

BIOENERGY BY-PRODUCTS AS ALTERNATIVE SOURCES OF PLANT NUTRIENTS FOR PRAIRIE SOILS

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ABSTRACT

Rapid growth in the bioenergy industry has created large amounts of the various by-products associated with bioenergy production. A proper method of utilizing these by-products has recently been sought, including their potential use as soil fertilizers or amendments. However, more knowledge is required to validate this option, as well as to make recommendations for their management. The objective of this research was to evaluate the effectiveness of bioenergy production by-products (BPB), mainly wet distillers' grains (WDG), thin stillage (TS), glycerol (GL), biochar (BC) and ash, as sources of plant nutrients in prairie soils with emphasis on their impact on soil biological and chemical properties and processes. This was addressed through a series of growth chamber and field studies. Under controlled environment conditions, the WDG, TS and GL with urea nitrogen (N) fertilizer increased N_2O emissions from soil, but not in excess of that produced from soil treated with urea alone. Microbial activity as indicated by emission of CO_2 was also increased significantly by WDG, TS and GL with N. The WDG and TS increased nutrient ion supply rates in soil, owing to their high content of readily available nutrient. In general, soil enzyme activity (alkaline phosphatase, dehydrogenase, protease) was significantly increased by WDG, TS and GL addition. These by-products also increased soil microbial biomass and microbial quotient with the exception of TS, likely related to its lower C content. Gasified dried distillers' grains ash (DDGA) was an effective source of plant available phosphorus (P) whereas gasified meat & bone meal ash (MBMA) had lesser effects on crop yield and P uptake compared to mineral fertilizer. The majority of P remaining in the soil following MBMA application was in stable and recalcitrant forms. In a two-yr field study, TS was an excellent source of nutrient in promoting crop yield and nutrient uptake that was greater than or similar to urea fertilizer, especially when injected. However, residual NO_3^- -N and available P accumulated in soil after the second year of TS application. Amendment with BC and GL resulted in limited effects on crop yield and nutrient uptake, with GL tending to reduce crop yield and N uptake due to microbial immobilization. Overall, the BPB amendments can be of benefit by enhancing biological activity, nutrient availability and crop growth, although their effect is greatly dependent on the form and composition.

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DEDICATION

I dedicate this dissertation work to the memory of my father, Dhawi Muslim Alotaibi (1930-2011), from whom I learned patience, endurance, and wisdom. He taught me how to face challenges and overcome difficulties; both of which are necessary requirements for success.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ANR	Apparent N recovery
APR	Apparent P recovery
BC	Biochar
BC+N	Biochar without N
BGM	Barley grains manure
BPB	Bioenergy production by-products
BR-TS	Broadcast and incorporated thin stillage
BRC	Broadcast and incorporated
BRTS-H	Broadcast and incorporated thin stillage at high rate
BRTS-L	Broadcast and incorporated thin stillage at low rate
BRTS-M	Broadcast and incorporated thin stillage at medium rate
DA	Dehydrated alfalfa
DDG	Dried distillers' grains
DDGA	Dried distillers' grains ash
DDGS	Dried distillers' grains with solubles
DG	Distillers' grains
DGM	Distillers' grains manure
EC	Electrical conductivity
G-N	Glycerol without N
G+N	Glycerol plus N
GL	Glycerol
INJ	Injected
INJ-TS	Injected thin stillage
INJTS-H	Injected thin stillage at high rate
INJTS-L	Injected thin stillage at low rate
INJTS-M	Injected thin stillage at medium rate
LSD	Least significant difference
MBC	Microbial biomass carbon
MBM	Meat & bone meal
MBMA	Meat & bone meal ash
MBN	Microbial biomass N
MP	Mineral phosphorus
MQ	Microbial quotient
OC	Organic carbon
PAMI	Prairie Agricultural Machinery Institute
PRST TM	Plant Root Simulator TM
SNK	Student-Newman-Keuls test
TPF	Triphenyle formazan
TS	Thin stillage
TTC	Triphenylterazolium
UR	Urea
UR-H	Urea applied at high rate
UR-L	Urea applied at low rate
UR-M	Urea applied at medium rate
WDG	Wet distillers' grains

1. GENERAL INTRODUCTION

1.1 Introduction

Uncertainty about global oil reserves and the sustainability of petroleum commodity supply, along with environmental concerns about greenhouse gas emissions has led to considerable interest in alternative sources of energy (Lehmann, 2007; Liew et al., 2014). Production of energy from organic materials grown on the land is an example of an alternative energy source that can reduce reliance on fossil fuels.

Various technologies have been implemented to produce different types of bioenergy. Some of these technologies are not new, but their application to certain types of organic materials as feedstock has not been widely evaluated or adopted. These technologies can be classified into three main categories: 1) biochemical 2) mechanical/chemical and 3) thermochemical processes (McKendry, 2002a). Biochemical conversion embraces two processes: fermentation and anaerobic digestion. Mechanical/chemical processes include transesterification of vegetable oil such as canola that results in production of biodiesel (McKendry, 2002a). The thermo-chemical conversion technology includes four processes: combustion, gasification, pyrolysis and liquefaction.

In addition to the bio-energy produced, each conversion process results in a specific by-product. The value and characteristics of these by-products vary according to the type of conversion process and feedstock converted (Cayuela, 2010). Bioenergy production by-products include, but are not limited to, distillers' grains (DS) and thin stillage (TS) generated from fermentation of sugar crops and starch crops, glycerol (GL) produced from transesterification of plant oil during biodiesel manufacture, ash resulting from gasification and biochar (BC)/charcoal produced from pyrolytic conversion. The composition of each by-product, including carbon (C) and plant nutrients such as nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) is dependent on the method of processing. Therefore, BC produced from pyrolysis and GL produced from biodiesel production via transesterification of vegetable plants are materials that are rich in carbon, while by-products generated from ethanol manufacturing contain considerable

amounts of N and P together with other nutrients and C. Ash materials produced from gasification are rich in P, K, calcium (Ca) and magnesium (Mg) but contain relatively little C, as this is lost as CO₂ during combustion.

Production of energy from renewable sources has grown rapidly, and it was estimated that in 2002 renewables contributed to 14% of the world's energy supply (McKendry, 2002b). Bioenergy manufacturing, especially ethanol, is the most common type of bioenergy product commercially produced on a large scale worldwide. In 2012, world ethanol production passed 100 billion L (RFA, 2013). In 2012, US ethanol refinery plants produced over 50 billion L of ethanol (RFA, 2013) and nearly 4 billion L of biodiesel (NBB, 2014). About 1.9 billion L of ethanol was produced in Canada in 2012 and expected to increase to approximately 2 billion L in 2013 (GAIN, 2013), with a significant portion of which produced in western provinces. Canadian biodiesel production was also estimated to reach about 0.5 billion L in 2013 (GAIN, 2013). The ethanol is derived from fermentation and distillation facilities that use sugar and starch crops as feedstock. It is also believed that ethanol will eventually be produced on a wide scale from lignocellulosic feedstocks, which are referred to as second generation feedstocks, including crop residues, processing wastes and biomass energy crops like willow and poplar (Gronowska et al., 2009). It was predicted that lignocellulosic feedstocks could produce approximately 229 billion L of ethanol in the U.S. by the year 2030 (De La Torre Ugarte et al., 2007). However, the advanced conversion technologies required to produce ethanol from lignocellulosic feedstocks are not available yet on a commercial scale, but are being researched and undergoing rapid development. The second generation bioenergy production by-products are not included here because they have not been produced in large quantities yet on a large scale.

As a function of the growing bioenergy production industry, there will be an abundance of by-products being generated. These by-products are organic, containing C and plant nutrients that are potentially valuable to soils and the production of crops; thus the potential uses of the by-products must include their application to soil.

Utilization of the by-products of bioenergy production as soil amendments can be a good strategy for recycling nutrients associated with production of feedstock, and thereby reduce reliance on mineral fertilizers for food production. Additionally, this potential use can help offset any negative impact on soil when crop residues are harvested for bioenergy production. Harvesting crop residues for bioenergy production is suggested to have implications on soil

quality and may jeopardize soil productivity through organic matter and nutrient loss (Lal, 2009). To compensate for the loss in nutrients that are harvested in crop and to maintain optimum crop production, there will be increased need for external organic matter and nutrient inputs to help maintain and improve fertility of the soil.

There is a myriad of research work that has been completed on land application of organic materials such as compost and manure (e.g. Eghball, 2002; Singer et al., 2004; Gale et al., 2006; Schoenau and Davis, 2006; Lithourgidis et al., 2007), paper mill biosolids (Aitken et al., 1998; Curnoe et al., 2006; N'Dayegamiye, 2006), and oily food wastes (Rashid and Voroney, 2004) and their effects on soils and crop growth. However, there has not been an attempt to evaluate the potential use of bioenergy production by-products as source of plant nutrients and soil amendments. Increased knowledge in this context is required to improve our understanding of bioenergy production by-products (BPB) potential contribution to soil enhancement and increased crop production.

1.2 Ph.D. Research Objectives

The general objective of the research presented in this dissertation was to evaluate the potential utilization of a range of by-products associated with bioenergy processing as alternative soil amendments to improve soil fertility and crop growth. A desired outcome of the series of studies undertaken was to improve our understanding of the influence of the by-products on important biological and chemical processes in soil. The tested bioenergy production by-products (BPB) included wet distillers' grains (WDG), thin stillage (TS), glycerol (GL), biochar (BC) and ash. A combination of controlled environment and field evaluations were used. The specific objectives were:

- To identify the direct impact of WDG, TS and GL amendment on greenhouse gas emissions and nutrient supply rates under controlled environment conditions.
- To investigate soil enzyme activity and microbial biomass content in soil receiving BPB and incubated under controlled environment conditions.
- To evaluate the effectiveness of ash derived from gasification of organic materials as a P fertilizer.
- To investigate the P fractions residing in the soil following ash application.

- To evaluate TS fertilizer potential under field conditions.
- To investigate changes in chemical and biological properties of soil receiving repeated application of TS.
- To examine direct and residual effects of BC and GL application on crop yield and selected chemical and biological properties of soil.

1.3 Dissertation structure

This dissertation reports on impacts of land application of BPB as an alternative option for their utilization. The dissertation is organized in a manuscript-based format consisting of 11 chapters. This chapter and the following one provide a general introduction and comprehensive literature review, respectively. The subsequent 7 chapters (3-9) report on specific research studies to address the objectives. These chapters are published, under review or submitted and under consideration in peer reviewed journals.

Chapter 1 is a general introduction to the dissertation and addresses the research questions, objectives and scope of the studies conducted.

Chapter 2 provides an in-depth review of the literature pertinent to the subject. It covers the background on selected BPB including their production processes, their existing methods of utilization and existing published studies on the feasibility of their usage as soil amendments.

Chapter 3 presents results of a growth chamber study conducted to investigate the direct impact of BPB addition on greenhouse gas emissions (N_2O and CO_2) and nutrient supply rate using plant root simulator (PRSTM) probes in a Brown Chernozemic soil.

Chapter 4 covers research with a similar experimental design to that of the study in Chapter 3, but with different objectives. In this chapter, response of selected soil enzyme activities (dehydrogenase, alkaline phosphatase, protease) and soil content of microbial biomass to application of BPB is reported.

Chapter 5 reports on the results of a short-term study carried out under controlled environment conditions aimed at evaluating the effectiveness of two type of ashes derived from gasification of

meat & bone meal and dried distillers' grains as a phosphorus fertilizer. In addition, this chapter also presents data regarding changes in soil chemical properties following ash application.

Chapter 6 expands on the study presented in the Chapter 5 by investigating the influence of the ash amendments on P forms residing in soil after crop harvest. Fractions of P residing in soil following ash application are revealed using a sequential chemical P extraction procedure to give a better understanding of the fate of ash P and formation of P compounds when added to soil.

Chapter 7 details a two-yr field study to evaluate the potential of using TS as a fertilizer in the field using advanced application techniques (band injection) that are usually employed to apply liquid animal manures. Comparisons of crop response to commercial fertilizer urea are made.

Chapter 8 extends the work reported on in Chapter 7 to include response of soil attributes to TS application. The effects of repeated application of TS on residual available N and P and changes in selected soil chemical and biological properties are made.

Chapter 9 reports on results of a three-yr field study in south central Saskatchewan to evaluate the direct and residual effects of BC and GL application on crop yield and nutrient uptake as well as changes in some chemical and biological properties of a Brown Chernozemic soil. In this study, amendments were applied only in the first year and the effects were monitored over a three-yr period.

Chapter 10 synthesizes and integrates the key findings of the individual research studies reported in this dissertation and concludes with some recommendations for future research work.

2. LITERATURE REVIEW¹

2.1. Bioenergy Production By-Products

2.1.1 Bioethanol production by-products

2.1.1.1. Distillers' grains

Bio-ethanol can be produced from grain using two milling processes: wet milling and dry-grind processing. In wet milling, the grain kernel is fractionated into primary components of germ, fiber and starch, resulting in several process streams and by-products (Raush and Belyea, 2006; Liu, 2011). The by-products associated with wet milling processes of corn are: corn gluten meal, corn gluten feed, crude corn oil and germ meal. In contrast to dry-grind mills, wet mills are corporate-owned because they require high capital investment and extensive equipment (Belyea et al., 2004). In the dry-grind process, the entire corn is subjected to fermentation; corn is not separated into individual fractions (Raush and Belyea, 2006; Liu, 2011). Dry-grind mills require less equipment, have lower capital investment, can be owned by producers and therefore can have a significant contribution to local economies (Singh et al., 2001). Since dry-grind mills were recently reported to represent approximately 90% of total ethanol production in the USA (RFA, 2010) and are the most common ethanol production facilities in western Canada, the focus here is placed upon the by-products generated from dry milling-based ethanol production.

In dry-grind milling as shown in Figure 2.1, cereal grain is processed by grinding and fermenting via yeast addition where grain starch is converted into ethanol and CO₂. Then, ethanol is distilled off followed by centrifugation to remove the remaining liquid. The by-products that are left over after fermentation, distillation and centrifugation include a liquid fraction named thin stillage and the solid fraction termed wet distillers' grains (WDG). Thin

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stillage (TS) can be partially dried/evaporated and become condensed distillers solubles. Condensed distillers solubles can be added back to the wet distillers' grains resulting in wet distillers' grains with solubles (WDGS) by-product. The WDGS can also be dried, making dried distillers' grains with solubles (DDGS). These by-products can differ slightly in terms of their nutrient content, partly as a function of heating/drying in the case of condensed distillers solubles and DDGS. However, all these by-products contain nearly all the nutrient originally present in the processed grains, but are now concentrated due to the starch removal. They contain fiber, fat, protein and minerals. Wet distillers' grains has a high moisture content; approximately 70%, affecting the time it can be stored without spoilage and the economic viability of transporting long distances from the ethanol production plant (Bonnardeaux, 2007). Unlike WDGS, DDGS has less moisture content; approximately 12%, making its shelf life indefinite and economically viable to ship to longer distances. However, drying WDG requires further energy and adds extra cost to ethanol production. Additionally, possible changes can occur during drying that might reduce nutritional value of distillers' grains (DG) when fed to animals (Ham et al., 1994).

The amount of DG resulting from the conversion of cereal grain to ethanol varies according to the types of grains and the processes used. For example, one tonne of wheat generates 372 L of ethanol and 457 kg WDG or 295 kg of DDGS whereas one tonne of corn produces 378 L of ethanol plus 479 kg WDG or 309 kg of DDGS (Bonnardeaux, 2007). In 2012, USA ethanol biorefinery produced more than 34 million Mg of DG (RFA, 2013). In 2009, ethanol plants in western Canada generated 0.46 million Mg of DDGS (University of Saskatchewan, 2009).

As a result of starch removal, the nutrient concentration in DG are approximately three times that in the original grains (Spiehs et al., 2002). Therefore, the DG has been traditionally used as animal feed due to its higher content of protein and nutrient (Ham et al., 1994; Raush and Belyea, 2006; Gibb et al., 2008; Harris et al., 2008). Use of DG as animal feed accounts for the second largest source of income for ethanol-producing plants, after ethanol marketing (Bonnardeaux, 2007). However, high concentrations of fiber and nutrient, especially P may impede the expansion of this market. High fibre concentration can limit the use of DG mainly to ruminant diets, and similarly high P content could pose manure disposal challenges for cattle producers (Raush and Belyea, 2006), due to a greater land area required for application to avoid P loading in the soil. This higher content of P, fiber and protein is greater than that needed in the

animal diets (Noureddini et al., 2008), and the excess nutrient in animal diets will be excreted in the urine and fecal material. Therefore, it is sometimes recommended to restrict inclusion of DG in animal diets to a certain ration, for example 20% of the diet (Hao et al., 2009). As a result, feeding DG to animals may not accommodate continued rise in DG production, as the rapid growth in the ethanol industry is expected to create a surplus of DDGS (Erickson et al., 2005; Rausch & Belyea, 2006). Therefore, alternative approaches to their utilization need to be considered, including direct land application as a fertilizer and soil amendment. This may create another outlet for utilization of ethanol by-products, as this is critical to sustain ethanol industry and could be another source of income to offset the expenses in ethanol production.

The option of land application of ethanol production by-products has received little attention, and very few studies have looked at this option in the past. In a pot study conducted with horticultural plants, DDGS was reported to suppress weeds when applied to the soil surface and incorporated (Boydston et al., 2008). In a field study conducted near Novelty, Missouri, Nelson et al. (2009) concluded that DDGS might be utilized as a valuable fertilizer to supply nutrient to corn crops. They reported that application of DDGS at a rate of 140 kg N ha⁻¹ resulted in similar corn yield to urea and anhydrous ammonia applied at the same rate of N. The DDGS did not have a significant effect on selected soil chemical properties such as soil organic matter, P, K, Ca or Mg content. In a growth chamber study carried out to determine the N mineralization rates and the amount of available N to plants from different types of organic amendments over a 120-d growing season, it was found that N availability from DDG for the 210-d period of incubation was 56%, equivalent to 251 kg plant-available N ha⁻¹ (Moore et al., 2010). This study also indicated that the mineralization rate of DDG organic N was slower compared to poultry litter amendment, and attributed this to compounds that are present in DDG that may have delayed the nitrification process and thereby increased NH₄-N accumulation in the soil.

Soil respiration and N release from soil amended with first generation bioenergy by-products, including DDGS, have also been recently investigated under controlled environment conditions (Cayuela et al., 2010). In this study, soil amended with DDGS and incubated for 60 d showed that more than 80% of DDGS added C was mineralized after 2 mo. Soil treated with DDGS did not show a significant increase in extractable N when sampled at day 7 and 15 and was only significant after 60 d of incubation. This indicates a slow release of DDGS-contained N into plant available inorganic forms, and suggests a potential for DG by-

products being utilized as a soil amendment that releases nutrient more slowly than mineral fertilizers when directly applied to arable soil. However, field studies are needed to verify their performance under different environmental conditions.

