
Soil Respiration and Nitrous Oxide Production in Distillers Grain and Glycerol Amended Soil

K. Alotaibi¹, J.J. Schoenau¹ and P. Qian¹

¹Department of Soil Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8

Key Words: Distillers grains, thin stillage, glycerol, carbon dioxide, nitrous oxide.

Abstract

Applying by-products of biofuel production to soil may be an alternative use to take direct advantage of nutrients and carbon contained within. Ethanol production results in distiller grain and biodiesel produces glycerol as by-product. However, no information currently exists on the effects of adding these amendments on evolution of carbon dioxide and nitrous oxide from soils, yet is important to complete our understanding of potential impacts of biofuel production on greenhouse gas budgets as well as soil quality. Pots containing soil amended with different rates of wet distillers grain, thin stillage, and glycerol were placed in incubation chambers and incubated for 10 days. Treatments of alfalfa powder and urea were added at the same rates of total N as the by-products for comparative purposes. Carbon dioxide and nitrous oxide evolved from amended soil was measured. The alfalfa powder and wet distillers grain resulted in the greatest evolution of CO₂ from the soil, with the thin stillage resulting in less CO₂ evolved per unit of nitrogen added. Addition of nitrogen fertilizer along with glycerol enhanced microbial activity and decomposition. Per unit of nitrogen added, urea tended to result in the greatest N₂O produced, followed by wet distillers grain and thin stillage, with glycerol and dehydrated alfalfa resulting in the lowest nitrous oxide production.

Introduction

Increased biofuel production as an alternative source of energy to oil is associated with a large amount of biofuel processing byproducts. These byproducts include wet distillers grain and thin stillage from ethanol production, and glycerol from biodiesel. Distillers grain and thin stillage are generated from ethanol production from grains involving the conversion of starch to alcohol through fermentation followed by distillation process (Bonnardeaux, 2007). Glycerol (also known as glycerin) is a byproduct of biodiesel production; mostly produced from soybean and canola oil. Fuel ethanol refineries produced approximately 13,101 million gallons worldwide and 211 million gallons in Canada in 2007 (RFA, 2007). As a result, a bushel of corn processed through an ethanol refinery produces approximately 2.8 gallons of ethanol and more than 17 pounds of byproduct distillers grain. Due to their nutritional value, wet distillers grains and thin stillage are considered to be a valuable feedstuff for animals; they contain relatively high

crude protein and phosphorus concentrations. Feeding to animals is the most common practice of current usage.

The fact that distiller grains and thin stillage contain essential plant nutrients such as nitrogen and phosphorus, similar to other byproducts such as animal manure, compost and paper mill residues, provides opportunity for their potential use as soil organic amendments or organic fertilizer. Their effect on soil respiration and nitrous oxide production is unknown, yet is an important consideration in constructing greenhouse gas budgets for biofuel production systems. Addition of organic materials to agricultural fields is an important source of CO₂ and N₂O (Akiyama and Tsuruta, 2003). Evolution of CO₂ is a result of microbial respiration during organic matter decomposition and carbon mineralization. Nitrous oxide emission from agricultural soil is mainly derived from the microbial processes of nitrification and denitrification in soils (Meng et al., 2005). The N₂O produced is governed by factors such as temperature, moisture content, amendment N content and form and the soil content of organic matter (Meng et al., 2005). Incorporation of C and N-rich substrates into soil can be a source of readily available C and N in the soil and is expected to influence the CO₂ and N₂O emissions (Flessa and Beese, 1995; Cochran et al., 1997; Eemke et al., 1999). However, organic substrate type and decomposition rate are considered to be important factors affecting CO₂ and N₂O emission (Aulakh et al., 1991; Mckenney et al., 1993; Shelp et al., 2000). There is no information available on how distillation by-products may influence the evolution of carbon dioxide and nitrous oxide when added directly to soil.

Glycerol (also known as glycerin) is a three carbon sugar alcohol made up of carbon, hydrogen and oxygen (C₃H₅(OH)₃). It is generated during the manufacture of biodiesel via transesterification of vegetable oils. The glycerol constitutes 10% of biodiesel production: every tonne of biodiesel generates 100 kg of glycerol. Consequently, this large amount produced has led to surplus glycerol which is disposed of by incineration (The Glycerol Challenge, 2007). The existing potential uses of glycerol include industrial, chemical and pharmaceutical preparations and potential for use in production of plastics is being explored. However, purification of crude glycerolis costly and the market of glycerol is already saturated (Groesbeck, et al., 2008). Glycerol has also been used as a feed ingredient in animal diets to reduce diet costs (Lammers et al., 2007; Groesbeck, et al., 2008). Another alternative use of glycerol is application to agricultural soil. However, this has received little attention, and the information about its application to soil is scarce (Schoenau, 2007). Qian and Schoenau (2008) found that application of glycerol at a high rate (10000 kg ha⁻¹) resulted in wheat yield reduction, and this might be attributed to microbial immobilization of available N.

