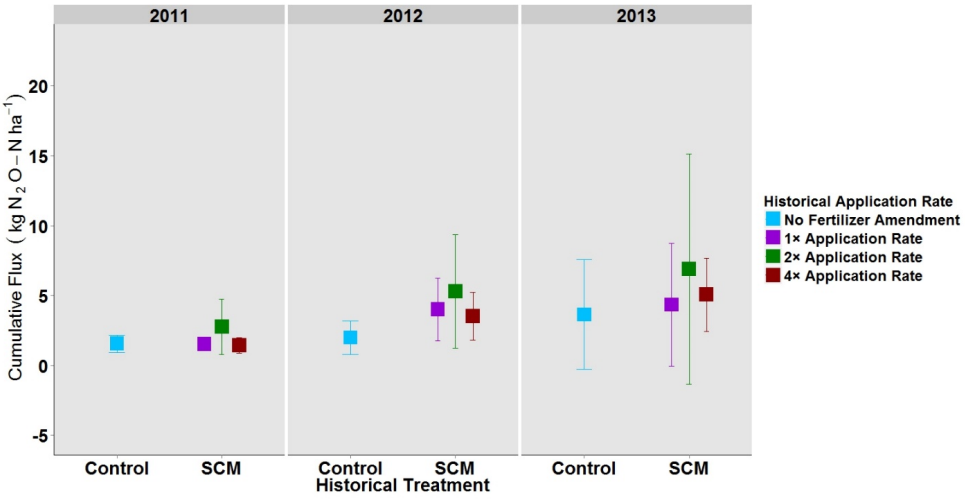

Figure A2. Cumulative N_2O flux of for the yearly historically applied solid cattle manure (SCM) plots for the 2011, 2012 and 2013 growing seasons.

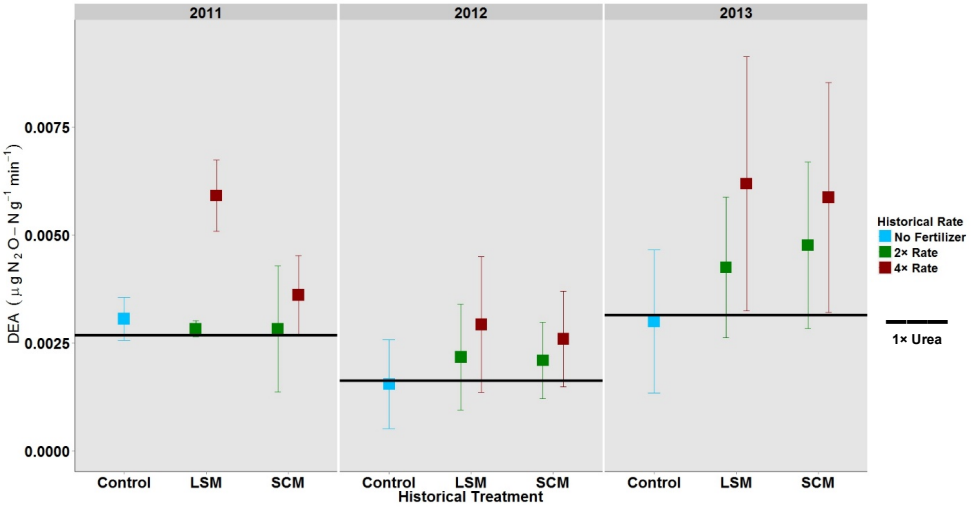

Figure A3. Cumulative denitrification enzyme activities for historically applied amendments and application rate for the historically applied control, liquid swine manure (LSM), solid cattle manure (SCM), and urea amendments.

References

Alef, K. 1995. Assay of Denitrification. Pages 284-286. Chapter 6 in Alef, K. and Nannipieri, P. (eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press Limited, London, U.K.

Helgason, B.L., F.L. Walley and J.J. Germida. 2010. No-till soil management increases microbial biomass and alters community profiles in soil aggregates. Appl. Soil Ecol. 46: 390–397. Stumborg, C., Schoenau, J. J., and Malhi, S. S. 2007. Nitrogen balance and accumulation pattern in three contrasting prairie soils receiving repeated applications of liquid swine and solid cattle manure. Nutr. Cycl. Agroecosyst. 78: 15-25.