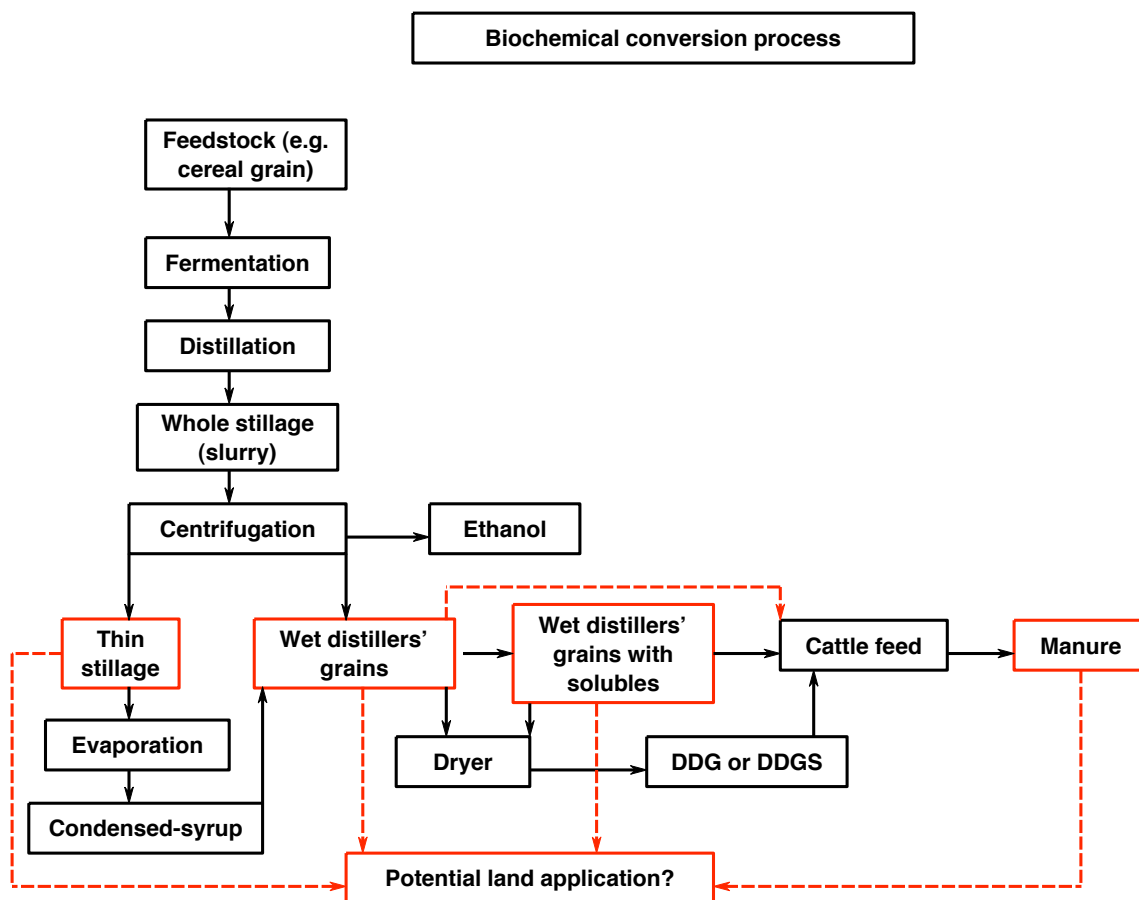


Fig. 2.1. Simplified process flow chart of cereal grain conversion to ethanol and associated by-products of distillers' grains and thin stillage. DDG: dried distillers' grains; DDGS: dried distillers' grains with solubles.

2.1.1.2. Thin stillage

Thin stillage (TS) is another major by-product associated with ethanol production. It can be defined as the aqueous by-product generated from the distillation of ethanol following fermentation of starch or sugar crops (Mustafa et al., 2000). The whole stillage, which contains solids from the grain along with added yeast and liquid from the water added during the process, is generated from fermentation and distillation processes (Fig. 2.1). The whole stillage is then

centrifuged to separate the liquid components called TS and the solid components called WDG. The TS is then further processed by evaporation to produce syrup which can be blended with WDG resulting in WDGS (Bonnardeaux, 2007). However, the evaporation process is costly and adds more expense to the cost of producing ethanol. Currently, one of the potential uses of TS is that it can be used as a partial or complete drinking water replacement for cattle (Mustafa et al., 2000). Approximately 6 L of TS is produced from one L of ethanol produced; a 190-million-L ethanol plant can produce about 1-3 million L of TS per day (AURI, 2008). It was also previously reported that up to 20 L of TS may be generated for each L of ethanol produced (van Haandel, 1994). There is growing interest in finding alternative uses for TS, including digestion to produce biogas; e.g. methane which can be used to power the ethanol plant, replacing natural gas, and recovering phosphate, ammonia and magnesium contained in TS to produce struvite pellets as a slow release 5-21-1 fertilizer (AURI, 2008). More research is required to investigate alternative methods to utilize this significant by-product stream associated with bioenergy production. As TS contains all essential plant nutrients which can promote crop production, and soluble organic matter that can stimulate soil biological activity, its direct application to agricultural soil might be a practical alternative. Equipment for land application of liquid by-products such as liquid manure is readily available and works well for direct injection of TS into soil (Fig. 2.2). However, the chemical characteristics of TS are variable, and will differ according to feedstock type and the treatments used in the bioenergy production plant. For example, TS generated from fermentation of cereal crops; e.g. corn or wheat, is different in its chemical properties to distillery wastewater generated from sugar cane, also known as vinasse. Vinasse is the most common feedstock in Brazil which has been investigated more extensively (Gemtos et al., 1999; Resende et al., 2006; España-Gamboa et al., 2011). Chemical composition of TS collected from a local ethanol production plant located in Saskatchewan, Canada showed that this by-product contains essential plant nutrients (Chapter 7). A significant portion, which was about 20% of total N, is in immediately plant available ammonium form, similar to that of liquid manure. The high content of soluble forms of nutrient in this by-product would promote rapid availability of nutrients to plants, as well as enable better ability to predict the availability when added to soil.



Fig. 2.2. Liquid manure application equipment (left) used during thin stillage injection (right) in a field trial in east-central Saskatchewan, Canada.

In an early field study carried out to investigate land application of TS generated from a sorghum grain feedstock in Texas, application of TS at a rate of 334 and 1040 kg N ha⁻¹ provided essential macronutrients to the soil, but not to levels that were thought to pose plant or animal toxicity issues (Jenkins et al., 1987). It was also reported in this study that TS application did not have a negative effect on grain sorghum yield. Residual effects of TS on wheat grown on the same plots after the grain sorghum harvest resulted in wheat yield on the TS-treated plots that was equal to or higher than plots treated with mineral fertilizer, indicating the slow release of N and the availability of nutrient from stillage organic matter persisting into the second season. In a parallel incubation experiment to examine TS N mineralization rates in comparison to composted cattle manure and fresh swine manure, it was shown that 27% of the applied N was mineralized, and this was about twice the amount of N mineralized from the composted cattle manure, but not as high as the mineralization rate of swine manure (Jenkins et al., 1987). However, there is limited documented information in recent years regarding fertilizer value of TS, especially as a soil amendment in prairie soils. A comprehensive understanding of the effects of TS application on soil properties, crop growth and nutrient recovery under field conditions is needed to provide recommendations about the potential use of this material as a soil amendment.

2.1.2. Biodiesel production by-products

2.1.2.1 Glycerol

Biodiesel is a biofuel that can be produced from renewable sources such as vegetable oils, animal fats or waste cooking oils. As a response to the increasing demand, biodiesel production has been growing dramatically over the past several years (Thompson and He, 2006; Manosak et al., 2011). It has some advantages over conventional diesel, including the sustainability of its feedstocks and lower emissions. Although biodiesel is considered as a sustainable, renewable and environmentally sound alternative to petroleum-based diesel fuel, its economic viability remains a major concern (Fan et al., 2010). Biodiesel manufacturing generates a principal by-product called glycerol (GL), also known as glycerin, (Fig. 2.3). It is generated during the manufacture of biodiesel via transesterification of vegetable oils that have been remained from oilseeds by crushing (Fig. 2.3). The GL constitutes 10% of biodiesel production: every tonne of biodiesel generates 100 kg of GL. The global production of biodiesel is estimated to reach over 140 billion L by 2016 with an average annual growth of 42%, which will result in approximately 15 billion L of crude GL being generated (Fan et al., 2010).

The large anticipated global increase in biodiesel production will lead to a surplus of glycerol, having environmental impact and will also affect the GL market (Tan et al., 2013). There is a wide range of applications for pure glycerol in pharmaceutical, food, cosmetic industries and many others; however, the refining of crude glycerol to a high purity is costly and may not be profitable for small and medium size biodiesel production plants; especially when the market for GL 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). Research is ongoing to explore alternative methods of crude GL utilization to improve the economic feasibility of the biodiesel industry. Some potential applications of crude GL have included combustion and thermo-chemical conversion (Kolesárová et al., 2010) and biological conversion or biological production of methane from crude GL using anaerobic sludge (Fountoulakis and Manios, 2009; Ma et al., 2008). Despite the existing uses of crude GL, more applications of this versatile by-product need to be developed to help sustain biodiesel production. One example of a potential use of GL is its direct application to soil as amendment. This potential has not been evaluated.

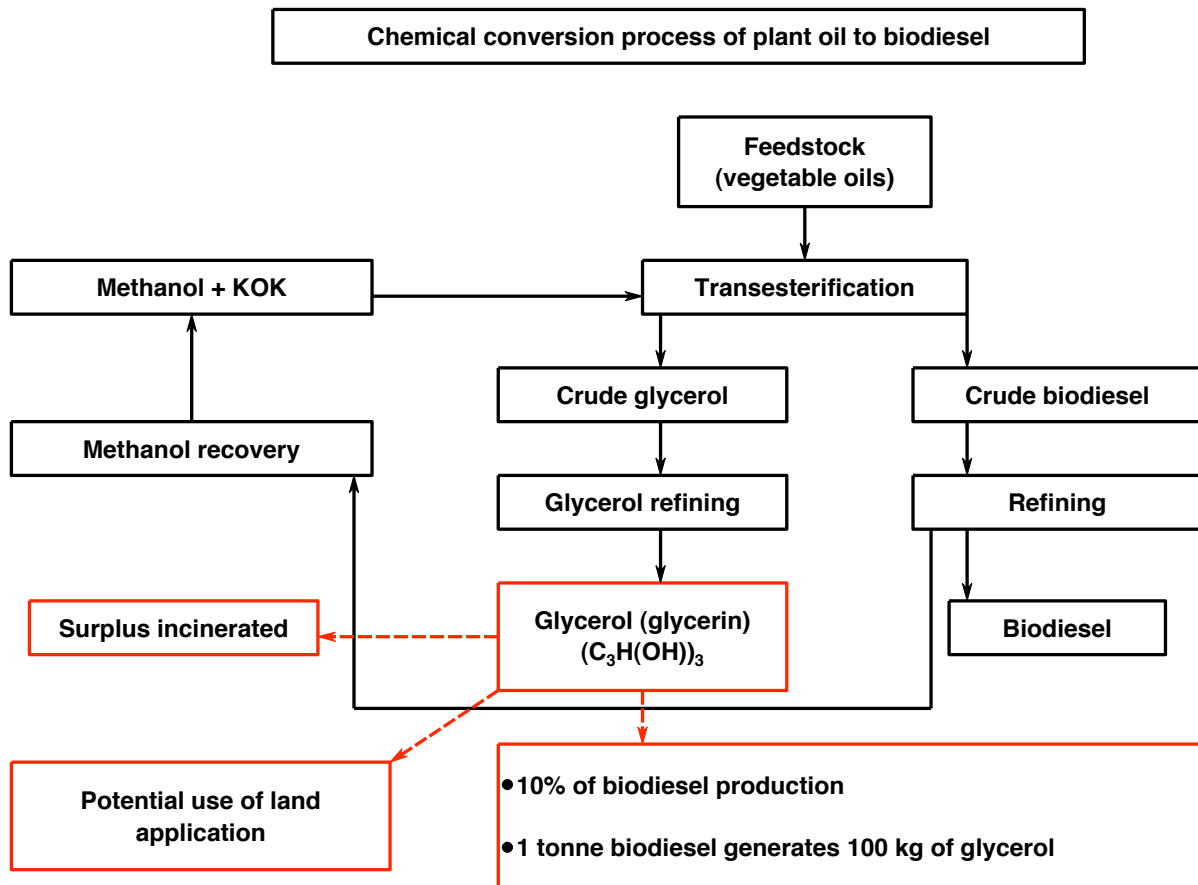


Fig. 2.3. Simplified process flow chart of vegetable oils conversion to biodiesel and associated by-product of glycerol.

Glycerol has no fertilizer value due to its lack of essential nutrient content, such as nitrogen and P. However, it is a concentrated C source, and could be used as a soil amendment to increase soil C content and build organic matter, especially in degraded soils that contain low organic matter due to the lack of organic inputs. It might also be used to prevent N losses via leaching and volatilization when combined with mineral N sources or liquid manure. This is related to its expected ability to tie up available N temporarily through microbial immobilization. In a study at the University of Saskatchewan, GL was obtained from a local biodiesel production plant and added to soil at a high rate (10000 kg ha⁻¹). A reduction in wheat yield and N plant uptake was observed, and this was attributed to microbial immobilization of available N (Qian et al., 2009). A recent study revealed that GL addition reduced N losses through N leaching,

relating this to its ability to immobilize available N (Redmile-Gordon et al., 2014). Research into GL application as soil amendment should be expanded to include its effects on soil physical, chemical and biological properties, including effects on microbial populations and activity along with plants growth.

2.1.3. Gasification and pyrolysis process by-products

Gasification and pyrolysis thermo-chemical technologies can be used to convert organic materials to energy. Thermal conversion such as incineration, combustion or gasification, of organic materials; e.g. wood to energy is not a new technology; however, its application to some types of organic materials is new and may be an effective strategy for organic waste recycling. There is a growing interest worldwide to develop such technologies to produce energy and at the same time reduce waste volume (McKendry, 2002a). Besides generating energy, gasification and pyrolysis technologies produce valuable by-products of ash resulting from gasification process and biochar (BC) resulting from pyrolysis process that can be utilized as soil amendments (Fig. 2.4).

2.1.3.1 Ash

The gasification process is defined as the thermo-chemical decomposition of organic materials under high temperature (800 – 900 °C) and in presence of oxygen (Ferreira et al., 2009). As shown in Figure 2.4, this process does not only produce biogas or syngas, such as CO, H₂, CH₄, CO₂, but it also produces ash as another end product. The ash contains all the P and K originally present in the gasified materials (Kuligowski and Poulsen, 2009), and the ash fraction comprises only about 1% of the raw waste mass. As such there is a significant reduction in processed waste volume and nutrient is significantly concentrated, especially P and K in ash generated, lowering cost of transport. The ash generated from gasification of organic materials contains a relatively high P content. Kuligowski et al. (2008) reported a P content of approximately 5.4% P; however, this is influenced by the type of materials gasified and their original P content. In our laboratory testing, ash from gasification of DDG and meat and bone meal were found to contain 19% and 18% of total P, respectively (Chapter 5).

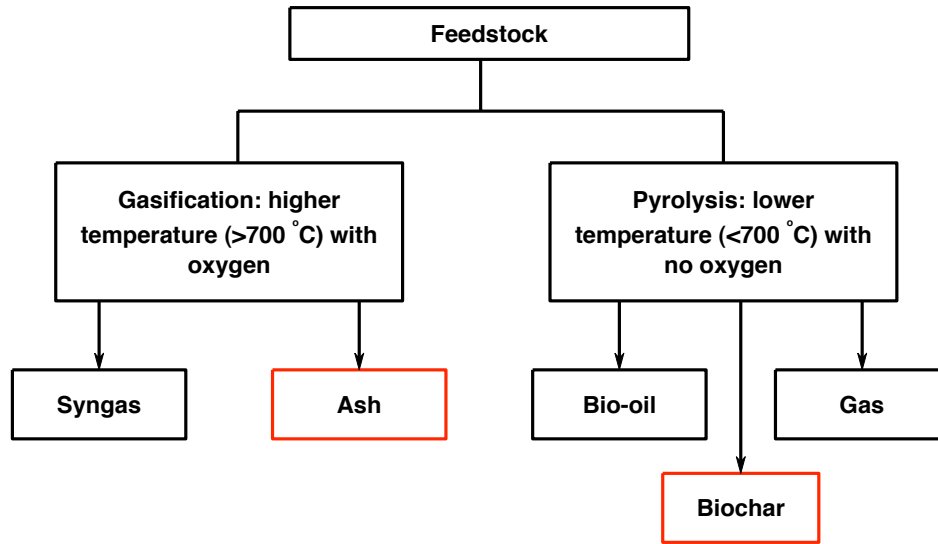


Fig. 2.4. Flow chart of conversion of organic materials to bioenergy through gasification and pyrolysis processes and associated by-products produced (ash and biochar).

Growing interest in producing bioenergy from sustainable sources through gasification has led to a large quantity of ash by-product being generated. For example, a power plant fueled with turkey litter in Minnesota, USA has capability to burn approximately 227000 Mg of turkey litter each year, resulting in approximately 45000 Mg ash being produced (Pagliari et al., 2010). It was estimated in Denmark that 1.5 million tons of pig manure gasified can result in 420000 tons of ash being produced if all the pig manure is thermally gasified (Kuligowski and Poulsen, 2009). In the light of the shrinking global phosphate rock reserves and increasing demand for P fertilizer in agricultural production, recycling P-rich ash would be a better option to replenish P-depleted soil. Therefore, there is a renewed interest in using ash as P fertilizer source. In the northeastern United States, most of the ash produced is land-applied (Campbell, 1990). The effects of ash on crop production and P nutrition vary depending on ash sources, gasified feedstock, tested crop and soil properties.

In a growth chamber study, alfalfa stem (*Medicago sativa*) gasification ash application to soil was found to be a potentially useful source of K and positively affected extractable soil P, but did not improve plant P nutrition (Mozaffari et al., 2002). Schiemenz and Eichler-Löbermann (2010) concluded that, based on results from pot experiments, crop biomass ashes can be effective source of P comparable to that of highly soluble triple superphosphate P fertilizer.

Wood ash application to soil increased K content in corn and winter wheat (*Triticum aestivum* L.) in greenhouse studies (Erich, 1991; Etiegni et al., 1991a) and alfalfa in field studies (Meyers and Kopecky, 1998). Plant P uptake and the most plant available resin extractable P fraction was increased in soil amended with poultry litter ash generated from combustion process, indicating the ashes may adequately substitute for mineral P fertilizers (Bachmann and Eichler-Löbermann, 2010). On a low-P soil, corn dry matter and shoot P uptake increased with increasing rate of turkey manure ash addition (Pagliari et al., 2010). However, by 52 days after emergence, corn dry matter in soil amended with turkey manure ash was lower compared to that of inorganic P fertilizer, and this was attributed to the initial slow release of plant available forms of P present in ash. In a two-yr field study, it was indicated that turkey manure ash can be an effective source of nutrients, especially P and K, for alfalfa production (Pagliari et al., 2009). Positive yield responses of alfalfa, bean (*Phaseolus vulgaris*), and fescue (*Festuca elatior*) to wood ash application were reported (Krejzl and Scanlon, 1996; Meyers and Kopecky, 1998; Muse and Mitchell, 1995). Ryegrass (*Lolium perenne* L.) yield was found to be higher in soil fertilized with coal ash (Matsi and Keramidas, 1999), and similarly sewage sludge ash addition increased yield of field corn and sweet corn (Bierman and Rosen, 1994). When applied at 12 or 25 t ha⁻¹ to an acid soil and with N fertilizer, wood ash significantly increased barley and canola seed yield by 50% and 124%, respectively in a field trial conducted northeast of Edmonton, Canada (Patterson et al., 2004). In addition to noted positive effects on crop production, ash application also influences soil chemical, physical and biological properties. Acid agricultural soils treated with wood ash in northwestern Alberta, Canada was found to have higher soil pH, microbial biomass content, C mineralization and also a change in the functional structure of bacterial communities was noted (Lupwayi et al., 2009).

Despite the observed positive responses of crop yields to ash application, there may be concern associated with ash application to agricultural soils related to content of heavy metals which might limit its use as a soil amendment. However, several studies reported no adverse effect of ash application on soil and plant content of heavy metals (Pagliari, 2010; Mozaffari et al., 2002; Schiemenz and Eichler-Löbermann, 2010; Pagliari et al., 2010; Pagliari et al., 2009; Codling et al., 2002; Mandre et al., 2010). This might be also clarified by conducting a long-term study with repeated application of different doses of ashes, and monitoring heavy metal toxicity and accumulation in soil and their potential transfer to the food and feed chain.

Table 2.1. Elemental analysis of oat hull-derived biochar (BC), glycerol (GL) from canola biodiesel product and ash from dried distillers' grains (DDGA) and meat & bone meal (MBMA) gasification. The by-products were chemically characterized at the University of Saskatchewan.

Parameter	BC	GL	DDGA	MBMA
	-----mg g ⁻¹ -----			
N	15.4	0.95	1.2	0.2
P	29.2	ND [§]	156	127
S	1.2	0.25	29.9	0.00
C	714	566	8.7	0.9

§ ND = not determined

2.1.3.2. Biochar

Pyrolysis is the thermal breakdown of organic materials in absence of oxygen (O₂) and at relatively low temperature (< 700 °C) (Lehmann and Joseph, 2009). As shown in Figure 2.4, this process converts organic materials into three by-products: 1) a liquid product called bio-oil (pyrolysis oil), 2) a non-condensable gas product called syngas or pyrolysis gas containing carbon monoxide (CO), carbon dioxide (CO₂), hydrogen (H₂), methane (CH₄) and higher molecular weight volatile hydrocarbon, and 3) a solid inert residue rich in carbon known as biochar (BC) when intended for soil application (McCarl et al., 2009). The BC generated during pyrolysis processes as a charcoal can be used as a fuel to produce heat, and as activated carbon used in purification processes (Horne and Williams, 1996), or as a soil amendment. The latter option for BC utilization is not a new concept, but it has recently received more attention. Research studies examining the agronomic and environmental potential benefits of BC application to soil have been reported, but studies on the effects of BC addition on agricultural productivity is still limited, especially in dry and temperate climates, as the majority of studies have been conducted in tropical regions (Blackwell et al., 2009). Biochar application is reported to improve plant production directly through supply of essential nutrient, or indirectly through improving soil quality and fertility. The ability of BC to retain nutrients in the soil and improve fertilizer use efficiency are regarded as examples of indirect BC nutrient value (Chan and Xu, 2009). The Amazonian Terra Preta soils containing high amounts of BC-like pyrogenic C were found to be more fertile compared to adjacent infertile soils, and this was related to their ability to retain nutrients (Glaser et al., 2001). The benefit of BC amended-soils will therefore be related at least in part to reduction in nutrient losses via volatilization, leaching and runoff.