To best manage by-products of an expanded biofuel production industry, a comprehensive knowledge of the impacts of adding biofuel processing byproducts to agricultural soils is needed. Thus, this research aimed to evaluate the potential uses of biofuel byproducts as soil amendments, and investigates their effect on soil respiration and nitrous oxide production under controlled environmental conditions. The effect of wet distillers grain, thin stillage and glycerol on CO₂ and N₂O production in a

Saskatchewan soil is examined in comparison to more conventional amendments including alfalfa and urea.

Methods and Materials

Experimental Design

The amendments used included three rates of urea, alfalfa powder, wet distiller grains, thin stillage and glycerol. The glycerol was applied with or without nitrogen (urea). A total amount of 800 g of soil was incubated in pots with a surface area of 113.04 cm². Urea treatments included three rates: 0.0864, 0.1728 and 0.3456 g/pot, equivalent to 100, 200 and 400 kg N ha⁻¹. Alfalfa pellets treatments were low rate: 1.5773; medium rate: 3.1564; and high rate: 6.3092 g/pot equivalent to 100, 200 and 400 kg N ha⁻¹, respectively. Three rates of wet distillers grains were applied to soil; 4.36, 8.72 and 17.44 g/pot and 8.512, 17.024 or 34.048 g/pot of thin stillage were added to soil. As for the other treatments these low, medium and high rates represented a total N addition rate of 100, 200 and 400 kg N ha⁻¹. The glycerol treatments included three rates with equivalency to 100 (low rate), 1000 (medium rate) or 10,000 kg glycerol ha⁻¹ (high rate) with or without 263.2 mg of urea (150 µg N g⁻¹ or 300 kg N / ha). A control that received no organic amendments was included. Each treatment was replicated four times.

Treatment application

A 650 g aliquot of soil was weighed first into each pot, and 50 g of soil was mixed with the amendment and spread on the soil surface on the pot. Then 150 ml of deionized water, which is required to bring soil moisture to field capacity level, was added and then 100 g of soil was placed on the top. In case of liquid or slurry amendments (glycerol, thin stillage) 700 g of soil was weighed into each pot, and then the amount of amendment was mixed well with 150 mL of deionized water and then added to soil. Then, 100 g of soil was placed on the top. All pots containing amended soil were placed on the bench in laboratory and remained in place for 6 hrs for stabilization.

Incubation setup

All the pots containing amended soil were placed into an airtight sealed containers that were created from two PVC pipes 15 cm in diameter and 15 cm long with caps on each end. The two-part PVC container was joined together by a rubber airtight flange fastened with hose clamps (Nelson, 2007). A rubber septum inserted into the cap was used to extract the gas samples. A 20-cm³ syringe needle was used to collect the gas sample and transfer it into a 10-cm³ evacuated vial. Sampling was done every two days at the same time of the day for 10 days. The incubation was carried out in an incubation chamber with electronically controlled environmental settings in which the chamber was set for 16 hr at 25 °C (day) and 8 hrs at 18°C (night). After each sampling time, the tops of the PVC containers were removed and allowed to remain open for 1 hr to allow natural airflow exchange between the chamber and the pots to ensure aerobic conditions. The collected

gas was analyzed for CO₂ and N₂O using gas chromatography. Due to limitations in number of PVC containers and space, the incubation was carried out in two sets. The first set of incubations included 10 treatments: three rates of urea, dehydrated alfalfa, and wet distillers grains in addition to a control treatment giving a total of 40 PVC containers. The second set of incubation included 12 treatments: three rates of glycerol with or without nitrogen, thin stillage and three rates of urea. Urea treatments were included for appropriate comparison in the two sets of incubations. A control that received no organic amendment was included as well.