In a pot experiment conducted near Manaus, Brazil, application of BC derived from wood applied at rates of 68 t ha⁻¹ to 135 t ha⁻¹ increased rice biomass production and cowpea by 17 and 43%, respectively, and this was attributed to improved P and K nutrition provided by the biochar (Lehmann et al., 2003). It should be noted that rates of 100 t ha⁻¹ would limit the distance such material could be economically transported. In a field trial conducted in Japan, BC addition increased height and volume of tea trees by 20 and 40%, respectively, and this was partly attributed to the ability of bamboo BC to keep the soil pH within the range suitable for tea tree growth (Hoshi, 2001). Biochar produced from paper mill sludge added at a rate of 10 t ha⁻¹ to an acidic soil from northern New South Wales, Australia in a pot experiment resulted in a 40% increase in wheat height (Van Swieten et al., 2007). The liming effect of BC may have promoted wheat growth by eliminating the toxic effects of soluble and exchangeable aluminium (Al) dominant in the acidic soils (Chan and Xu, 2009).

The chemical and physical characteristics of BC vary according to temperature used during pyrolysis and the type of feedstocks used. In laboratory testing (Table 2.1), BC derived from oat hull showed a high content of C (71%), with low N (1.5%) and P (3%) content. The positive effects of BC application on plant production are not necessarily related to plant nutrition; it has been reported that BC behaved as sorptive agent and removed organic compounds such as phenolics released from humus (Wardle et al., 1998); improved soil physical properties, like water holding capacity (Iswaran et al., 1980) and reduced soil strength (Chan et al., 2007).

Green waste BC applied at rates up to 100 t ha⁻¹ in a combination with 100 kg N ha⁻¹ increased radish dry matter by up to 266%, but this increase was not observed at the same rate of N in absence of BC (Chan et al., 2007). This indicates an ability of BC to improve N fertilizer use efficiency. It was also demonstrated in another study that BC was able to protect applied fertilizer against leaching, resulting in increased fertilizer use efficiency (Lehmann et al., 2003).

Despite the positive response of different crops to BC addition that has been documented in studies recently published, it was reported earlier that BC addition at 5 t ha⁻¹ and 15 t ha⁻¹ resulted in soybean yield reductions of 37 and 71%, respectively (Kishimoto and Sugiura, 1985). This reduction in soybean yield was attributed to micronutrient deficiency as a consequence of pH increases. It was also shown that long-term productivity of woody plants was inhibited on

charcoal hearths, and this was mainly attributed to higher pH and exchangeable base cations that were observed in charcoal hearth soils compared to nonhearth soils (Mikan and Abrams, 1995).

In addition to agronomic potential benefits observed with BC application, BC might be considered as part of the solution for existing environmental issues such as reduction of mineral fertilizer losses through leaching and runoff, mitigation of greenhouse gases emissions, C sequestration, and potential application as sorbents in remediation of contaminated soils, and managing agricultural, urban and industrial wastes.

It has been shown that BC was capable of retaining nutrients from applied mineral fertilizers due to a high sorptive surface area, reducing leaching and thereby increasing fertilizer use efficiency (Lehmann et al., 2003). Therefore, BC application could have environmental benefit through reduction of nutrients losses by its ability to absorb nutrients such as phosphate and ammonium that would cause eutrophication and pose pollution risks (Lehmann et al., 2003; Lehmann, 2007). Biochar was also found to absorb pesticides before they reached water bodies (Takagi and Yoshida, 2003). As for its effect on greenhouse gas emissions reduction, BC addition to soil could help mitigate greenhouse gas emissions through its ability to reduce N₂O emissions and increase CH₄ uptake from soil (Rondon et al., 2006; Yanai et al., 2007; Singh et al., 2010). In a seven-day incubation study, addition of biowaste charcoal at a rate of 10% wt/wt (approximately equivalent to 180 t ha⁻¹) to a grassland soil rich in organic matter suppressed N₂O emissions by 90%, but the emission of N₂O increased when the same soil was rewetted (Yanai et al., 2007). In a parallel trial in the same experimental set, these authors applied ash derived from the same BC feedstock (pH 11.6) to the same soil and found no N₂O suppression when ash was added, suggesting that alkalinity properties of BC (pH factor) was not the reason behind reduced N₂O emissions reduction. The mechanism responsible for N₂O emissions reduction when BC was added is still unclear (Yanai et al., 2007; Van Swieten et al., 2009; Singh et al., 2010). Despite the few short-term incubation studies conducted under controlled conditions (Yanai et al., 2007; Singh et al., 2010), there is, however, a dearth of field studies on the potential of BC to mitigate greenhouse gas emissions.

2.2. Manure derived from cattle fed ethanol by-products

Ethanol production by-products including DG and TS can be an excellent source of protein and nutrients when included in livestock diets (Mustafa et al., 2000). However, unlike DG, TS has not routinely been used in animal diets except as a source of water.

Distillers' grains have commonly been used as animal feed in the wet or dry form and are recognized as an excellent source of protein and phosphorus (Erickson et al., 2005; Harris et al., 2008). Similarly, TS can be fed alone or in a combination with DG (Mustafa et al., 2000). However, due to its liquid nature and for better utilization by beef cattle, TS can replace water as a fluid source for animals (Mustafa et al., 2000). The co-location of an ethanol plant with a feedlot, such as exists near Lanigan, Saskatchewan, allows TS to be used as a cattle watering source and WDG to be used as feed.

In cattle feeding, only about 10% of the N and only about 20% of the P in the feed is retained in the beef animal, and the rest is excreted in feces and urine (Bierman et al., 1999). Therefore, it is expected that manure produced from animals fed DG will have different characteristics compared to that derived from animals fed regular grains. In particular, owing to higher contents of N and P in DG, the DG derived manure will also have higher content of nutrients, especially N, P and S (Hao et al., 2009). Recycling these nutrients back to soil through manure application will partially compensate for nutrients remained from the soil (Benke et al., 2010). This option will then, to some extent, help maintain soil quality and productivity.

In general, animal manure has been shown to be a valuable source of plant nutrients, making an effective contribution to improved chemical and physical properties of soil as well as biological activity and plant nutrition (Schoenau and Davis, 2006; Edmeades, 2003). Many manure characteristics and its agronomic value are influenced by livestock diet (Eghball, 2002; Hao et al., 2009). For example, the capacity of manure to increase pH of acid soils is affected by the addition of CaCO_3 to the diet (Eghball, 1999). Diet modification could also influence odor by affecting the various volatile fatty acid content (Hao et al., 2005). Reduction in protein and P intake was shown to result in a significant reduction in N and P concentration in manure and therefore decrease ammonia volatilization and P accumulation in manure amended soils (Satter et al., 2002; Maguire et al., 2007). In a study at Lethbridge, Alberta to evaluate the effect of including wheat DDGS in finishing feedlot cattle diets on composition of manure, Hao et al., (2009) concluded that including 40 and 60% wheat DDGS in feedlot cattle diets resulted in

significant increases in water soluble NH_4^+ -N, total N, and total P in animal manure. Similarly, Spiehs and Varel (2009) reported that increasing amount of WDGS in feedlot cattle diets resulted in increased P, N and S content in cattle manure. Increased inclusion of DG in animal diets from 0% to 40% (DM basis) increased diet crude protein from 13% to 18.7% and P in the diets from 0.29% to 0.49% (Bremer et al., 2008). This increased the excreted N and P by 51% and 90%, respectively, as a consequence. It was also previously documented that cattle consuming dietary N (Cole et al., 2005; Archibeque et al., 2007), P (Benson et al., 2006; Luebke et al., 2008), and S (Fron et al., 1990) in excess of nutrients needs will result in extra nutrients being excreted in urine and feces. In terms of TS-derived manure, there is a lack of studies examining manure composition as affected by TS inclusion in animal diets.

Distillers' grains inclusion in animal diets does not only increase the amount of nutrient excreted, but it also can influence the forms of these nutrient. Water soluble P tended to increase when WDGS was included in the diet (Spiehs and Varel, 2009). Similarly, Ebeling et al. (2002) revealed that higher dissolved reactive P was observed in manure obtained from animals fed higher P concentration diets. Therefore, this could enhance nutrient availability and crop growth response when manure is land-applied, but also increase potential for transport of P in runoff.

Greater concentration of nutrient in manure produced by animal fed DG can increase animal manure value as organic fertilizer by reducing handling and transportation costs per unit of nutrient. Moreover, recycling these nutrients back to soil through manure application will partially compensate for nutrients removed with grains and straw sent for bioenergy production and that otherwise will have to be replaced by mineral fertilizers. Land application will help maintain soil quality and productivity especially in land areas experiencing high demand for bioenergy and food production. However, application rates need to be adjusted for higher nutrient content that may be encountered to avoid excessive application rates of nutrients that can have environmental implications including water quality degradation through nutrient runoff and leaching.

1. GREENHOUSE GAS EMISSIONS AND NUTRIENT SUPPLY RATES IN SOIL AMENDED WITH BIOENERGY PRODUCTION BY-PRODUCTS

3.1 Preface

The sustained rise in bioenergy production from renewable sources has created a surplus of the by-products associated with the bioenergy industry. As documented in the Literature Review (Chapter 2), recycling of these materials by application to agricultural soils is a possible option for their usage. However, there is limited information on the impacts of land application on soil biological processes and nutrient cycling. The main goal of this study was to investigate the direct effects of adding selected bioenergy production by-products (BPB) on greenhouse gas emissions (N_2O , CO_2) and nutrient supply rates ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$) using Plant Root Simulator (PRSTM) resin membrane probes over a short incubation period. Initiation of this research with a growth chamber experiment conducted under optimum controlled conditions is necessary to provide information that can improve our understanding of greenhouse gas emissions and nutrient turnover as a function of the application of bioenergy production by-products. The information here is used to help understand results of longer-term field research studies that are covered in subsequent chapters.

Chapter 3 has been published as: Alotaibi, K.D. and J.J. Schoenau. 2013. Greenhouse gas emissions and nutrient supply rates in soil amended with biofuel production by-products. *Biol.Fertil Soils* 49: 129-141. J.J. Schoenau's contributions to this work were: guidance during the experimental setup, financial coverage of research expenses and manuscript editing.

3.2 Abstract

Ethanol production results in distillers' grains, and biodiesel produces glycerol as a by-product. However, there is limited information on the effects of their addition on the evolution of N_2O and CO_2 from soils, yet it is important to understanding the impacts of bioenergy production on greenhouse gas budgets. The objective of this study was to evaluate the direct effects of adding wet distillers' grains (WDG), thin stillage (TS) and glycerol at three rates on greenhouse gas emissions (N_2O , CO_2) and nutrient supply rates in a cultivated soil from the Canadian prairies. The WDG and TS application rates were: 100, 200 or 400 kg N ha⁻¹ whereas glycerol was applied at: 40, 400 or 4000 kg C ha⁻¹ applied alone (G-N) or in a combination with 300 kg N ha⁻¹ (G+N). In addition, conventional amendments of urea (UR) and dehydrated alfalfa (DA) were added at the same rates of total N as the by-products for comparative purposes. The production of N_2O and CO_2 was measured over an incubation period of 10 days in incubation chambers and Plant Root Simulator (PRSTM) resin membrane probes were used to measure nutrient (NH_4^+ -N, NO_3^- -N, PO_4^{3-} -P) supply rates in the soil during incubation. Per unit of N added, UR tended to result in the greatest N_2O production, followed by WDG and TS, with glycerol and DA resulting in the lowest N_2O production. Cumulative N_2O production increased with increasing rate of N-containing amendments and was greatest at the highest rate of UR. Addition of urea with glycerol contributed to a higher rate of N_2O emission, especially at the low rate of glycerol. The DA and WDG resulted in the greatest evolution of CO_2 from the soil, with the TS resulting in less CO_2 evolved per unit of N added. Addition of N fertilizer along with glycerol enhanced microbial activity and decomposition. The amendments had significant impacts on release of available nutrient, with the UR treatments providing the highest NO_3^- -N supply rate. The TS treatments supplied the highest rate of NH_4^+ -N, followed by WDG compared to the other amendments. The WDG treatments were able to provide the greatest supply of PO_4^{3-} -P supply in comparison to the other amendments. Microbial N immobilization was associated with G-N treatments. This study showed that the investigated bioenergy by-products can be suitable soil amendments as a result of their ability to supply nutrients, and N_2O emissions that did not exceed that of the conventional urea fertilizer.

3.3 Introduction

Bioenergy production is associated with a large amount of bioenergy processing by-products. These by-products include wet distillers' grains (WDG) and thin stillage (TS) from ethanol production, and glycerol from biodiesel. Both WDG and TS 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 by-product of biodiesel production; mostly produced from soybean and canola oil via transesterification process. Due to their nutritional value, WDG and TS are considered to be a valuable feedstuff for animals (Ham et al., 1994; Harris et al., 2008). However, increased ethanol production may result in a surplus of distillers' grains (DG) (Rausch and Belyea, 2006); therefore, alternative uses need to be sought, including consideration of land application as a fertilizer.

Large amounts of produced biodiesel have led to a surplus of glycerol (GL), which is disposed of by incineration (The Glycerol Challenge, 2007). The existing potential uses of GL include industrial, chemical and pharmaceutical preparations and potential for use in production of plastics is being explored. However, purification of crude GL is costly and the market of GL 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 GL is application to agricultural soil. However, this has received little attention, and the information about its application to soil is scarce. Qian and Schoenau (2008) found that application of GL at a high rate (10000 kg ha^{-1}) resulted in wheat yield reduction, and this might be attributed to microbial immobilization of available N.

The by-products discussed above contain C that can improve soil organic matter content and enhance microbial activity. They also contain essential plant nutrients such as N and P, especially DG and TS by-products. These characteristics suggest opportunity for their potential use as soil organic amendments to improve soil fertility and quality. However, information regarding utilization of these by-products as soil amendments is scarce. In general, addition of organic materials to agricultural fields is an important source of CO_2 and N_2O (Akiyama and Tsuruta, 2003) as a result of microbial respiration and nitrification and denitrification in soils, which depend on 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; Lemke et al., 1999). However, the CO₂ and N₂O emissions depend on chemical composition of added materials and their rate of microbial breakdown in soil (Aulakh et al., 1991; Mckenney et al., 1993; Shelp et al., 2000).

To best manage by-products of an expanded bioenergy production industry, a comprehensive knowledge of the impacts of adding bioenergy processing by-products to agricultural soils is needed. Thus, this research evaluates the effect of a range of by-products on N₂O production, soil respiration and release of nutrients into available ionic forms under controlled environment conditions in a cultivated soil typical of the Canadian prairies. The effect of WDG, TS and glycerol on N₂O and CO₂ production in a Saskatchewan soil is examined in comparison to more conventional N containing amendments including alfalfa and urea.

3.4 Materials and Methods

3.4.1 Soils

The soil used for the growth chamber study was collected from the surface layer (0–20 cm) of a cultivated (cereal-legume-oilseed rotation) Brown Chernozem (U.S. equivalent: Aridic Haploboroll) in south-central Saskatchewan, Canada. Three soil samples were collected from the same depth and mixed to produce a composite soil sample. This composite soil was mechanically mixed using a stationary mixer (Bouldin & Lawson Inc., McMinnville, Tennessee) to provide a homogenized sample and then stored at 20 °C until its use. The soil was used for incubation within 2 wk of its collection to reduce the impact of storage on the biological assessments (Zelles et al., 1991). Selected chemical properties of the soil are given in Table 3.1.

3.4.2 Bioenergy by-products preparation

The bioenergy by-products examined in this study included: wet distillers' grains (WDG), thin stillage (TS) and glycerol. In addition to these by-products, urea (UR) and dehydrated alfalfa (DA) were also included for comparative purposes. The by-products of WDG (solid) and TS (liquid) were obtained from a wheat-based ethanol production facility at Lanigan, Saskatchewan. Glycerol (GL) by-product, a thick syrupy liquid from canola-based biodiesel production, was obtained from Milligan Biotech at Foam Lake, Saskatchewan. Alfalfa used in

this study was a dried dehydrated powder obtained from MCN Bioproducts Inc., Saskatoon, Saskatchewan. All by-products were sub-sampled for composition analyses and characterization, and then stored at 4 °C until use. Selected characteristics of the by-products used in the controlled environment chamber experiment are given in Table 3.2.

Table 3.1. Selected properties of cultivated Brown Chernozem soil used in the experiment.

Property	Value
Organic C (mg g ⁻¹)	18.9
Total N (mg g ⁻¹)	1.1
Total P (mg g ⁻¹)	0.5
NaHCO ₃ -extractable P (mg kg ⁻¹)	5.73
NO ₃ -N (mg kg ⁻¹)	1.69
NH ₄ -N (mg kg ⁻¹)	5.34
pH	7.9
EC (mS cm ⁻¹)	0.29

Table 3.2. Selected properties of organic amendments used in the experiment.

Amendments [¶]	OC	Total N	Total P	S	pH	C:N	C:S	MC [#]
	-----mg g ⁻¹ -----							%
DA	432	25	2.2	2.8	ND [§]	17:1	153:1	10
WDG	511	37	4.2	13.7	ND	14:1	36:1	74
TS	449	73 [‡]	13.3	14.6	3.8	6.20	31.10	92.5

¶ DA denotes dehydrated alfalfa; WDG denotes wet distillers' grains; TS denotes thin stillage. All nutrient content rates are on dry weight basis.

§ ND, not determined.

MC, moisture content.

‡ 21% of total N in TS is in NH₄⁺ form on a wet basis.

3.4.3 Treatment application

Homogenized field-moist soil (650 g) was placed into each 1-L cylindrical plastic pot of 12.2 cm height × 12 cm diameter (tapered) with a surface area of 113.04 cm². Then, 50 g of soil were mixed with the required rate of amendment application and spread on the soil surface; then 150 mL of deionized water, which is adequate to bring soil moisture to field capacity, was added followed by 100 g of soil placed on top. In case of liquid or slurry substrates (TS, GL), 700 g of soil were 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 were placed on the top. Therefore, a total amount of 800 g of soil were incubated in each pot. The N-containing amendments (UR, DA, WDG, TS) used in this study were applied at three rates: low, medium and high, providing 100, 200 and 400 kg N ha⁻¹, respectively, assuming incorporation of the amendments to soil depth of 15 cm. The GL amendment was also applied at three rates: low, medium and high, providing 40, 400 and 4000 kg C ha⁻¹, respectively, given a C content of the glycerol of 40% C by weight. The GL was applied based on its total C content because it does not contain N. Each rate of GL was applied alone or combined with one rate of urea, equivalent to 300 kg N ha⁻¹. This rate of N will result in a C:N ratio of 13:1 when combined with the highest rate of glycerol-C which will ensure that N is not a limiting factor and will thereby stimulate microbial activity to utilize glycerol C. A control that received no organic amendment was included as well. Each treatment was replicated four times in a completely randomized design. Moisture contents in the pots were constantly maintained at or near field capacity by measuring weight loss on a daily basis, and deionized water was added when needed.

3.4.4 Nutrient availability measurement

The bioavailable NO₃⁻-N, NH₄⁺-N, and PO₄⁻³-P supply rates in the soil were determined using Plant Root Simulator (PRSTM) resin membrane probes as ion sinks as described by Qian and Schoenau (2002). Briefly, the PRSTM anion probes were initially soaked in distilled water for 24 h. Then, the probes were charged for 2 h in 0.5 M NaHCO₃ to saturate the exchange sites with bicarbonate as the counter ion, and this was repeated four times. The probes were then washed twice and stored in distilled water until their insertion into the soil. The PRSTM cation probes were charged by soaking in 0.5 M HCL two times for 2 h to saturate the exchange sites with H⁺ ions and then washed and stored as described above. Then, the anion and cation probes were inserted into the pots containing amended soil and remained installed in the pots for the entire 10 d period of incubation. At the end of incubation, the probes were removed from the soil and placed into plastic ZiplockTM bags and transported to the laboratory. Then, the probes were washed thoroughly to remove all remaining soil particles and placed into a clean ZiplockTM bag in which each probe was placed in a separate ZiplockTM bag. A 20-mL aliquot of 0.5 M HCL was added to each bag containing the probe and shaken for 1 h to elute the sorbed ions from the membrane surface. The eluent was then placed in a 7 dram vial, capped, and stored at 4°C until it

was colorimetrically analyzed for NO_3^- -N, NH_4^+ -N and PO_4^{3-} -P using Technicon™ Auto-Analyzer II.

3.4.5 Gas emissions sampling

All the pots containing amended soil and the PRS™ probes were equilibrated for about 24 h under laboratory conditions. Then, they were placed into 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 et al., 2007). A rubber septum inserted into the cap was used to extract the gas samples. The incubation was conducted as described by Nelson et al. (2007). The airtight sealed containers were moved to a growth chamber, which was electronically set for 16 h at 25 °C (day) and 8 h at 18°C (night), and incubated for a period of 10 d. For gas sampling, 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 noon over the 10 d period. After each sampling, the tops of the PVC containers were removed and allowed to remain open for 1 h to allow natural airflow exchange to ensure aerobic conditions. The collected gas was analyzed for CO₂ and N₂O using a gas chromatograph. The CO₂ was determined using a Varian CP-2003 micro-GC with twin micro-thermal conductivity detectors. The N₂O was determined using a Varian CP-3800 gas chromatograph with an electron capture detector set at 370 °C (Nelson et al., 2007).

To ensure that the incubation condition remained aerobic when containers were sealed for 2 d, a preliminary experiment was conducted to test the effect of different times of opening and sealing of PVC containers on the O₂ content. We found that sealing the containers for 1 h, 3 h or 2 d before sampling did not cause a decline in the O₂ content; however, O₂ concentration started to decrease after keeping the containers closed for 3 d. Therefore, we decided to maintain containers sealed for 2 d before sampling.

A short term incubation was chosen because greatest response of microbiological indicators to addition of organic materials is expected to occur within a few days of incubation following application. It was also demonstrated that the PRS™ probes can be adequate in providing reliable results when incubated into soil for a short period of 2 wk or less (Qian and Schoenau, 2002).

3.4.6 Statistical Analyses

The experimental treatments consisted of 6 amendments (UR, DA, WDG, TS, G-N and G+N) with three application rates (low, medium and high) in addition to a control where no amendment was added. This design provided a factorial arrangement. The measured parameters included GHG emissions (N_2O and CO_2), that were repeatedly measured and nutrient supply rates ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$), that were measured at the end. Due to the sequential nature of GHG sampling from the experimental unit (PVC chamber), repeated measures analysis was conducted using the PROC MIXED procedure with restricted maximum likelihood (REML) of the SAS software, version 9.2 (SAS Institute, Cary, NC) as reported by Littell et al. (1998). Covariance structures were compared objectively using the Akaike information criterion and the Bayesian information criterion to find the best covariance model, and it was determined that the unstructured UN covariance structure provided the best fit for our model with the least complexity. The effects of treatments, day of sampling and their interaction with N_2O and CO_2 emissions were tested using the above described model. The effects of treatments (amendment types), rate and their interaction on cumulative N_2O and CO_2 , calculated for the entire period of incubation, and nutrient supply rates ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$) were also tested using two-way ANOVA procedure. For this analysis, the control treatment was omitted as there was only one level of this factor. Before applying the statistical analysis, data were checked and subjected to normal distribution test using Shapiro-Wilk at $P < 0.05$. This test revealed that nutrient supply rates variables ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$) were not normally distributed and, to achieve uniformity, these variables were log-transformed, after which transformed data were used for statistical analysis; however, the raw data are reported here. Treatments means (averaged across the rates of application) were separated by the Student-Newman-Keuls test. The effects were declared statistically significant at $P < 0.05$.

3.5 Results

3.5.1 Nitrous oxide evolution

Treatment, day and their interaction significantly affected N_2O evolution rates ($P < 0.001$). Generally, N_2O evolution rates were much lower than CO_2 evolution rates. The

application of amendments had variable effects on N₂O fluxes according to the amendment type and rate of application (Fig. 3.1).

Addition of UR increased N₂O production rates, and this increase was rate-dependant, with the greatest emission observed at high rate of application (Fig. 3.1). The effect was delayed until day 4, reaching the peak at day 8 for high rate treatment and day 6 for medium rate treatment. The total cumulative N₂O emission for the 10 d of incubation for UR treatment was the highest in comparison to other treatments, especially at the medium and high rate of application (Fig. 3.2). When averaged across the rates of application, N₂O produced from soil treated with UR was significantly higher than that from other treatments (Fig. 3.2).

Dehydrated alfalfa treatment had a small effect on N₂O production rates over the course of the incubation (Fig. 3.1), especially when applied at medium and high rates. The total cumulative N₂O production from DA treatment was similar amongst the three rates of application and did not significantly ($P > 0.05$) differ from G-N treatment (Fig. 3.2). However, it was significantly ($P < 0.001$) lower than UR, TS and WDG treatments.

Wet distillers' grains addition contributed to enhanced N₂O evolution rates above the control over the entire duration of the incubation (Fig. 3.1). The effect of WDG treatment remained constant for all the rates of application until day 6 and increased rapidly, particularly at medium and high rates (Fig. 3.1). The total cumulative N₂O evolved from soil treated with WDG was similar for medium and high rates of application (Fig. 3.2). When averaged across the three rates of application, it was significantly ($P < 0.001$) lower than UR treatment and higher than the other treatments with the exception of TS treatment, which did not significantly differ from WDG treatment (Fig. 3.2).

Thin stillage treatment had a significant effect on the rates of N₂O evolved from the amended soil (Fig. 3.1). The effect was delayed until day 2. Soil treated with TS applied at a high rate produced higher N₂O compared to medium or low rate. Nitrous oxide evolution peaked at day 6 for the low and medium rate treatments followed by a rapid decline, and at day 8 for high rate treatment. The total cumulative N₂O emission from soil treated with TS was relatively similar for low and medium rates and higher at the high rate of application (Fig. 3.2). It was not different from that of WDG treatment, significantly ($P < 0.001$) lower than UR treatment and higher than all the other treatments (DA, G-N, G+N), when averaged across the three rates of application (Fig. 3.2).

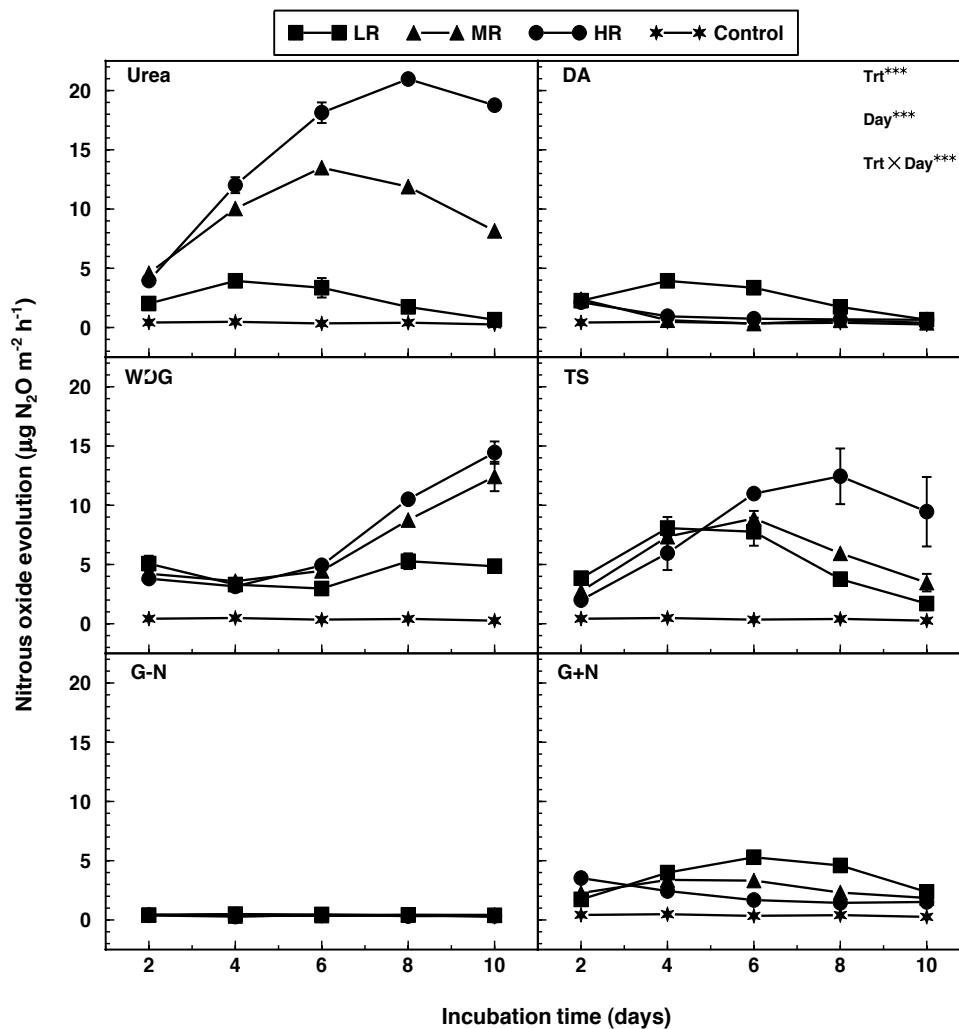


Fig. 3.1. Nitrous oxide emissions from soil amended with three rates (low, LR; medium, MR; high, H) of urea (UR), dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without N (G-N) and glycerol with N (G+N). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

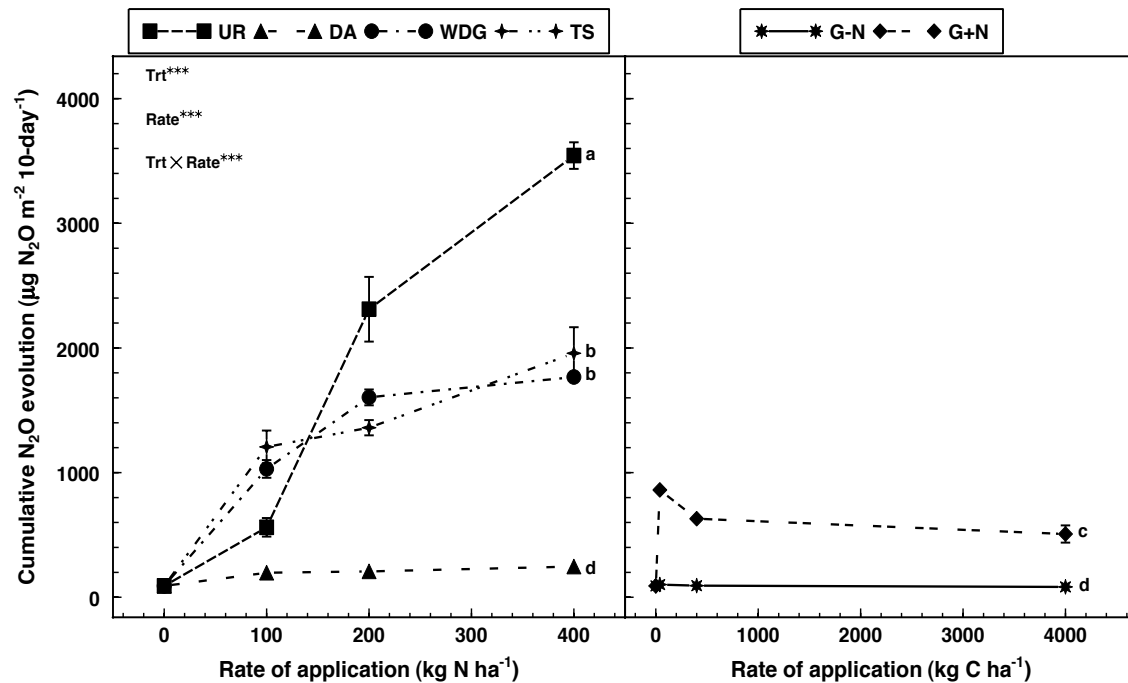


Fig. 3.2. Cumulative N₂O emissions, calculated for the entire period of incubation, from soil amended with three rates (low, LR; medium, MR; high, H) of urea (UR), dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without N (G-N) and glycerol with N (G+N). Points sharing the same letter among amendment type are not significantly different according to SNK test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note that the statistical analysis was conducted on all amendments regardless of the basis of their application whether it is N or C and the same for means separation for all amendment types. However, after the analysis, glycerol treatments were considered separately to allow for better comparisons among its rate of application.

Glycerol applied alone did not have any effect on N₂O evolution when applied at any rate and the evolved N₂O from the amendment soil remained low throughout. However, this was not the case when GL was applied with N, since this treatment stimulated N₂O evolution (Fig. 3.1). This increase was specifically observed with the low rate of application especially after the first measurement period, reaching the peak at day 6. Increasing G+N amendment rate tended to decrease N₂O production rates particularly after day 2 (Fig. 3.1). The total cumulative N₂O emission for the entire period of incubation study for G+N treatment was higher at the low rate and similar for the medium and high rates of application (Fig. 3.2). When averaged across the rates of application, cumulative N₂O emission of soil treated with G+N was significantly ($P < 0.001$) higher than that of glycerol treated soil (Fig. 3.2). It was also higher than that of the DA treatment.

3.5.2 Carbon dioxide evolution

Treatment, day and their interaction significantly impacted CO₂ evolution from soil ($P < 0.001$). Amendments produced different CO₂ fluxes, depending on the type and rate of amendment (Fig. 3.3).

Urea treatment had a significant impact on CO₂ evolution compared to the control (Fig. 3.3), with no significant rate effect observed among urea-N rates during the entire period of incubation. All urea –N rates showed similar effect on CO₂ production. The effect was delayed until day 6, with CO₂ evolution not reaching a peak until day 8 and 10. The total cumulative CO₂ emission from the urea treatment was the least among the other treatments, especially at the medium and high rates (Fig. 3.4), and this was also significantly different from other type of amendment, when averaged across the rate of application (Fig. 3.4).

Carbon dioxide evolution rates in soil amended with dehydrated alfalfa were higher than that of the control at any rate of application (Fig. 3.3). The evolution of CO₂ increased with increasing DA rates when compared to the control. The low rate of DA treatment showed similar CO₂ fluxes for the period 2-10 d. For the medium and high rates of DA, peaks of CO₂ evolution were observed at day 2 and 4 and declined thereafter. For the entire period of incubation, the total cumulative CO₂ emission from DA treatment was the greatest in comparison to the other amendments (Fig. 3.4). When averaged across the rate of application, the cumulative CO₂

emission for the DA treatment was similar to WDG treatment, but significantly ($P < 0.001$) higher than the other amendments (Fig. 3.4).

The WDG treatment effect on CO₂ production followed a similar pattern of DA treatment (Fig. 3.3), and the effect increased with increasing the amendment rate. For the medium and high rates of application, the CO₂ evolution rates peaked on day 4 and declined thereafter. Together with DA, WDG treatment showed the greatest cumulative CO₂ fluxes for the entire experimental period (Fig.3.4), compared to other amendments.

The enhanced evolution of CO₂ from soil treated with TS was of short duration (Fig. 3.3), with the peak of CO₂ production at day 2 and 4 followed by a rapid decline. The highest rate of CO₂ evolution was obtained with the high TS rate followed by medium and low rates. The control treatment remained the lowest over the course of incubation. The total cumulative CO₂ for TS treatment increased with increasing amendment rate (Fig. 3.4). It was significantly ($P < 0.001$) higher than UR treatment and lower than DA and WDG, when averaged across the rate of application (Fig. 3.4).

The impact of G-N on CO₂ evolution rates was only evident with high rate of application (Fig. 3.3). The highest rate of CO₂ evolved from GL-N amended soil was observed at the first two measurement periods and decreased after day 4. Soil treated with high rate of G-N showed the highest rate of CO₂ evolved during the period of incubation whereas medium and low rates were not significantly ($P > 0.05$) different from the control. For the entire period of incubation (10 d), the total cumulative CO₂ for the G-N treatment increased with increasing the amendment rate, and it was not significantly ($P > 0.05$) different from the urea treatment, when averaged across the three rates of application (Fig. 3.4).

Addition of GL with N resulted in higher rates of CO₂ evolution when compared to G-N treatment (Fig. 3.3). This increase in CO₂ emission was particularly evident at the high rate of glycerol application, reaching the peak at day 4 and then levelled off after day 6. The total cumulative CO₂ emission from soil treated with G+N increased with increasing rate of application (Fig. 3.4). It was significantly ($P < 0.001$) higher than G-N and UR treatments, when averaged across the rates of application (Fig. 3.4).

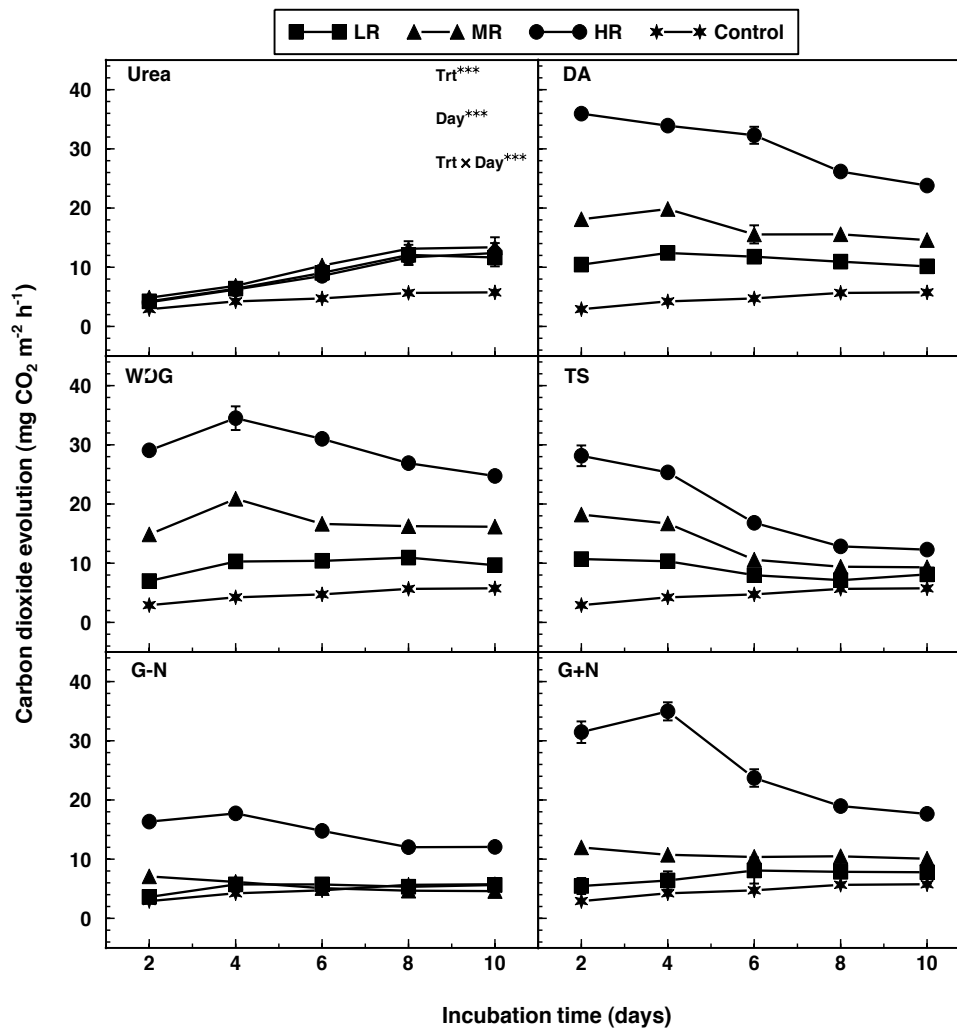


Fig. 3.3. Carbon dioxide emissions from soil amended with 3 rates (low, LR; medium, MR; high, H) of urea (UR), dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without N (G-N) and glycerol with N (G+N). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

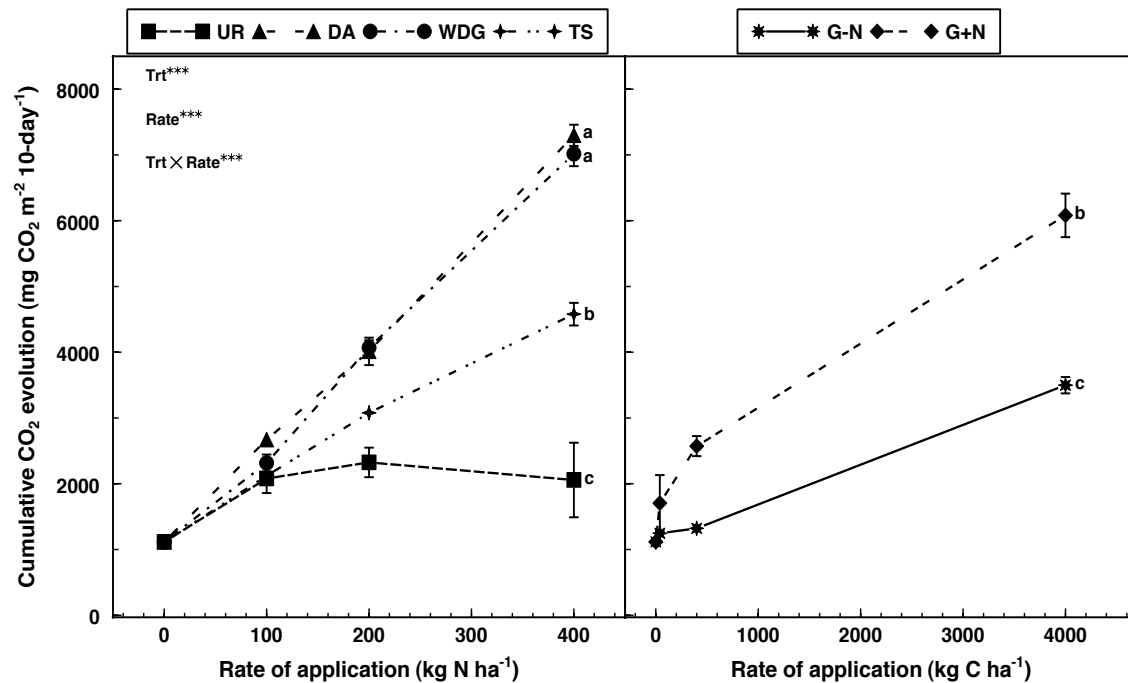


Fig. 3.4. Cumulative CO₂ emissions, calculated for the entire period of incubation, from soil amended with three rates (low, LR; medium, MR; high, H) of urea (UR), dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without N (G-N) and glycerol with N (G+N). Points sharing the same letter among amendment type are not significantly different according to SNK test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note that the statistical analysis was conducted on all amendments regardless of the basis of their application whether it is N or C and the same for means separation for all amendment types. However, after the analysis, glycerol treatments were considered separately to allow for better comparisons among its rate of application.