Results and Discussion

Carbon Dioxide Evolution (Soil Respiration) $\mu\text{g CO}_2 \text{ cm}^{-2} \text{ hr}^{-1}$

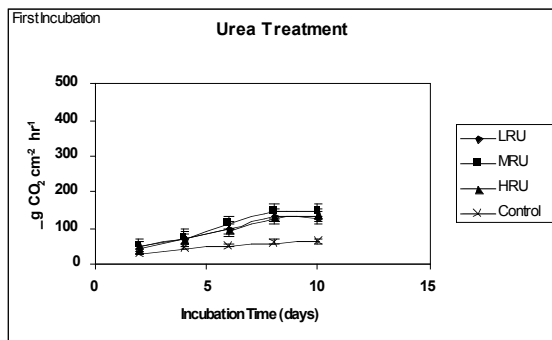


Figure 1. Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on CO₂ evolution from soil during the first set of incubation.

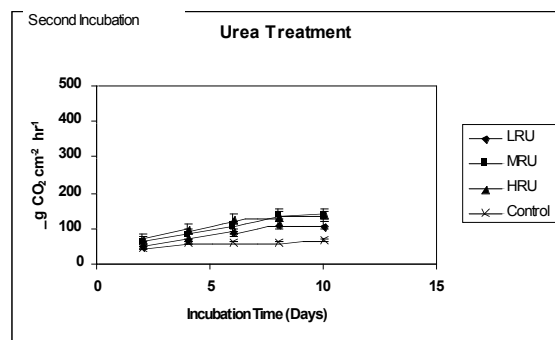


Figure 2. Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on CO₂ evolution from soil during the second set of incubation.

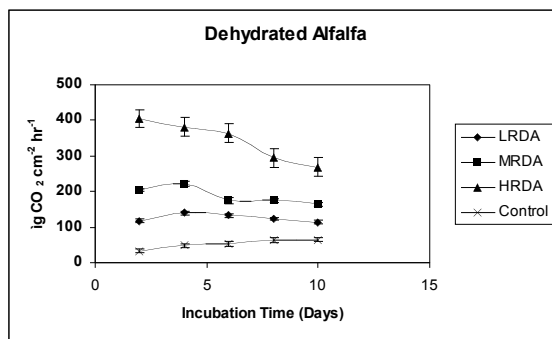


Figure 3. Carbon dioxide evolved from soil amended with three rates of dehydrated alfalfa: high rate (HR); medium rate (MR) and low rate (LR).

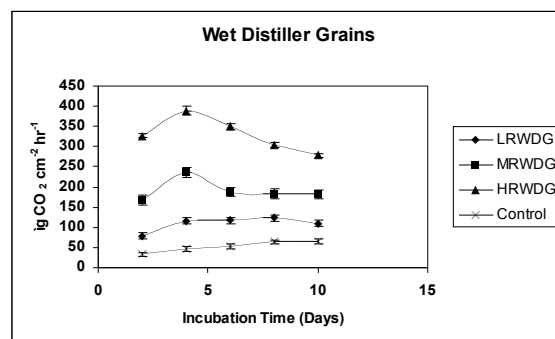


Figure 4. Carbon dioxide evolved from soil amended with three rates of wet distillers grains: high rate (HR), medium rate (MR) and low rate (LR).