3.5.3 Soil nutrient availability

3.5.3.1 Soil NO_3^- -N supply rate

Treatment, rate and their interaction had a significant effect on NO_3^- -N supply rate in the amended soil ($P < 0.0001$), and the effect was variable among treatments, depending on amendment type and rate (Fig. 3.5). Urea treatment showed the greatest NO_3^- -N supply that increased with increasing rate of application (Fig. 3.5). It was significantly ($P < 0.01$) greater than all other treatments when averaged across the rates of application. The NO_3^- -N supply in soil amended with DA was very low, compared to UR treatment (Fig. 3.5). The highest supply of NO_3^- -N in soil amended with different rates of DA was obtained at the low rate ($15.1 \mu\text{g NO}_3^-$ -N cm^{-2}) compared to the control ($5.3 \mu\text{g NO}_3^-$ -N cm^{-2}) and declined with the higher rates of application. The DA NO_3^- -N supply was significantly ($P < 0.001$) lower than UR, WDG, G+N and TS treatments and slightly but significantly ($P < 0.001$) higher than the G-N treatment (Fig. 3.5). Both WDG and TS treatments were almost identical in their effect on soil NO_3^- -N availability (Fig. 3.5), which was significantly ($P < 0.001$) lower than the UR treatment and G+N, but higher than the DA and G-N treatments. Glycerol applied alone at any rate showed the lowest supply of NO_3^- -N (Fig. 3.5). However, when supplemented with N, GL addition at the low rate showed enhanced NO_3^- -N availability, compared to the other rates of GL application (Fig. 3.5). Increasing GL application rates reduced NO_3^- -N supply. After a 10 d incubation, the treatments in order of descending NO_3^- -N supply were urea > G+N > WDG \geq TS > DA > G-N.

3.5.3.2 Soil NH_4^+ -N supply rate

Ammonium-N supply rate was significantly affected by amendment type, rate and their interaction ($P < 0.001$). The NH_4^+ -N supply was the highest in soil amended with GL applied at the low rate combined with 300 kg N ha^{-1} as urea (Fig. 3.5). When averaged across the three rates of application, NH_4^+ -N supply of the G+N treatment was significantly higher than all the other treatments. The TS by-product application increased soil NH_4^+ -N availability (Fig. 3.5), and the effect was increased with increasing rate in the TS. The NH_4^+ -N supply rate in the TS treatment was significantly ($P < 0.001$) higher than WDG, UR, DA and G-N treatments, but significantly ($P < 0.05$) lower than the G+N treatment, when averaged across the three rates of

application (Fig. 3.5). Application of WDG, UR, and DA amendments at low and medium rates had a relatively similar effect on NH_4^+ -N availability (Fig. 3.5); however, at the high rate, WDG and UR amendments had higher NH_4^+ -N supply and were different whereas the DA amendment remained low even at the high rate of application and did not significantly ($P > 0.05$) differ from G-N treatment. Both DA and G-N treatments had the lowest supply rate of NH_4^+ -N, and they were not significantly different from each other. After a 10 d incubation, the treatments in order of descending NH_4^+ -N availability were $\text{G+N} > \text{TS} > \text{WDG} \geq \text{UR} > \text{DA} \geq \text{G-N}$.

3.5.3.3 Soil PO_4^- -P supply rate

The PO_4 -P supply rate in the incubated soil over the 10 d of incubation was significantly affected by treatment ($P < 0.0001$) and rate of application ($P < 0.01$). Addition of WDG by-product resulted in the greatest PO_4 -P supply, especially when applied at medium and high rate (Fig. 3.5). When averaged across the three rates of application, this treatment resulted in significantly ($P < 0.01$) higher PO_4 -P release than the other treatments (DA, G-N, UR and G+N) with the exception of the TS treatment, which did not significantly ($P < 0.05$) differ from the WDG treatment. The TS treatment increased PO_4 -P supply, showing higher PO_4 -P availability, compared to UR, G-N and G+N treatments (Fig. 3.5). Addition of DA at any rate provided higher PO_4 -P availability especially at the low rate, compared to control whereas urea application showed a negative impact on PO_4 -P supply in soil (Fig. 3.5). Similarly, GL addition with or without N reduced PO_4 -P availability. In presence of N, GL addition resulted in lowest supply of PO_4 -P in soil (Fig. 3.5). After a 10 d incubation, the treatments in order of decreasing PO_4 -P availability were $\text{WDG} \geq \text{TS} \geq \text{DA} > \text{G-N} \geq \text{UR} > \text{G+N}$.

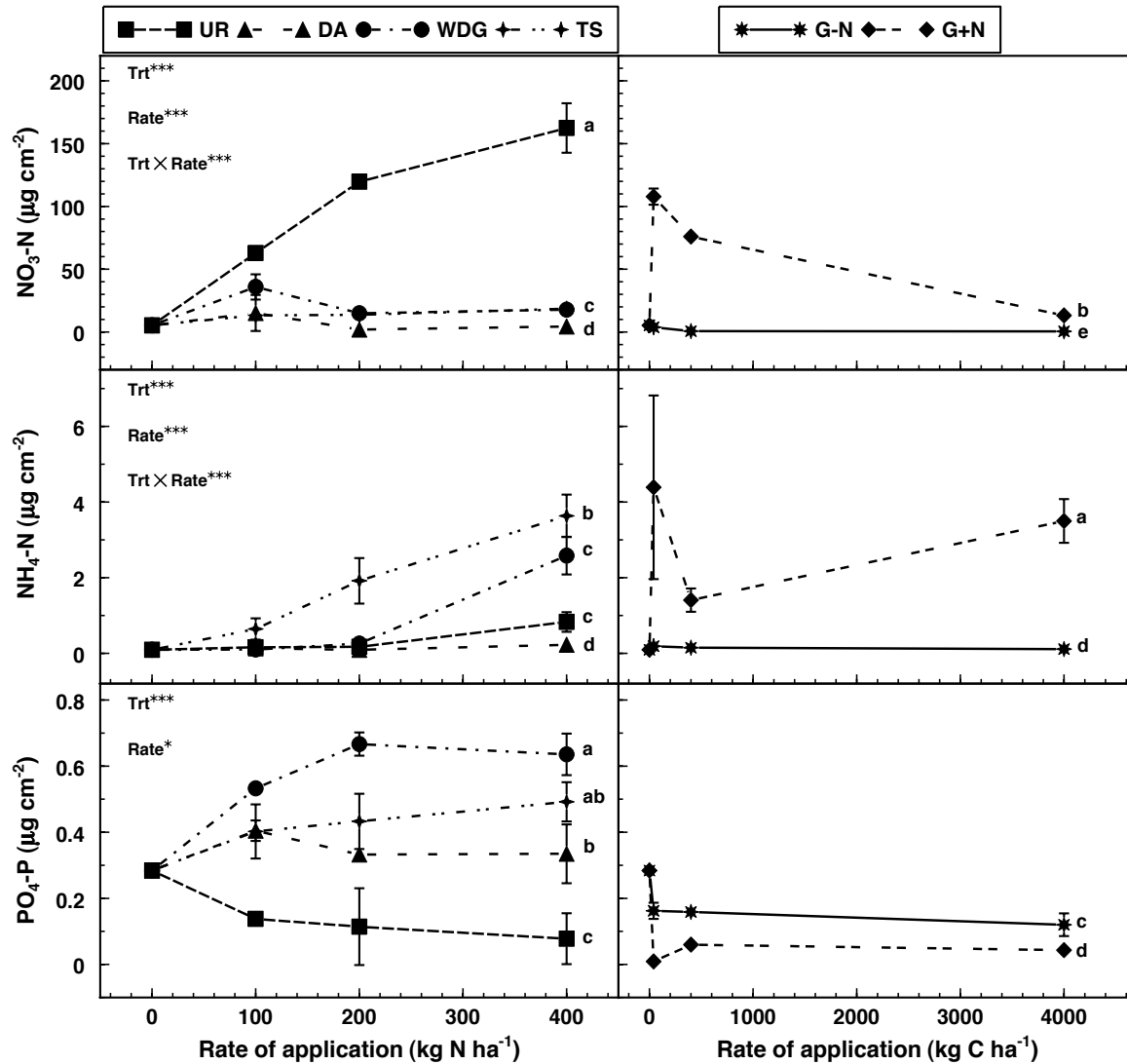


Fig. 3.5. Nutrient supply rates ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$) as measured by Plant Root Simulator (PRSTM) resin membrane probes for a 10-day incubation in soil amended with three rates (low, LR; medium, MR; high, H) of urea (UR), dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without N (G-N) and glycerol with N (G+N). Points sharing the same letter among amendment type are not significantly different according to SNK test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note that the statistical analysis was conducted on all amendments regardless of the basis of their application whether it is N or C and the same for means separation for all amendment types. However, after the analysis, glycerol treatments were considered separately to allow for better comparisons among its rate of application.

3.6 Discussion

3.6.1 Effect on N₂O evolution

Nitrous oxide (N₂O) is produced by nitrification and denitrification and can contribute to global warming (Granli and Bøckman, 1994). Both nitrification and denitrification depend on the availability of inorganic N, soil temperature and factors that affect the redox potential of the soil such as texture, moisture and organic C content (Granli and Bøckman, 1994; Skiba and Smith 2000). Organic materials, such as animal manure, crop residues, municipal solid wastes and composts can enhance emissions of CO₂ and N₂O to the atmosphere compared to inorganic fertilizers (Jones et al., 2005; Ding et al., 2007; Johnson et al. 2007). In the present study, addition of bioenergy production by-products, with the exception of GL without N increased N₂O production compared to the unamended control, probably due to stimulation of microbial activity. However, each by-product type showed a different N₂O production response, owing to its chemical composition. With the exception of the WDG treatment, fluxes of N₂O from the amended soil surface generally increased over the first few days of the incubation, followed by a levelling off or decrease. Rates of N₂O production were the highest and sustained over the longest period at the high rate of urea addition, as expected. Of the organic amendments, the WDG and TS produced the highest rates of N₂O per unit of N added, probably due to a greater net release of NH₄⁺-N by mineralization because of a narrow C:N ratio and more easily decomposed organic materials, with higher nitrification rates than other treatments.

In this study, organic materials with relatively higher C:N ratio exhibited low N₂O production, as with DA treatment, or showed a delayed effect on N₂O emission, as with WDG treatment, thus confirming previous findings (Khalil et al., 2002; Flessa and Beese, 1995). The low N₂O emission from soil treated with DA might be attributable to microbial immobilization of inorganic N, affecting ammonium availability and nitrification. This explanation is supported by the extremely low inorganic N (NH₄⁺-N and NO₃⁻-N) availability in DA treated soil measured for the entire period of incubation (Fig. 3.5). In comparison to the DA treatment, the application of WDG and TS led to higher N₂O production, probably due to differences in the decomposition of these two by-products (WDG and TS) and therefore release of NH₄⁺-N (mineralization). However, each of these two by-products showed a different pattern of N₂O fluxes.

Approximately 21% of total N in TS amendment is in $\text{NH}_4^+\text{-N}$ form, and can be an important contributor to microbial growth and activity. In a short incubation study to evaluate the role of $\text{NH}_4^+\text{-N}$ and the concomitant increase in N_2O emission under different conditions of C and N supply, Azam et al. (2002) found increased N_2O loss with the amount of applied $\text{NH}_4^+\text{-N}$, as did the consumption of O_2 and evolution of CO_2 .

Thin stillage slurry resembles, in terms of nutrient ($\text{NH}_4^+\text{-N}$) and dry matter content, liquid swine manure; therefore, it may be appropriate to compare the TS treatment effects to previous findings from liquid animal manure experiments. Velthof et al. (2003) reported a rapid increase in N_2O emission during the first few days of laboratory incubation after liquid pig manure application, and attributed this to increased denitrification of soil $\text{NO}_3^-\text{-N}$ associated with the addition of easily decomposable organic substrates. Similarly, Chadwick et al. (2000) reported a peak in N_2O evolution in the first days after manure addition. The increase in N_2O evolution during the first days after TS application is presumably related to a presence of the large amount of easily degradable C compounds such as organic acids in addition to the amount of water that may help to distribute the C and N better through the soil matrix, leading to higher rates of N_2O loss from partially anaerobic microsites, as observed from soil amended with low molecular weight substrates (Velthof et al., 2003). The decline in N_2O emission in TS treatment at the end of incubation may indicate the depletion of soluble C and N. Moreover, the very low C:N ratio together with the presence of soluble nutrients that can be utilized by soil microorganisms in the TS amendment may have also accelerated the microbial decomposition process.

The higher C:N ratio of WDG amendment could have a significant influence on N availability and thus N_2O production. The relatively slow onset of N_2O production, which remained at the relatively similar level among all the rates of application until day 6, is probably due to an initial immobilization of N, resulting in a low N_2O emission at the beginning of incubation. Velthof et al. (2003) indicated that soil amended with organic materials, such as animal manures with a C:N ratio of higher than 15, may result in an initial microbial immobilization of N. Cayuela et al. (2010) also reported that soil amended with dried distillers' grains with solubles led to microbial immobilization of N during the first stages of decomposition and attributed this to the high content of labile organic matter in this by-product.

As expected, the G-N treatments resulted in very little N_2O produced at any rate of application, due to N immobilization. However, treatments of G+N increased N_2O production, but levels were still low compared to WDG and TS treatments, and closer to flux rates observed for DA, probably due to the addition of N with GL stimulated soil microbial activity, decomposition of soil organic matter, and possibly denitrification. This is in line with other findings which demonstrated that incorporation of crop residues with urea resulted in higher N_2O emission compared to that of crop residues applied alone (Huang et al., 2004). The reduction in N_2O production by increasing glycerol C addition is likely related to C:N ratio increase and greater N immobilization. In comparison to urea treatments applied alone, GL addition at any rate with urea was able to reduce N_2O emission. This demonstrates the ability of GL to conserve soil N through microbial immobilization and thus reduce N_2O production.

The origin of N_2O emissions in the present study is assumed to be related to nitrification since soil samples were incubated at moderate moisture (e.g. near field capacity) and the PVC headspace was maintained aerobic for the entire period of incubation. These conditions are considered to be favorable for nitrifying microorganisms (Skiba and Smith, 2000). However, several findings indicated that denitrification can take place under aerobic conditions. Müller et al. (2004) reported that the NO_3^- -N reduction through denitrification is the predominant mechanism responsible for N_2O production even under aerobic soil conditions in a temperate grassland soil. Thus, despite the favorable conditions for nitrification, organic amendments addition might have promoted microbial growth and activity, with stimulation of microbial respiration and oxygen consumption with creation of temporary anaerobic microsites (Göek and Ottow, 1988; Cannavo et al., 2004; Huang et al., 2004). McKenney et al. (1993) also found that addition of organic amendments resulted in higher denitrification rates in aerobic than in anaerobic conditions. In a field study, Meijide et al. (2007) reported that nitrification was the main process responsible for N_2O emissions from plots treated with urea whereas N_2O emissions from organic fertilizer treatments were caused by denitrification.

3.6.2 Effect on CO_2 evolution

Addition of substrates rich in C can affect CO_2 emission from soil, which is governed by the substrate quality and C availability (Schimel and Holland, 2005). In the present study, all amendments significantly enhanced CO_2 emission, but their impact varied considerably

according to amendment type and rate of application. The microbial breakdown of organic materials added to soils largely depends on the rate of decomposition of each of the C-containing materials (Reddy et al., 1980; Ajwa and Tabatabai, 1994) and is also influenced by the content of N, S, soluble C, lignin and carbohydrates (Herman et al., 1977; Reinertsen et al., 1984; Janzen and Kucey, 1988). Carbon dioxide produced by the urea treated soils may be derived from enzymatic (urease) hydrolysis of the urea to CO₂ and ammonia, and stimulation of heterotrophic microbial activity (Serrano-Silva et al., 2011). However, higher fluxes of CO₂ were observed from the N containing amendment treatments than urea at equivalent rates of added N, probably due to the effect of the addition of substrate C for microbial decomposition along with N in the amendment treatments. Similar findings were observed by Muhammad et al. (2011).

Generally, fluxes of CO₂ were greatest for the amendments at day 2 and 4 and then declined, presumably related to microbial consumption of easily degradable C fractions present in the amendment. Similarly, in a 30-d incubation, Ajwa and Tabatabai (1994) reported that more than 50% of the total CO₂ evolved from soils treated with crop residues occurred in the first 6 days of the incubation and related the greater increase in CO₂ evolution that initially occurred for some organic residues to their higher content of decomposable organic C. The decrease in CO₂ evolution from the soil with time was greater in the TS treatment compared to other amendments, probably due to the higher contents of available organic C in the TS. Jarecki et al. (2008) also found that addition of swine manure slurry to soil resulted in a peak of CO₂ emissions after 4 days of slurry application, followed by a rapid decline.

The DA and WDG resulted in the greatest CO₂ evolution per unit of N added, owing to a higher C content relative to N than TS or UR. The CO₂ flux from G-N was low, especially at medium and high rates, compared to other amendments, due to lack of N restricting microbial respiration and decomposition of substrate C. When N fertilizer was added, CO₂ evolution rates were significantly increased, as the N fertilizer supplied the N needed for microbial growth. This is in agreement with previous findings that mineral N availability had a positive impact on increased microbial decomposition of organic residues (Recous et al. 1995; Sakala et al., 2000).

3.6.3 Effect on soil nutrient availability

Organic amendments such as animal manures, composted materials from municipal, industrial and agricultural operations, meat, blood and bone meal, and paper mill biosolids (e.g.

Edmeades, 2003; Schoenau and Davis, 2006; Hargreaves et al., 2008; Diacono and Montemurro, 2010; Quilty and Cattle, 2011) have shown to increase contents of plant available nutrients in soil. A few studies have recently assessed the impact of bioenergy by-products addition on crop yield, N mineralization and C and N dynamics (Nelson et al., 2009; Cayuela et al., 2010; Moore et al., 2010; Qian et al., 2011). Incubation of soil treated with different bioenergy by-products under controlled environment conditions for a period of 30 d led to an increase in N and P content (Galvez et al., 2011).

Ammonium supply rates were generally low for the low and medium rate urea treatments, indicating that NH_4^+ -N formed from urea hydrolysis was rapidly converted to nitrate through nitrification. Nitrification is likely the dominant mechanism and source of N_2O in UR treatments.

High NH_4^+ -N supply rates were observed sometimes at high rates of some amendments like WDG and TS, probably due to their content of NH_4^+ -N. Indeed, 21% of the total N content was present as NH_4^+ -N in the TS whereas NH_4^+ -N was formed by decomposition or hydrolysis of other amendments like DA and UR. Similarly, addition of DDG and mustard meal by-products to soils increased NH_4^+ -N concentrations initially through ammonification, and these by-products maintained higher NH_4^+ -N concentrations for a longer period of incubation, than other organic amendments, indicating some nitrification inhibition associated with these by-products (Moore et al., 2010). Therefore, the supply rate of NO_3^- -N over the 10-day incubation was limited in the WDG and DA treated soils. The release of available N from these amendments may also have been limited by microbial immobilization, due to the C:N ratio that was higher in DA than WDG. This also corresponded with low N_2O emission rates from the DA amendment. More available N, and N_2O , would likely be released in following weeks as microbial decomposition of the amendments proceeded, converting C to CO_2 and therefore narrowing C:N value suitable to microbial N mineralization (Moore et al., 2010). This is in line with findings by Galvez et al. (2011) in which alkaline soils amended with different types of bioenergy by-products, including bioethanol residues from wheat starch and rapeseed meal from biodiesel production, showed that NO_3^- -N concentrations increased with time during a 30-d incubation.

The GL amendment alone immobilized nearly all soil available N as shown by the low supply rates of NO_3^- -N and NH_4^+ -N and this N immobilization was decreased by adding urea

with GL. This suggests the significant role that GL can play in preserving fertilizer N through microbial immobilization, and thereby its positive effect on N₂O emissions reduction as previously discussed.

As expected, UR addition reduced soil phosphate supply rates over the 10-d incubation, likely due to microbial utilization/immobilization of soil P as a result of the stimulated microbial growth by the addition of N. This is particularly evident in the GL treated soil, in which GL promoted high N immobilization as described above. Of the organic amendments, the WDG and TS were most effective at increasing soil supply rates of available P. The greatest effect of WDG on available P is consistent with the higher content of total P in this by-product.

3.7 Conclusion

The impact of adding bioenergy production by-products to soil on increasing N₂O and CO₂ emissions and influencing nutrient availability was evident in this study. However, their effect on N₂O emissions was less than that of urea fertilizer, when applied at the same N rate. This mitigates some environmental concerns associated with their utilization as soil amendments. The bioenergy by-products were not recalcitrant to microbial decomposition, showing a decomposition rate similar to, or somewhat less than, that of the DA reference amendment. This supports their potential value as slow release fertilizers that can increase soil N and P availability. As expected, the by-product of GL cannot be used as a source of nutrient directly due to its lack of N, but its application indicates that this by-product could have a significant influence on conserving inorganic N under high loss potential environmental conditions via stimulating microbial immobilization process.

4. ENZYMATIC ACTIVITY AND MICROBIAL BIOMASS IN SOIL AMENDED WITH BIOENERGY BY-PRODUCTS

4.1 Preface

Given the importance of biological indicators in evaluating changes in soil quality when introducing new management practices, enzyme activity and microbial biomass in soil treated with bioenergy production by-products (BPB) were investigated and are reported on in this chapter. In Chapter 3, the effects of amendment on cycling of nutrients from BPB into greenhouse gases and plant available ionic forms under controlled conditions was covered. Enzyme activity and microbial biomass parameters have frequently been used as sensitive indicators of the general ability of the soil to carry out nutrient turnover and of overall soil quality and health. Therefore, the main objective of this study was to investigate the direct effects of BPB application on selected enzyme (alkaline phosphatase, protease, dehydrogenase) activity and microbial biomass C and N content over a short period of incubation. The data presented in this chapter is obtained from the same experimental set reported in Chapter 3.

This chapter has been published as: Alotaibi, K.D. and J.J. Schoenau. 2011. Enzymatic activity and microbial biomass in soil amended with bioenergy production byproducts. *Appl. Soil Ecol.* 48: 227-235. The contribution of the co-author, J.J. Schoenau, is greatly appreciated, and included providing suggestions and guidelines during the entire course of this study in addition to manuscript editing.

4.2 Abstract

This study examined the effect of addition of by-products generated from the bioenergy industry on soil quality as measured by response of soil enzyme activity, microbial biomass C (MBC) and N (MBN) content and microbial quotient (MQ) to the addition of these materials. By-products of ethanol manufacture utilized included: wet distillers' grains (WDG) and thin stillage (TS) that were applied at three rates of N (100, 200, and 400 kg N ha⁻¹), whereas glycerol (GL), a by-product of biodiesel production, was applied at three rates of C (40, 400, 4000 kg C ha⁻¹) alone (G-N) or combined with 300 kg N ha⁻¹ as urea (G+N). Urea and dehydrated alfalfa (DA) were applied at the same N rates as WDG and TS, as reference amendments. With the exception of TS and G-N, alkaline phosphatase activity was significantly enhanced by WDG, G+N, urea and DA addition, especially with low and medium rates. All amendments significantly increased dehydrogenase activity, but the rate effect was variable among all treatments. Protease activity was also enhanced by all amendment additions, with TS being less effective than others. All amendments with exception of TS significantly increased MBC, MBN and MQ. TS had no effect on these parameters, which is attributed to less organic carbon added relative to nitrogen in this amendment. Overall, addition of bioenergy processing by-products to soil stimulated microbial growth and enzyme activity; supporting their overall use and value as soil amendments to enhance soil biological activity and recycle plant nutrients.

4.3 Introduction

Increased demand for renewable sources of energy has driven the need to consider bioenergy as an alternative to fossil fuel. Ethanol production from cereal grain (mainly corn) involves conversion of starch to ethanol through fermentation followed by distillation. The by-products of these processes are wet distillers' grains (WDG) comprised of coarse grain particles and thin stillage (TS) containing yeast cells, soluble nutrients and very small grain particles (Bonnardeaux, 2007). Due to their nutritional value of relatively high protein, phosphorus concentration and other minerals, distillers' grains (DG) have commonly been used as animal feed (Ham et al., 1994; Bonnardeaux, 2006; Harris et al., 2008). However, expanded

ethanol production may result in a surplus of DG (Rausch and Belyea, 2006); therefore, alternative approaches of their utilization are needed, including consideration of land application.

The manufacture of biodiesel from oilseed like canola is accompanied by a primary by-product of glycerol (GL), produced via transesterification of oils from plants (The Glycerol Challenge, 2007). One tonne of biodiesel produced is associated with 100 kg of GL as a by-product (The Glycerol Challenge, 2007). Traditional uses of GL include food additive, industrial chemical and pharmaceutical preparations, as well as new uses such as addition to animal feeds (Groesbeck et al., 2008). As GL is a carbon-rich substrate, it may have utility as a soil amendment; however, there is limited information on effects of GL when land applied as a carbon containing soil amendment.

The bioenergy production by-products discussed above can be deemed somewhat similar to other organic amendments such as animal manures, paper mill biosolids, sewage sludge, compost and crop residues; especially in terms of their low content of essential plant nutrients compared to commercial inorganic fertilizer. Many studies have investigated the effect of such traditional amendments on nutrient availability (Lupwayi et al., 2005; Schoenau and Davis, 2006) and soil enzyme activity (Mandal et al., 2007; Fernández et al., 2009). Enzymes are considered to be a key soil component catalyzing important transformations related to nutrient turnover. As such their activity in soil can be revealing and they are attractive as one measure of soil health (Dick, 1997). Other microbial indices that have been suggested as soil health indicators are microbial biomass and microbial quotient, which is the proportion of total soil organic C represented by microbial biomass C (Sparling, 1997). The amount of microbial biomass in soils typically reflects total organic matter content (Sparling, 1997) and is an active fraction of soil organic matter. Microbial biomass carbon comprises 1-5% (w/w) of total soil organic C, while microbial nitrogen comprises 1-6% of total soil organic N (Jenkinson and Ladd, 1981; Sparling, 1985; Wardle, 1992).

Microbial biomass C, phosphatase and dehydrogenase activities have all been found to be higher in soils treated with animal manure (Parham et al., 2002; Masto, 2006). Rose et al. (2006) found that application of different composts to soil significantly enhanced enzyme activities. However, Fernández et al. (2009) reported that application of composted or non-composted sewage sludge caused decreases in microbial biomass C and enzyme activities. Soil amended with crop residues (straw and cotton), animal by-products (meat bone meal and blood meal)

produced a significant increase in soil microbial biomass C and enzymatic activities (Cayuela, 2009).

It is clear that addition of organic materials to soil can have variable effects on enzyme activity and microbial biomass. However, information on impact of application of bioenergy production by-products is scarce. As these organic materials are rich in carbon and other nutrients, their application to soil should stimulate microbial activity and therefore contribute to nutrient turnover. Therefore, the objective of this study was to investigate the effect of applying bioenergy by-products at different rates on activity of three selected enzymes (alkaline phosphatase, dehydrogenase and protease), microbial biomass C, microbial biomass N, and microbial quotient in comparison to urea and alfalfa as conventional amendments, in order to understand how these materials may be better utilized on a large scale as alternative soil amendments.

4.4 Materials and Methods

4.4.1 Experimental design

4.4.1.1 Soil and by-products preparation

The soil selected for the incubation study was a field fresh soil collected from the surface layer (0-15 cm) of a cultivated Brown Chernozem (U.S. equivalent: Aridic Haploboroll) in south-central Saskatchewan, Canada. Wet distillers' grains (WDG) and thin stillage (TS) were obtained from a wheat-based ethanol production facility at Lanigan, Saskatchewan. Glycerol (GL), a thick syrupy liquid from canola-based biodiesel production, was obtained from Milligan Biotech at Foam Lake, Saskatchewan. Alfalfa used in this study for comparison was a dried dehydrated (DA) powder obtained from MCN Bioproducts Inc., Saskatoon, Saskatchewan. All by-products were sub-sampled for composition characterization and then stored at 4 °C until use. Selected characteristics of the soil and by-products used in the controlled environment chamber experiment are given in Table 4.1 and Table 4.2 respectively. The amendment treatments of urea, DA, WDG, TS and GL were applied at three rates. Glycerol treatments included an application alone (G-N) and with nitrogen (G+N). The three rates were equivalent to 100, 200 or 400 kg N ha⁻¹ for all amendments with exception of GL; referred to as low, medium and high rate respectively in this study. The three rates of GL (low, medium and high) were 100, 1000 and

10000 kg GL ha⁻¹ respectively, equivalent to 40, 400, and 4000 kg C ha⁻¹ or 20, 200 and 2000 µg C g⁻¹ soil respectively, given a C content of the GL of 40% C by weight. The three rates of glycerol C addition were chosen along a range of rates of other amendments commonly used on the Canadian Prairies such as manures at 10 – 15 kg tonnes ha⁻¹ and mineral fertilizer at 100 kg ha⁻¹. The rates of application were determined based on nitrogen content of amendments, except glycerol in which the rate was selected according to C content since it does not contain N. Each rate of GL was applied alone or combined with 300 kg N ha⁻¹ of urea. The C:N ratio of GL plus urea is 13:1 for 10000 kg GL ha⁻¹ (4000 kg C ha⁻¹) combined with 300 kg urea-N ha⁻¹ treatment. This will ensure that N is not a limiting factor and will thereby promote microbial activity to decompose/utilize GL carbon.

Table 4.1. Selected properties of soil used in the experiment.

Properties	Value
Organic C (mg g ⁻¹)	18.9
Total N (mg g ⁻¹)	1.1
Total P (mg g ⁻¹)	0.5
Extractable P (mg kg ⁻¹)	5.7
Extractable NO ₃ -N (mg kg ⁻¹)	1.7
pH	7.9
EC (mS cm ⁻¹)	0.3

Table 4.2. Selected properties of organic amendments used in the experiment.

Amendments [¶]	OC	Total N	Total P	S	pH	C:N	C:S	MC [#]
	-----mg g ⁻¹ -----							%
DA	432	25	2.2	2.8	ND [§]	17:1	153:1	10
WDG	511	37	4.2	13.7	ND	14:1	36:1	74
TS	449	73 [‡]	13.3	14.6	3.8	6.20	31.10	92.5

¶ DA denotes dehydrated alfalfa; WDG denotes wet distillers' grains; TS denotes thin stillage. All nutrient content rates are on dry weight basis.

§ ND, not determined.

MC, moisture content.

‡ 21% of total N in TS is in NH₄⁺ form on a wet basis.

4.4.1.2 Incubation set-up

Field-moist soil samples were weighed (650 g) and placed in 1-L pots (12.2 cm height × 12 cm diameter). Three rates of urea (0.0864, 0.1728 and 0.3456 g pot⁻¹), DA (1.5773, 3.1564, and

6.3092 g pot⁻¹), WDG (4.36, 8.72 and 17.44 g pot⁻¹), TS (8.512, 17.024 or 34.048 g pot⁻¹) and the GL treatments with or without 263.2 mg of urea (150 µg N g⁻¹ or 300 kg N ha⁻¹) were prepared. A control that received no organic amendment was included. In preparation of the solid amendment treatments, first, 50 g of soil was mixed with the amendment and spread on the soil surface. Then 150 mL of deionized water, which is sufficient to bring soil moisture to field capacity level, was added and then 100 g of soil was placed on top. In case of liquid or slurry amendments (GL, TS), 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. Some of the liquid amendment may have infiltrated below the depth of placement. Solid organic materials were mixed with smaller quantity of soil first to ensure that they are homogeneously incorporated and evenly distributed in the surface soil in the pots. Addition of 100 g of soil to cover amendments was intended to represent a shallow layer of soil covering the amendment that would happen if they were injected or banded in the field as a possible method of their application. Each treatment was replicated four times. All pots containing amended soil were placed on a bench in laboratory and allowed to remain in place for 6 h prior to incubation. Pots containing amended soils were then incubated for a period of 10 d in a growth chamber with electronically controlled environmental settings in which the chamber was set for 16 h at 25 °C (day) and 8 h at 18°C (night). A short-term incubation was chosen because greatest response of biological indicators to addition of organic materials is expected to occur within a few days of incubation following application. Moisture contents in the pots were constantly maintained by measuring weight loss on a daily basis, and deionized water was added when needed. At the end of incubation, soils were removed, air-dried and then coarse sieved (< 2 mm) for preparation to determine enzymatic activity and microbial biomass C and N in each treatment.

4.4.2 Enzyme assays

Alkaline phosphatase activity was determined using p-nitrophenyl phosphatase substrate made in a buffer solution with pH = 11 as described by Alef et al. (1995). Briefly, 1 g of moist soil was treated with 0.25 mL of toluene, 4 mL of modified buffer (pH 11), 1 mL of p-nitrophenyl phosphate made in the same buffer, mixed and incubated for 1 h at 37 °C. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL of 0.5 M NaOH were added, and contents were

mixed and filtered through a filter paper. The absorbance in the filtrate was then measured at 400 nm using a spectrophotometer.

Dehydrogenase activity was determined by the reduction of 2,3,5-triphenylterazolium chloride (TTC) to triphenyl formazan (TPF) as described by Casida et al. (1964) and slightly modified by Serra-Wittling et al. (1995). In particular, 3 g of air-dried soil (< 2 mm) was incubated with 3 mL water and 3 mL TTC at 37 °C for 24 h in darkness. After incubation, 10 mL of methanol was added, and contents were mixed and filtered through a glass fiber filter. Additional methanol was added until the reddish color disappeared from the filter. The filtrate was then diluted with methanol to a 100-mL volume. The intensity of reddish color caused by the reduction of TTC to TPF was then measured using a spectrophotometer at 485 nm.

Protease activity was measured based on a method described by Alef and Nannipieri (1995). The activity was estimated by determination of amino acids released from 1 g of moist and sieved soil sample (< 2 mm) incubated with sodium caseinate (2%) for 2 h at 50 °C using Folin-Ciocalteu reagent. Centrifuged and filtered mixtures were read in a spectrophotometer at 700 nm.

Enzyme assays were conducted in duplicate with one control where the same procedure for enzyme assay was followed but the measurement substrate was added to soil after incubation and the value subtracted from a sample value.

4.4.3 Microbial biomass analyses

The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by fumigation extraction method as described by Voroney et al. (2008). Briefly, two 25 g portions of sieved soil (< 2 mm) that were preincubated at 50% water holding capacity were weighed out. One sample portion (25 g) was fumigated with ethanol-free CHCl_3 for 24 h under vacuum and then extracted with 0.5 M K_2SO_4 (1:2 soil: extractant ratio). The other sample portion was extracted immediately. Total organic C and N from fumigated and non-fumigated (control) soil extract were analyzed using a CN analyzer (TOC-V_{CPH}-TN Shimadzu). The non-fumigated control values were subtracted from fumigated values, and MBC and MBN were calculated using K_{EC} factor of 0.45 for MBC (Wu et al., 1990; Joergensen, 1996) and K_{EC} factor of 0.54 for the MBN (Joergensen and Mueller 1996).

To calculate microbial quotient (MQ), organic carbon (OC) in air-dried soil (< 2 mm) was determined using Leco CR-12 Carbon Analyzer. Then, microbial quotient (MQ) was calculated as MBC/OC (%).

4.4.4 Statistical Analyses

The experiment was set up as a completely randomized design. The treatments were arranged as a complete factorial. It consisted of six amendment treatments with three levels plus a control. Shapiro-Wilk test was used for testing whether data come from a normal distribution. Data corresponded to required assumptions and did not need to be transformed; therefore, statistical analysis was conducted on the raw data. The effects of organic amendments, rate and their interaction on activity of each selected enzyme, MBC, MB N and MQ were carried out using the General Linear Model (GLM) procedure in SAS software, version 9.2 (SAS Institute, Cary, NC). Main and interaction effects of amendment type and application rate were determined after excluding control data from the dataset in order to obtain factorial combination of various factors including 6 types of amendments and 3 rates of application. Means of the control and the three rates of each organic amendment application were separated by Fisher's protected LSD at $P < 0.05$ to test if they are significantly different. The effects were declared statistically significant at $P < 0.05$.

4.5 Results

4.5.1 Enzyme activity

Phosphatase activity was significantly influenced by amendment ($P < 0.001$), rate ($P < 0.05$) and their interaction ($P < 0.001$). The greatest phosphatase activity was obtained with dehydrated alfalfa applied at the low rate and activity of this enzyme decreased with increasing rate (Fig. 4.1). All rates of DA were significantly different from the control. Urea treatments significantly increased phosphatase activity, compared to the control, and similar results were obtained for WDG treatments, although the high rate of WDG was not significantly different from the control (Fig. 4.1). Neither TS nor G-N at any rate differed significantly from the control (Fig.4.1).

Dehydrogenase activity was significantly influenced by amendments ($P < 0.001$), rate ($P < 0.001$) and their interaction ($P < 0.01$). The highest value for dehydrogenase activity was observed when thin stillage was applied, followed by dehydrated alfalfa, WDG, and urea (Fig. 4.2). The activity of dehydrogenase enzyme was higher when GL was applied with N compared to without N (Fig. 4.2).

Amendment ($P < 0.001$), rate ($P < 0.001$) and their interaction ($P < 0.001$) also significantly affected protease activity in the amended soil. Urea, DA and WDG amendments showed a similar pattern in their effect on protease activity, in which the tyrosine value increased with increasing rate (Fig. 4.3). However, the effect of TS was only significant when applied at a low rate (Fig. 4.3). Glycerol addition greatly stimulated protease activity whether in absence or presence of N (Fig. 4.3).

4.5.2 Microbial biomass C

The content of MBC was higher in all amendment treatments compared to the control, with the exception of TS. The DA treatment significantly increased MBC content, with no significant differences observed among the DA rates of application (Fig. 4.4). The MBC content in soil treated with WDG applied at any rate was significantly higher than that of the control, with greatest content of MBC obtained with the high rate of WDG (Fig. 4.4). However, TS had no significant effect on MBC when applied at any rate (Fig. 4.4). The MBC content in soil treated with GL in the absence of N was significantly higher than that of the control, but with no significant differences observed among rates of application. In presence of N, GL addition also enhanced MBC content in soil (Fig. 4.4), with the high rate of GL plus N having the highest MBC.