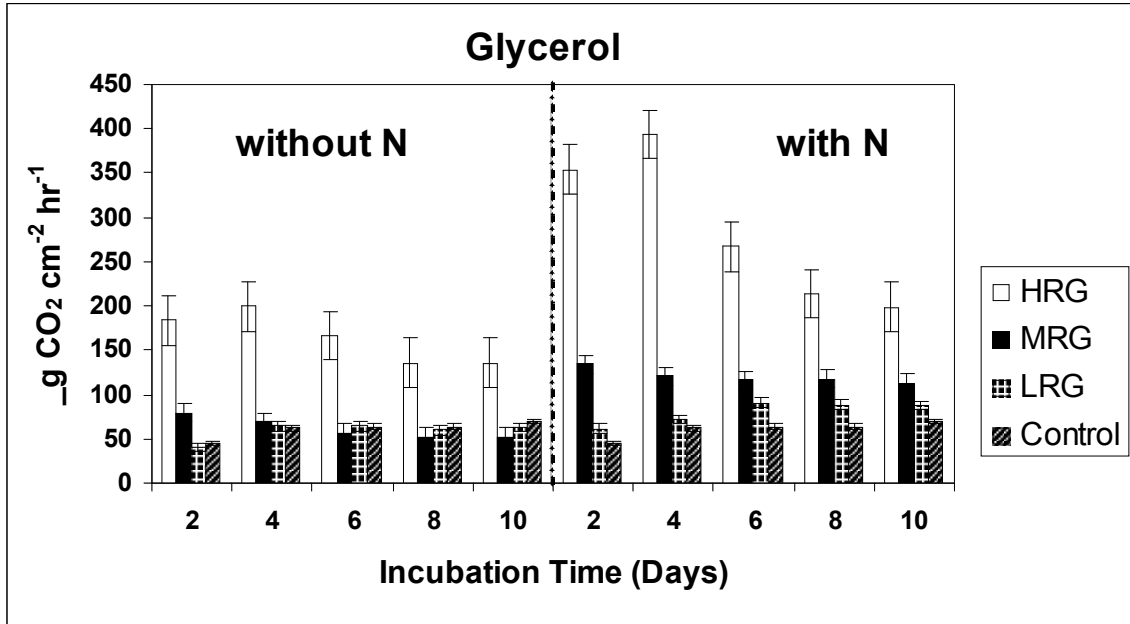


Figure 5. Carbon dioxide evolved from soil amended with three rates of glycerol: high rate (HR), medium rate (MR) and low rate (LR) with or without N.

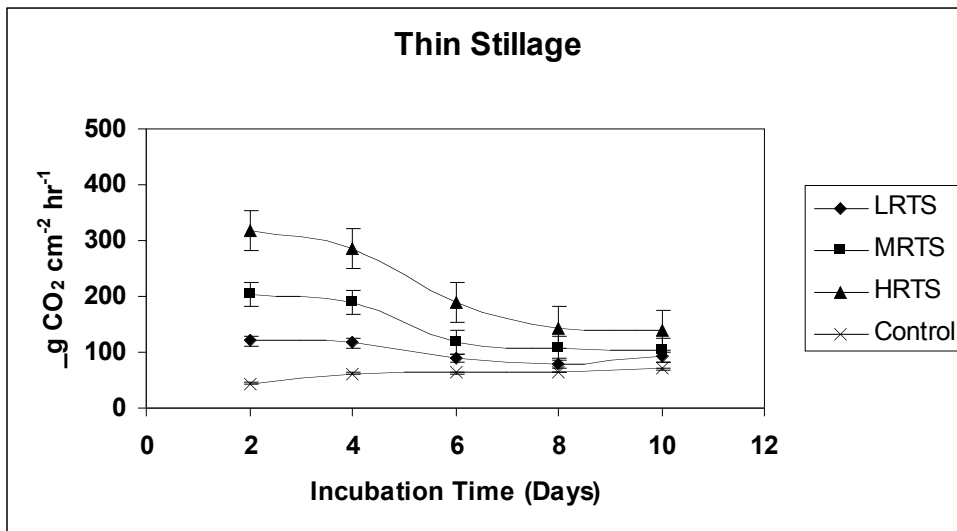


Figure 6. Carbon dioxide evolved from soil amended with three rates of thin stillage: high rate (HR), medium rate (MR) and low rate (LR).

Amendment with urea stimulated microbial activity in the soil, as shown by significantly higher rates of carbon dioxide production in the urea amended soils than the control in both incubation experiments (Figures 1 and 2). The effect was delayed, with CO₂ evolution not reaching a peak until days 8 and 10. This CO₂ is likely derived from enzymatic (urease) hydrolysis of the urea to CO₂ and ammonia, as well as stimulation of heterotrophic microbial activity. All of the organic amendments stimulated microbial activity in the soil, with elevated CO₂ production compared to the unamended control (Figures 3-6). There were generally higher fluxes of CO₂ in the N containing amendment treatments than urea at equivalent rates of added N, especially for the medium and high amendment rates. The rate effect was much more apparent for the organic amendments than for urea, and reflects the effect of the addition of substrate carbon for microbial decomposition along with nitrogen in the amendment treatments. Generally, fluxes of CO₂ were greatest for the amendments at the two day measurement period and declined thereafter, presumably related to microbial consumption of the amendment. The decrease in CO₂ evolution from the soil with time was greater in the thin stillage treatment (Fig. 6) compared to other amendments. This can be explained by more of the organic carbon in the thin stillage being present in soluble, low molecular weight forms that would undergo more rapid decomposition and depletion. The dehy alfalfa and wet distillers grain resulted in the greatest CO₂ evolution per unit of N added, owing to a higher carbon content relative to N than thin stillage or urea. CO₂ flux from glycerol amended soil was low compared to other amendments, due to lack of nitrogen restricting microbial activity. When N fertilizer was added, carbon dioxide evolution rates were significantly increased, as the N fertilizer supplied the N needed for microbial growth.