4.5.3 Microbial biomass N

The content of MBN in soils treated with DA or WDG at different rates increased with increasing rate and was highest in WDG-treated soil at the high rate (Fig. 4.5). Only the medium rate of urea produced significantly higher MBN than the control. Glycerol alone and TS did not have a significant effect (Fig. 4.5).

4.5.4 Microbial quotient

With the exception of TS, the amendments had a significant effect on MQ ($P < 0.001$), whereas rate and interaction effects were not significant ($P > 0.05$). The highest value was observed under WDG applied at the high rate (Fig. 4.6). Regardless of the application rate, all amendments except the TS significantly increased the MQ ratio (Fig. 4.6).

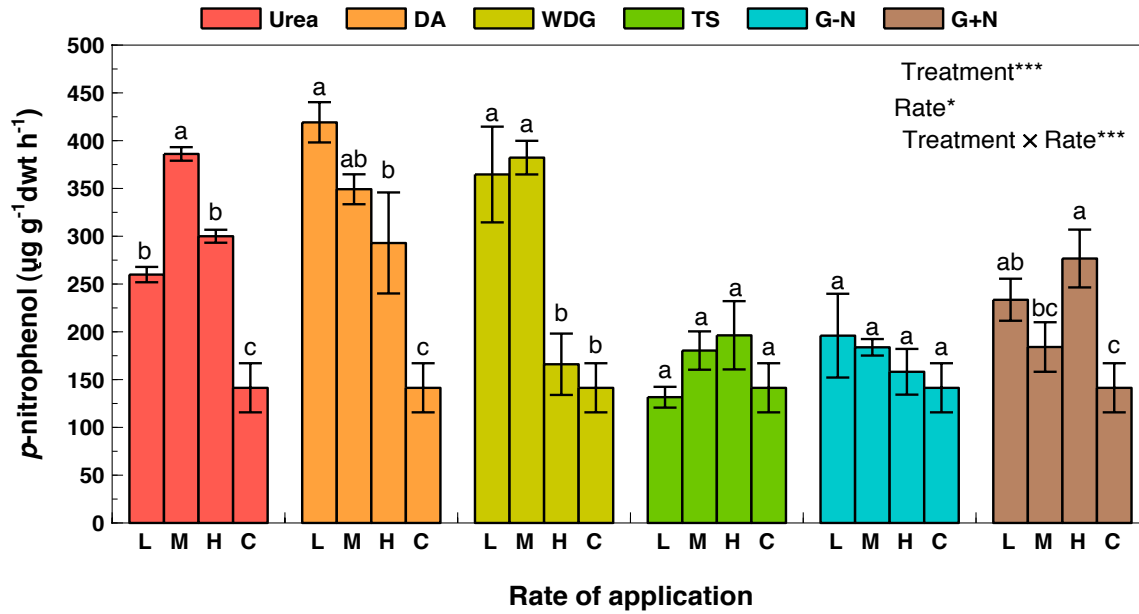


Fig. 4.1. Alkaline phosphatase enzyme activity in soil amended with three rates (low, L; medium, M; high, H; control, C) of urea, dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without nitrogen (G-N) and glycerol with nitrogen (G+N). Bars sharing the same letter within each amendment treatment are not significantly different according to LSD test ($P < 0.05$). Error bars represent standard error of the mean ($n=4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

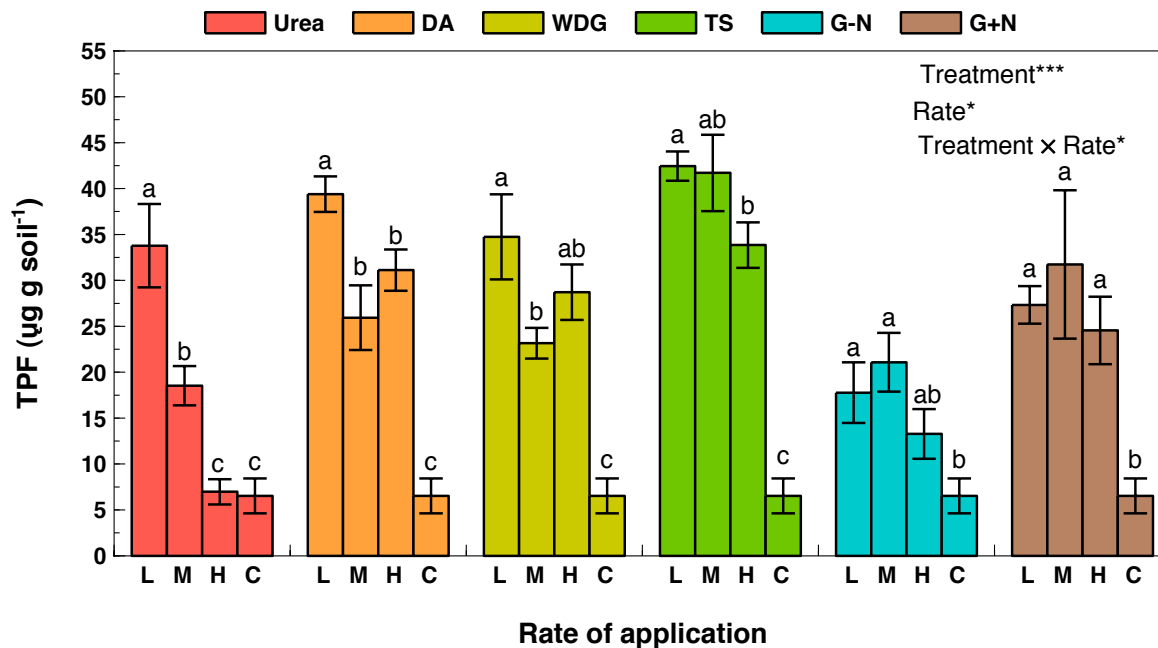


Fig. 4.2. Dehydrogenase enzyme activity in soil amended with three rates (low, L; medium, M; high, H; control, C) of urea, dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without nitrogen (G-N) and glycerol with nitrogen (G+N). Bars sharing the same letter within each amendment treatment are not significantly different according to LSD test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

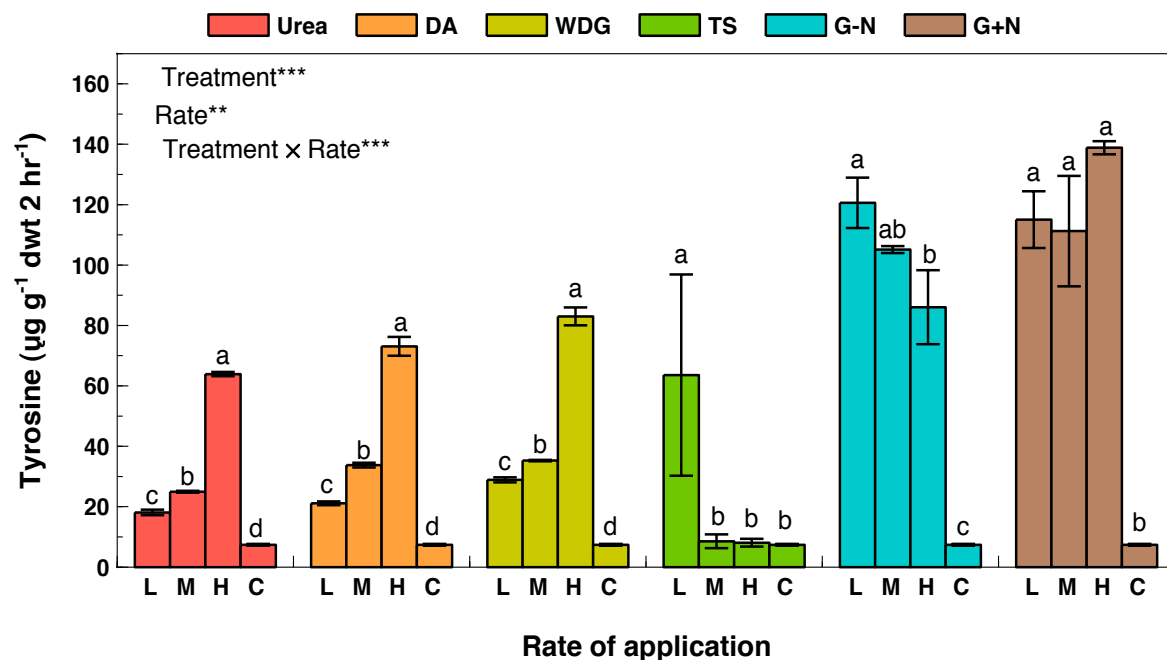


Fig. 4.3. Protease enzyme activity in soil amended with three rates (low, L; medium, M; high, H; control, C) of urea, dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without nitrogen (G-N) and glycerol with nitrogen (G+N). Bars sharing the same letter within each amendment treatment are not significantly different according to LSD test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

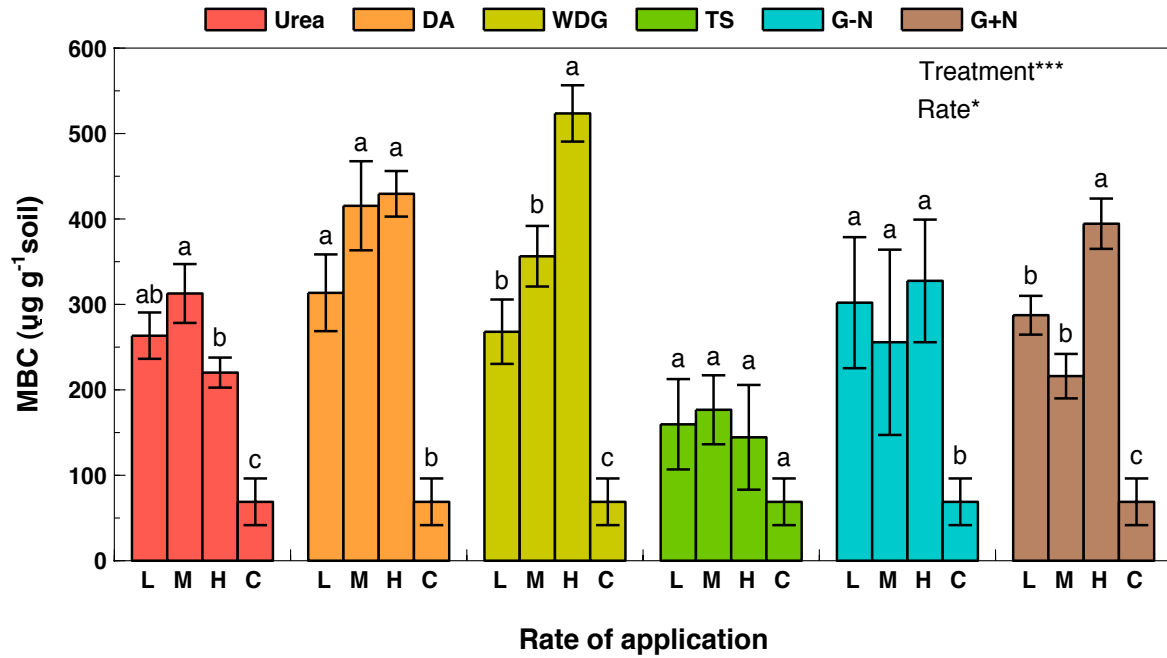


Fig. 4.4. Microbial biomass carbon (MBC) in soil amended with three rates (low, L; medium, M; high, H; control, C) of urea, dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without nitrogen (G-N) and glycerol with nitrogen (G+N). Bars sharing the same letter within each amendment treatment are not significantly different according to LSD test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

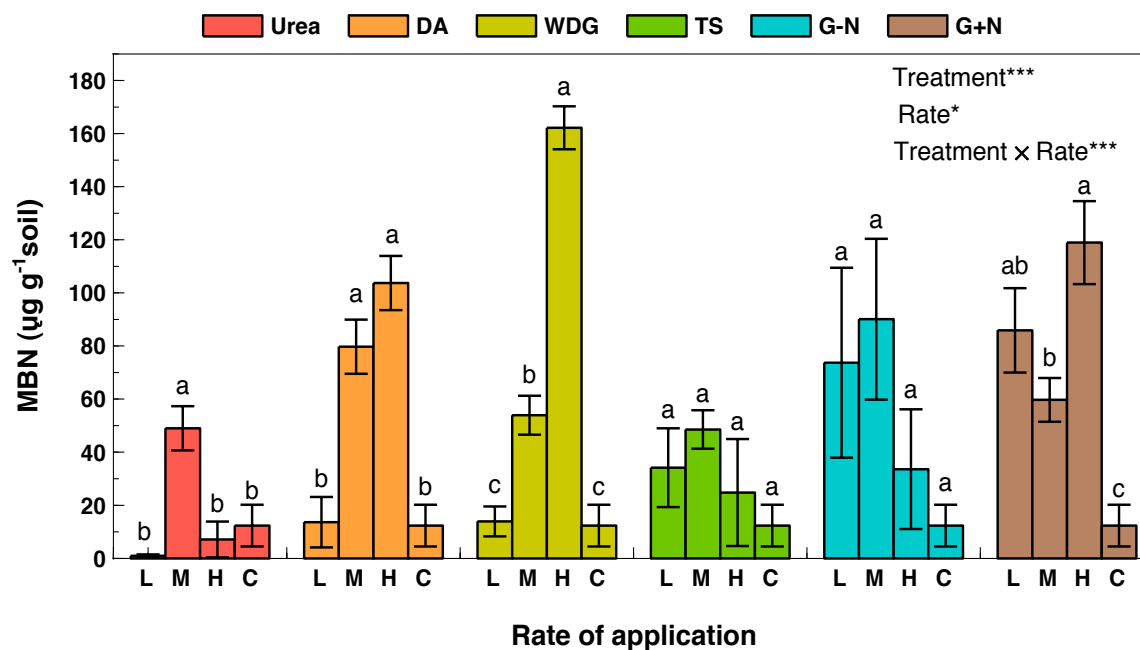


Fig. 4.5. Microbial biomass nitrogen (MBN) in soil amended with three rates (low, L; medium, M; high, H; control, C) of urea, dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without nitrogen (G-N) and glycerol with nitrogen (G+N). Bars sharing the same letter within each amendment treatment are not significantly different according to LSD test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

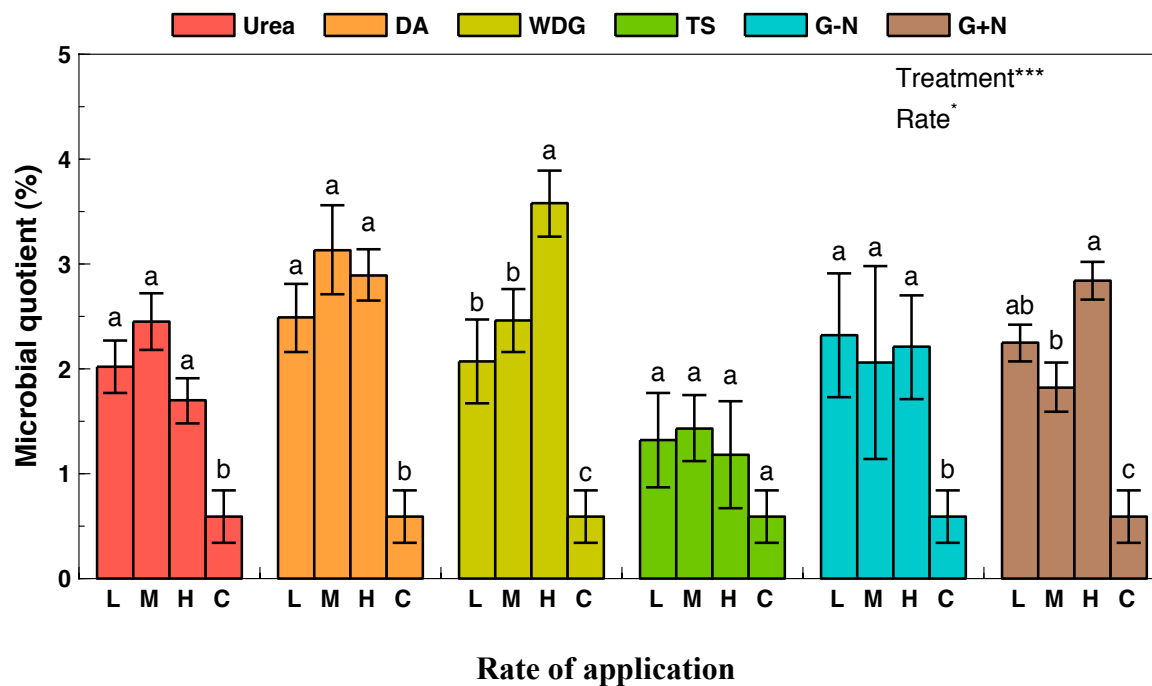


Fig. 4.6. Microbial quotient (MQ) in soil amended with three rates (low, L; medium, M; high, H; control, C) of urea, dehydrated alfalfa (DA), wet distillers' grains (WDG), thin stillage (TS), glycerol without nitrogen (G-N) and glycerol with nitrogen (G+N). Bars sharing the same letter within each amendment treatment are not significantly different according to LSD test ($P < 0.05$). Error bars represent standard error of the mean ($n = 4$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4.6 Discussion

4.6.1 Enzyme activity

Soil enzymes play a vital role in numerous essential functions related to nutrient cycling. The activity of these enzymes is a reflection of microbial activity in soil, and therefore they act as soil quality indicators (Dick and Wang, 2000). Extracellular phosphatase enzyme activity functions in soil P cycling through hydrolysis of organic phosphate compounds (Skujins and Burns, 1976), and is expected to be influenced by P containing amendments (Marianari et al., 2000). With exception of TS and G-N treatments, addition of bioenergy processing by-products stimulated microbial growth and activity as reflected by their significant effect on phosphatase activity. This could be in part due to the relatively high content of organic P, especially in WDG and DA amendments. This is supported by other workers who found that an elevated phosphatase activity reflects presence of organic P and the lack of available P to soil microorganisms (Skujins, 1967; Nannipieri et al., 1979; Bol et al., 2003). Similarly, phosphatase activity was also found to be significantly higher in soil treated with different composts (Rose et al., 2006). Phosphatase activity was not affected by TS addition, and this might be due to the relatively high concentration of available soluble P as has been shown with pig slurry (Plaza et al., 2004) or dairy slurry addition (Bol et al., 2003). In a growth chamber study, Schoenau et al. (2009) revealed that soil amended with TS showed a higher level of available P than that of the control or urea-treated soil. Another possible explanation is that the acidic nature of TS might have contributed to the absence of significant impact on phosphatase activity. Similarly, Bardgett et al. (1995) reported that addition of acidic silage effluent had no consistent effect on both phosphatase and urease activity.

The dehydrogenase enzyme activity in the soil reflects the functioning of microbial redox systems (Trevors, 1984) that are involved in oxidation of soil organic matter (Skujins and Burns, 1976). Therefore, it has been frequently used for assessing management influences on soil quality (Gil-Sotres, 2005). Incorporation of bioenergy by-products into soils will promote microbial growth, explaining the significant increase in dehydrogenase activity we observed. The enhancement of dehydrogenase activity in soil treated with bioenergy by-products was higher or relatively similar to that of DA or urea. Rate of amendment application did have inconsistent

effects on dehydrogenase activity. Our findings are in agreement with other studies that have revealed that soil amended with organic fertilizer, landfill effluents and industrial waste had higher dehydrogenase activity than unamended equivalents (Bardgett et al., 1995; Pascual et al., 1999; Langer and Gunther, 2001).