Nitrous Oxide (N₂O) Evolution ug N₂O cm⁻² hr⁻¹

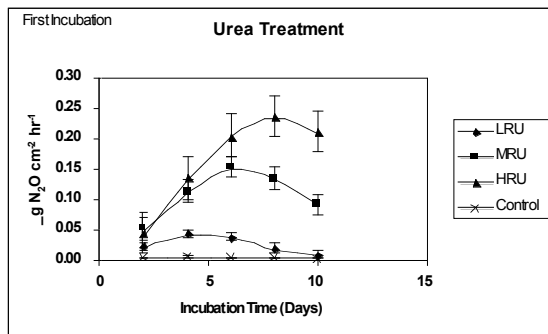


Figure 7. Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on N₂O evolution from soil during the first set.

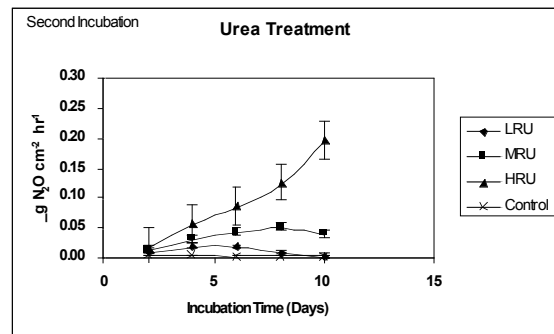


Figure 8. Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on N₂O evolution from soil during the second set.

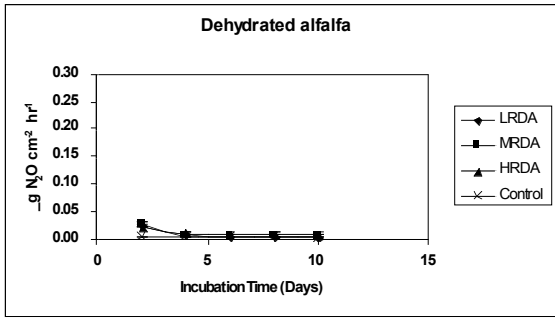


Figure 9. Nitrous oxide evolved from soil amended with three rates of dehydrated alfalfa : high rate (HR); medium rate (MR) and low rate (LR).

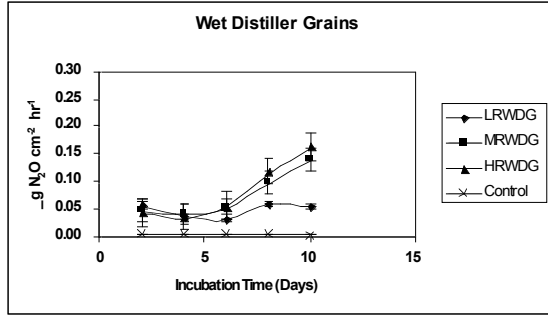


Figure 10. Nitrous oxide evolved from soil amended with three rates of wet distillers grain: high rate (HR), medium rate (MR) and low rate (LR).

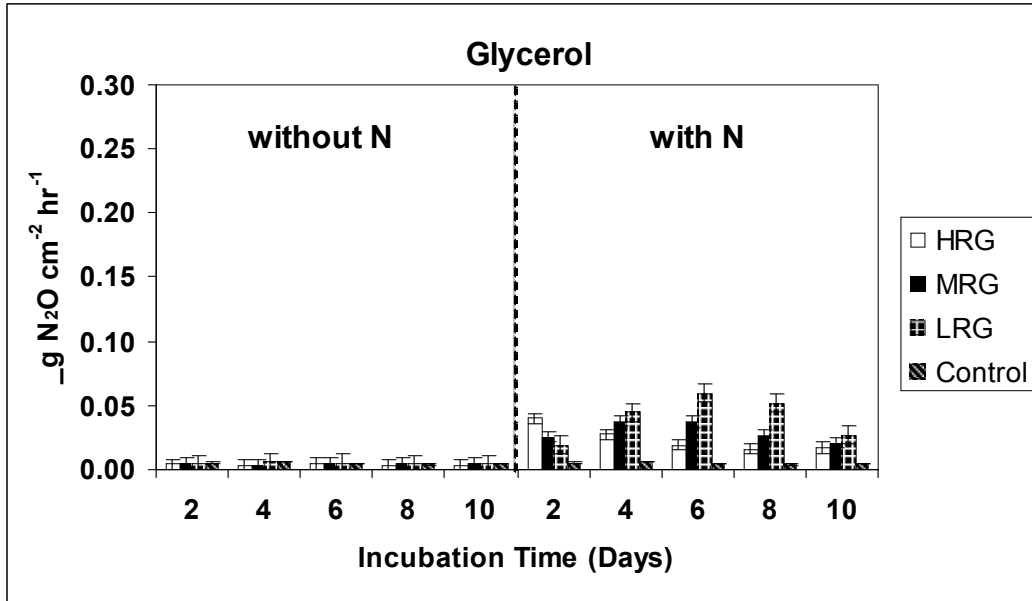


Figure 11. Nitrous oxide evolved from soil amended with three rates of glycerol; high rate (HR), medium rate (MR) and low rate (LR) with or without N

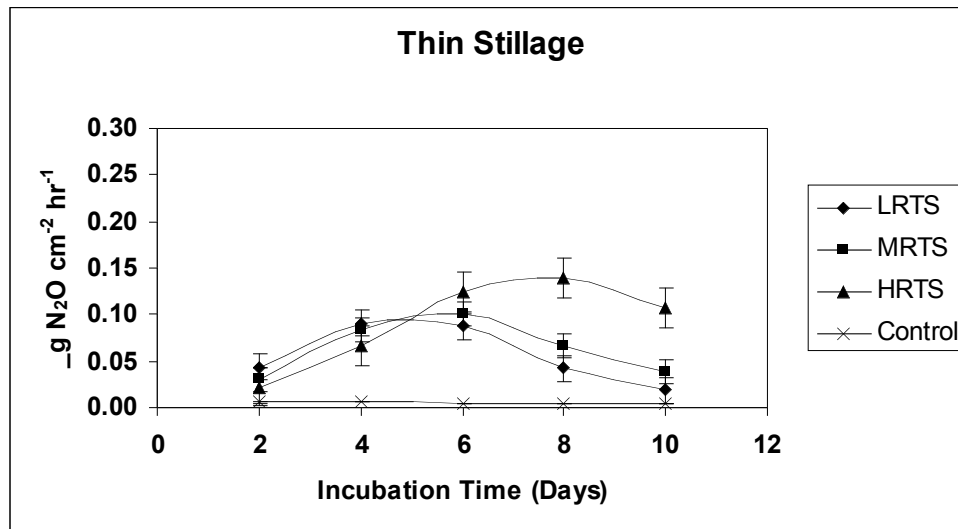


Figure 12. Nitrous oxide evolved from soil amended with three rates of thin stillage; high rate (HR), medium rate (MR) and low rate (LR).

Rates of evolution of N₂O were about 1000 times less than for CO₂ over the ten days of the incubation. Fluxes of N₂O from the soil surface generally increased over the first few days of the incubation, followed by a leveling off or decrease (Fig. 7 and 8). Rates of N₂O production were the highest and sustained over the longest period at the high rate of urea addition, as expected. As the nitrate content of the initial soil was quite low, and the moisture content was at field capacity or less, it would appear that the N₂O evolution observed over this time period is originating from the nitrification process. Of the organic amendments, the wet distillers grain and thin stillage produced the highest rates of N₂O production per unit of N added (Figs. 10 and 12). This can be attributed to a greater net release of ammonium by mineralization, due to a narrow C:N ratio and more easily decomposed organic materials, to produce ammonium that was subsequently nitrified to nitrate, producing N₂O. Of the N containing amendments, the dehydrated alfalfa produced low amounts of N₂O, with total production of N₂O over the ten days that was significantly less than wet distillers grain or thin stillage. As expected, the glycerol without added N resulted in very little N₂O produced (Figure 11), since immobilization is not anticipated to be associated with nitrous oxide production. The treatments where urea N fertilizer was added with the glycerol resulted in elevated N₂O production, but levels were still low compared to wet distillers grain and thin stillage, and closer to flux rates observed for dehydrated alfalfa.

Conclusions

- Per unit of N added, the dehydrated alfalfa and wet distillers grain resulted in the greatest evolution of carbon dioxide from the soil. This is explained by these amendments containing the largest amounts of carbon per unit of nitrogen added that acts as substrate for microbial decomposition. The stimulation of microbial activity by thin

stillage was less and of a shorter duration, due to less carbon added that was more easily decomposed compared to the other organic amendments.

- Addition of nitrogen fertilizer along with glycerol enhances microbial activity and decomposition.
- Per unit of N added, urea tended to result in the greatest production of N₂O, followed by wet distillers grain and thin stillage, with glycerol and dehydrated alfalfa resulting in the lowest nitrous oxide production.

Acknowledgements: Financial support from Saskatchewan Agriculture Development Fund and ABIP Feed Opportunities for Biofuel Industry Program is greatly appreciated.

References

- Akiyama, H. and H. Tsuruta. 2003. Nitrous oxide, nitric oxide, and nitrogen dioxide fluxes from soils after manure and urea application. *J. Environ. Qual.* 32:423-431.
- Aulakh, M.S., Doran, J.W., Walter, D.T., Mosier, A.R., Francis, D.D. 1991. Crop residue type and placement effects on denitrification and mineralization. *Soil Sci. Soc. Am. J.* 55:1020-1025.
- Bonnardeaux, J. 2007. Potential uses for distillers grains. Department of Agriculture and Food, Western Australia, March 2007, p.1.
- Cochran, V.L., Sparrow, E.B., Schlentner, S.F., Knight, C.W. 1997. Long-term tillage and crop residue management in the subarctic: fluxes of methane and nitrous oxide. *Can. J. Soil.Sci.* 77:565-570.
- Fless, H., Beese, F. 1995. Effect of sugarbeet residues on soil redox potential and nitrous oxide emission. *Soil Sci. Soc. Am. J.* 59:1044-1051.
- Groesbeck, C.N., L.J. McKinney, J.M. DeRouchey, M.D. Tokach, R.D. Goodband, S.S. Dritz, J.L. Nelseen, A.W. Duttlinger, A.C. Fahrenholz and K.C. Behnke. 2008. Effect of crude glycerol on pellet mill production and nursery pig growth performance. *J. Anim. Sci.* 86:2228-2236.
- Lammers, P.J., M.S. Honeyman, B.J. Kerr, T.E. Weber and K. Bregendahl. 2007. Growth performance and carcass characteristics of growing pigs fed crude glycerol. *J. Anim. Sci.* 85 (Suppl.1): 508. (Abstr.).
- Lemake, R.L., Izaurralde, R.C., Nyborg, M., Solberg, E.D. 1999. Tillage and N source influence soil-emitted nitrous oxide in the Alberta Parkland region. *Can. J. Soil Sci.* 79:15-24.
- McKenney, D.J., Wang, S.W., Drury, C.F., Findlay, W.I. 1993. Denitrification and mineralization in soil amended with legume, grass, and corn residues. *Soil Sci. Soc. Am. J.* 57:1013-1020.
- Meng, L., W. Ding and Z. Cai. 2005. Long-term application of organic manure and nitrogen fertilizer on N₂O emissions, soil quality and crop production in a sandy loam soil. *Soil Biol. Biochem.* 37:2037-2045.
- Nelson, J.D.J., J.J. Schoenau, S.S. Malhi, K.S. Gill. 2007. Burning and cultivation effects on greenhouse gas emissions and nutrients in wetland soils from Saskatchewan, Canada. *Nutr. Cycl. Agroecosyst.* 78:291-303.

- Qian, P. and J.J. Schoenau. 2008. Effect of Soil Amendment with Alfalfa Pellets and Glycerol on Nutrition and Growth of Wheat. Proceedings of the 2008 Soils and Crops Workshop, February 28-29, Saskatoon, Saskatchewan. (on CD)
- RFA. 2007. Industry Statistics (online). Available at <http://www.ethanolrfa.org/industry/statistics/> (verified 2 Nov. 2008).
- Schoenau, J.J. 2007. Potential impacts of biofuel production on soils. Proceedings of Capturing Feed Grain & Forage Opportunities-2008, December 11&12, Red Deer, Alberta, 119-122.
- Shelp, M.L., Beauchamp, E.G., Thurell, G.W. 2000. Nitrous oxide emissions from soil amended with glucose, alfalfa, or corn residues. Commun. Soil Sci. Plant Anal. 31:877-892.
- The Glycerol Challenge, 2007. Available at <http://www.theglycerolchallenge.org> (verified 13 Sep 2008).