The protease enzyme hydrolyses N compounds to NH_4^+ using low molecular weight substrates (Tabatabai, 1982; Alef and Nannipieri, 1995). The significant increase in protease activity of bioenergy by-product amended soils is possibly due to stimulation of microbial activity by enhancing resource availability, especially with high rates of addition. The enhancement of protease activity in treated soils could be explained by addition of N containing organic substrates. This is in agreement with another study that showed a significant enhancement of protease activity by sewage sludge and cattle manure compost addition (Rose et al., 2006). However, greatest protease activity was observed with GL treatments and the GL itself does not contain N. The high content of labile low molecular weight carbon in GL would be readily available and utilized by soil microorganisms as a source of energy, thereby stimulating the microbial population to hydrolyze soil organic N to NH_4^+ via protease enzyme. This may be an appropriate interpretation of elevated protease activity shown with GL-amended soil. The limited effect of TS application may be due to the fact that 20% of total N present in this material is in available form of NH_4^+ , which may inhibit protease activity at high rates. This interpretation is supported by other researchers who found limited effect of pig slurry addition on urease activity and attributed this to the large portion of NH_4^+ incorporated with this amendment (Béline et al., 1998; Plaza et al., 2004). Microbial production of enzymes occurs at expense of growth and metabolism if available nutrients are scarce (Koch, 1985; Allison and Vitousek, 2005), and it has been reported that activities of nutrient-releasing enzymes are often negatively correlated with concentrations of available nutrients (Pelletier and Sygush, 1990; Chróst, 1991; Sinsabaugh and Moorhead, 1994).

4.6.2 Microbial biomass carbon

The soil microbial biomass plays a vital role in breakdown of organic materials added to soil, thereby enhancing nutrient cycling and availability to plants (Moore et al., 2000). The soil microbial biomass is the most labile pool of organic matter, and can give an early indication of changes in total soil organic matter content long before changes in total soil C and N can be

reliably detected (Powlson et al, 1987). The increase in MBC content observed in soil amended with bioenergy by-products can be attributed in part to input of readily decomposable nutrient containing substrates, as shown in other studies of soil amended with organic materials such as animal manures, crop residues and silage effluent (Martens et al., 1992; Wardle, 1992; Kandeler and Eder, 1993; Bardgett et al., 1995). Thin stillage addition was less effective in enhancing MBC than other amendments. This effect was consistent with its limited effect on activity of phosphatase and protease enzymes. The DA and WDG treatments had a higher MBC content, and this is most likely related to a wider C:N. Fauci and Dick (1994) reported that soil amended with poultry manure showed less impact on MBC compared to beef manure or pea vine amendments and related this to the narrow C:N ratio of 8.3 of the poultry manure, where C was apparently limiting MBC accumulation in the poultry manure treatment. Lack of response to rate of C substrate addition as GL suggests that microbial growth is not limited by C at the higher rates but may be limited by N.

4.6.3 Microbial biomass nitrogen

In a field experiment, Zaman et al. (2002) reported that soil amended with organic amendments, such as dairy shed effluent exhibited a more significant impact on soil MBN than soil treated with chemical fertilizer (NH_4Cl) and attributed this to C, N, and other energy rich nutrient, such as $\text{PO}_4\text{-P}$, and $\text{SO}_4\text{-S}$ present in the organic amendment. When averaged across the three rates, urea addition was least effective in terms of its impact on MBN. Unlike the medium rate, high rate and low rate application of urea did not show a significant impact on MBN. The high rate of urea-N was actually suppressing microbial activity, resulting in less N tied up in their cells. The WDG, G+N and DA contributed most to soil MBN increase. This is probably due to readily available nutrient substrates and C that would have a significant impact on microbial growth and activity. This is in agreement with other studies of soil amended with different types of organic materials such as sugarcane trash, press mud, mustard oil cake and cow dung (Paul and Solaiman, 2004); straw incorporation (Powlson et al, 1987); and application of dairy shed effluent (Zaman et al., 2002) reporting that soil amended with organic materials had higher MBN content compared to unamended soil.

4.6.4 Microbial quotient

Microbial quotient is a useful measure of soil quality change and soil organic matter state after addition of organic materials, and has been reported to be a better indicator than either MBC or total C (Sparling, 1992; 1997). Jenkinson and Ladd (1981) reported that MBC typically comprises 1-5% of soil organic C. Similarly, in the current study, MQ content was within this range in all treated-soils; with the lowest value in the unamended control and the highest value in the high rate WDG-amended soil. It is believed that the larger the MQ is, the more active and susceptible is the organic matter to change (Sparling, 1992).

4.7 Conclusion

Generally, a significant positive response of microbial parameters measured in this study to addition of the by-products indicates that amendment has a beneficial impact on soil quality as it relates to microbial growth and activity. The results of this study would support the potential use of these organic materials as soil amendments. Long-term controlled environment and field experiments are needed to assess the agronomic value of these materials as organic fertilizers to increase soil nutrient availability and crop growth, particularly as the bioenergy industry expands and availability of its by-products increases.

5. POSSIBLE UTILIZATION OF ASH FROM MEAT & BONE MEAL AND DRIED DISTILLERS' GRAINS GASIFICATION AS A PHOSPHORUS FERTILIZER: CROP GROWTH RESPONSE AND CHANGES IN SOIL CHEMICAL PROPERTIES

5.1 Preface

Ash is a by-product generated during gasification/combustion of organic materials to produce biogas. Unlike the amendments investigated in Chapters 3 and 4, the ash by-product lacks C and N content due to its production conditions, but a high content of phosphorus makes it a good candidate for being a P fertilizer. Ash will primarily affect soil chemical conditions rather than biological properties like the organic BPs evaluated in Chapters 3 and 4. This study evaluated the effectiveness of gasified meat & bone meal ash (MBMA) and dried distillers' grains ash (DDGA) as a phosphorus source for canola in a growth chamber experiment. Changes in selected soil chemical properties were also assessed at the end of the experiment.

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5.2 Abstract

The gasification process is regarded as a viable option for treatment of various types of organic waste. Its application to meat and bone meal (MBM) and dried distillers' grains (DDG) is a new concept that is recently receiving more attention as a possible strategy for safe recycling of these materials to benefit from their energy and nutrient value. This process produces ash that is rich in phosphorus (P) and may be suitable for utilization as a P fertilizer. Therefore, the objective of this study was to evaluate the effectiveness of these specific types of ashes as P fertilizers via evaluating their direct effect on canola growth, P uptake, apparent P recovery and changes in selected soil chemical properties after their addition to a P deficient soil. A growth chamber controlled environment experiment was set up to meet the study objective. The experimental treatments included meat and bone meal ash (MBMA) and dried distillers' grains ash (DDGA) applied at three rates (25, 50 or 100 kg P ha⁻¹) in comparison to a mineral (mono-calcium phosphate) fertilizer (MP) applied at the same rates in addition to non-P treated soil (control). After a growth period of five wk, the DDGA was the most effective ash type and provided biomass yield, P uptake and apparent P recovery better or similar to that of MP, indicating high availability of its P. The MBMA had a limited effect on measured crop variables, suggesting that a significant portion of this ash P is present in insoluble form and is not as readily available for plant uptake. This was also indicated by its lesser effect on enhancing extractable available P remaining in soil after harvest in comparison to MP or DDGA. Application of all ash material caused a slight but significant change in soil content of inorganic N as well as soil pH and EC ; however, this change was more evident with DDGA treatments. Ash derived from gasified DDG was the most effective P fertilizer and was comparable to mineral fertilizer. The results of this study demonstrated that the effectiveness of organic material ash as a P fertilizer is controlled by the type of gasified feedstock. The positive results obtained from this study should stimulate further research on utilization of these ashes as a source of P for different crops in different soil types, especially repeated application under field conditions.

5.3 Introduction

Rapid growth in energy demand coupled with uncertainty of fossil fuels reserves and attempts to mitigate greenhouse gas emissions have stimulated the production of energy from biomass. Conversion of biomass to energy can be achieved via various types of technologies, some of which are thermochemical conversion processes, such as combustion, gasification and pyrolysis. Gasification is a process that can be employed to convert biomass (Balas et al., 2007) and meat production waste (Fedorowicz et al., 2007) to energy. Gasification process is defined as the thermal-chemical decomposition of organic materials under high temperature (800-900°C) and in presence of oxygen (Ferreira et al., 2009). This technology is not a new concept, but its application to certain types of organic materials as feedstocks has not been widely evaluated or adopted. For example, biomass gasification as a means of meat and bone meal (MBM) and dried distillers' grains (DDG) recycling and energy recovery has recently gained interest (Fedorowicz et al., 2007; Tavasoli et al., 2009; Cascarosa et al., 2012;).

The MBM, a by-product of rendering industries, is obtained after eliminating fat from mammal carcasses during the cooking process followed by drying and crushing (Cascarosa et al., 2012). The DDG is the by-product resulting from the conversion of cereal grain starch to ethanol through fermentation and distillation processes. In addition to generation of biogas or syngas, such as CO, N₂, CH₄, CO₂, the gasification of MBM and DDG also produces a valuable by-product of ash. Thus, progressive adaptation of thermochemical treatment of biomass waste has led to a large quantity of ash by-product being generated (Kuligowski and Poulsen, 2009; Pagliari et al., 2010). Therefore, best uses of this ash need to be explored, including its utilization as a phosphorus (P) fertilizer. An attractive option is to recycle P-rich ash to replenish P-depleted soil, especially in the light of the shrinking global phosphate rock reserves and increasing demand for P fertilizer in agricultural production. The ash by-product contains higher concentrations of the P and K than what is originally present in the gasified materials (Kuligowski and Poulsen, 2009), and the ash fraction constitutes only about 1% of the raw waste mass. As such there is a significant reduction in processed waste volume and nutrient is significantly concentrated, especially P and K in ash generated, lowering cost of transport.

However, the fertilizer value of ash may vary depending on feedstock and gasification conditions, tested crop and soil properties. Ashes derived from different organic materials, such as poultry litter, turkey manure and crop residues were found to have a positive effect on crop

yield and soil properties when utilized as P fertilizer source (Codling et al., 2002; Pagliari et al., 2009; Schiemenz and Eichler-Löbermann, 2010). However, evaluation of ashes derived from MBM and DDG as P fertilizer is lacking and deserves attention. The MBMA and DDGA were characterized for their high content of P, and were suggested as a potential phosphate source for agricultural soil (Deydier et al., 2005; Coutand et al., 2008). Since gasification of MBM and DDG is a new concept and the produced ash is rich in P and is suitable for land application, more information is needed to determine the value of these specific ash types as a P source for crop growth. Therefore, the objective of this study was to evaluate canola growth and changes in selected soil chemical properties after the application of different rates MBMA and DDGA in comparison to conventional soluble P fertilizer (mono-calcium phosphate), under controlled environment conditions.

5.4 Materials and Methods

5.4.1 Soils

The soil used for this study was collected from the surface layer (0–20 cm) of a cultivated (cereal-legume-oilseed rotation) Brown Chernozem (U.S. equivalent: Aridic Haploboroll) in south-central Saskatchewan, Canada. This soil type (Brown Chernozem) was selected for this particular study because it was relatively low in its content of plant available P and is typical of the south-central prairie region of Saskatchewan. Bulk soils were collected from the same depth and combined to give a composite soil sample. Then, the composite soil was brought to the soil processing unit at the University of Saskatchewan and mechanically mixed using a stationary mixer to provide a homogenized sample, followed by air-drying and storage at 20°C until its use. Soil was analyzed for its basic characteristics immediately prior to experiment initiation. The soil has organic C content of 19 mg C g⁻¹, pH of 7.2, electrical conductivity of 0.19 dS m⁻¹, NO₃⁻-N of 9.7 mg kg⁻¹, NH₄⁺-N of 6.1 mg kg⁻¹, and available P and K of 7.3 and 450 mg kg⁻¹, respectively.

5.4.2 Ash production and procurement

The MBM ash (MBMA) was produced from gasification of bovine MBM cracklings that was supplied by Saskatoon Processing Ltd., Saskatoon, SK, Canada. The DDG ash (DDGA) was

produced from gasification of DDG that was provided by a wheat-based ethanol production facility near Lanigan, Saskatchewan. First, the MBM and DDG products were ground in the lab. Then, they were gasified at atmospheric pressure in a two-stage fixed bed reactor using a fluidized bed gasification pilot system developed by the Fluidization Laboratory of Saskatchewan (FLASKTM) at the University of Saskatchewan, Department of Chemical Engineering to produce syngas from biomasses and other carbonaceous materials (Campbell et al., 2012). The materials were gasified at a temperature of 650 – 850°C. Details of the gasification process were previously given by Campbell et al. (2012). The MBMA and DDGA were collected and ground to pass through a 600 µm sieve to ensure appropriate homogeneity. The resulting ash was then stored in the lab until its use. Prior to ash application to soil, a representative sample of each ash type was collected and sent to a commercial laboratory (ALS Laboratory Group, Saskatoon, SK) for chemical composition analyses. The commercial laboratory used a standard method that is widely employed to analyze solid waste materials, such as animal manures (Peters et al., 1998). Basic properties of both ashes are given in Table 5.1.

Table 5.1. Basic characteristics of dried distillers’ grains ash (DDGA) and meat and bone meal ash (MBMA). All contents are expressed on a dry weight basis.

Parameter (total)	Ash type	
	DDGA	MBMA
	mg g ⁻¹	
C	8.7	0.9
N	1.4	2.4
P	187	177
K	149	29
S	8	4
Na	75	66
Ca	79	247
Mg	54	11
Cu	0.2	0.1
Fe	6.2	3.6
Mn	1.7	0.1
Zn	1.2	0.7
C:N	6.2	0.4

5.4.3 Treatment application

The experiment was set up to include ten treatments, consisting of three P sources: 1) soluble MP fertilizer as mono-calcium phosphate, 2) MBMA, and 3) DDGA applied at three rates: 0.013, 0.025 and 0.050 g P pot⁻¹. These rates are equivalent to 25, 50 and 100 kg P ha⁻¹, referred to as low (L), medium (M), and high (H) rate, respectively. A control treatment that received no P was included. Each treatment received 200 kg N ha⁻¹ as urea, including the control, to supply adequate amount of N to ensure that N was not a limiting factor for plant growth. Moreover, as soil used in this experiment shows deficiency in S, and canola crop requirement of S is high (Malhi et al., 2005), each treatment was supplied with a basal application of 40 kg S ha⁻¹ as K₂SO₄ solution, including the control. Each treatment was replicated four times in a completely randomized design.

In the experimental set-up, 800 g sample of homogenized field-moist soil was weighed and placed into each 1-L cylindrical plastic pot of 12.2 cm height × 12 cm diameter (tapered). P fertilizers (MP, MBMA, DDGA) were mixed with 50 g of soil, followed by addition of urea to this amount of soil, and the mixture was spread on the surface of the soils already weighed into each pot. Potassium sulfate (K₂SO₄) solution (1.25% S) was then added to soil in an amount that provided 20 µg S g⁻¹, equivalent to 40 kg S ha⁻¹. Then 100 g of soil was added on top to cover all amendments. Pots containing amended soils were equilibrated for 24 h, after which 10 seeds of Argentine canola (*Brassica napus* L.L. 5030) were sown in each pot, followed by addition of 50 g soil to each pot to cover the soil and to bring the total soil weight to 1000 g. Deionized water was added to bring soil moisture to 80% of field capacity. This moisture content was maintained for the entire duration of study by daily watering. The pots were then moved to the growth chamber that was set at 22°C day and 13°C night, with an 18-h d length and 6-h night length. Moisture content was checked on a daily basis by weight loss, and water was added if necessary. After plant emergence, seedlings were thinned to keep the three most robust plants per pot.

After five week of growth, the total aboveground biomass was harvested, dried at 50°C and weighed for dry matter yield determination. Plant samples were then ground and prepared for determination of total P content. Total P was measured by digesting the ground plant samples in sulfuric acid-peroxide (H₂SO₄-H₂O₂) using a temperature-controlled digestion block (Thomas et al., 1967), followed by automated colorimetry for determining P using a Technicon Autoanalyzer

II (Technicon Industrial Systems, 1973). Total P uptake was then calculated from plant P content and total dry matter yield. Apparent P recovery was calculated as follows:

$$\text{Apparent phosphorus recovery} = \frac{TPUTP - TPUC}{\text{Total P applied}} \times 100$$

where TPUTP denotes total P uptake for a given treatment pot, TPUC is the total P uptake in control pot and total P applied is the amount of P applied for a given rate of P application.

After canola plant harvest, soils were removed, air-dried and ground to pass a 2-mm sieve prior to laboratory analysis. The air-dried and sieved soil samples were then analyzed for available P, K, inorganic N (NH_4^+ -N and NO_3^- -N), organic C, pH and electrical conductivity (EC). Soil pH was measured at the end of the growth period in the Department of Soil Science laboratory, and by ALS Labs Saskatoon for the initial soil characterization. Available P and K were determined by a modified Kelowna method (Qian et al., 1994). Exchangeable NH_4^+ -N and NO_3^- -N were extracted by shaking 5 g of soil with 50 mL of 2 M KCl for 1 h on rotary shaker, followed by filtration. The NH_4^+ -N and NO_3^- -N content in the KCl extracts were measured colorimetrically using a Technicon Autoanalyzer II (Keeney and Nelson, 1982). The organic C content was determined using a LECO CR-12 combustion carbon analyzer (LECO Corporation, St. Joseph, MI) set at 840°C (Wang and Anderson, 1998). The pH and electrical conductivity were measured in 1:1 soil:water suspension.

5.4.4 Statistical analyses

The experiment was a completely randomized design with a complete factorial arrangement. It consisted of three amendment types (factor) with three rates of addition along with a control. Before conducting the statistical analysis, the data was subjected to Shapiro-Wilk test for data normality check. This test revealed that the response variables of soil NO_3^- -N, available P, pH and EC exhibited lack of normality, and this was addressed by log-transformation. The effects of P sources (MP, MBMA and DDGA), rate (control, L, M and H) and their interaction on soil and crop variables were conducted using two-way ANOVA procedure. Treatment effects were considered significant at a probability level of $P < 0.1$, and the means were separated at $P \leq 0.05$. Treatment means separation was conducted using

Student-Newman-Keuls (SNK) test. Statistical analysis was carried out using the statistical software R (version 2.13.0; R Development Core Team, 2011).

5.5 Results

5.5.1 Ash characteristics

Both of the ash types exhibited low content of total C and total N, with DDGA showing slightly higher content of C (see Table 5.1). The total N content in MBMA was approximately two times higher than that in DDGA. The dominant plant macronutrient in DDGA was P (187 mg g⁻¹), followed by K (149 mg g⁻¹), Ca (79 mg g⁻¹), Mg (54 mg g⁻¹) and S (8 mg g⁻¹) was the lowest. However, the dominant nutrient in MBMA was Ca (247 mg g⁻¹), which was about three times higher than that in DDGA, followed by P (177 mg g⁻¹), K (29 mg g⁻¹), Mg (11 mg g⁻¹) and S (4 mg g⁻¹) which was the least prevalent nutrient present in the MBMA. The Na content in both ashes was 75 and 66 mg g⁻¹ in DDGA and MBMA, respectively. The DDGA had a higher content of Mn, Zn, Fe and Cu compared to that in MBMA.

5.5.2 Crop response

Phosphorus source and rate of P application had a significant impact on total biomass yield of canola, and there was no significant interaction of P source and rate of P application (Table 5.2). The response of canola crop to fertilization with the ashes was more evident with DDGA, compared to MBMA (Fig. 5.1).

Phosphorus source, rate and their interaction were highly significant in their effect on P uptake (see Table 5.3). The P uptake significantly increased with increasing rate of application of all three amendments (Fig. 5.2). All treatments resulted in significantly higher P uptake than the control. The P uptake with DDGA application was higher than under MBMA application at all application rates; they were similar to those with MP application at the low and medium application rate, but higher at the high application rate (see Fig. 5.2).

The P recovery by the canola plants was significantly influenced by the two factors of P source and rate of application, but there was no significant interaction (see Table 5.3). Overall, the P recovery was similar between MP and DDGA (Fig. 5.3). The canola recovered

significantly less P from the MBMA than the other two sources. For MP and DDGA treatments, P recovery decreased with increasing rate of P application.

Table 5.2. Significance levels of treatment factors of P source, rate and interactions for analysis of crop variables (yield, P uptake and apparent P recovery).

	Source of variation	F value	P value
Yield	P Source	2.79	0.079
	Rate	3.45	0.046
	P Source \times Rate	0.92	0.465
P uptake	P Source	102.72	<0.001
	Rate	125.49	<0.001
	P Source \times Rate	4.91	<0.001
P recovery	P Source	3.96	0.031
	Rate	3.55	0.042
	P Source \times Rate	0.22	0.924

5.5.3 Soil response

The content of available P remaining in the soil after crop harvest was significantly influenced by P source and rate of application, and their interaction was significant (Table 5.3). All P treatments resulted in significantly higher extractable available P than the control (see Table 5.3). The available phosphorus level in soil increased significantly with increasing rate of application for DDGA and MP treatments, but not for MBMA treatment. The DDGA and MP treatments provided the highest content of residual available P, when applied at the high rate. The MBMA effect on residual extractable available P was reduced in comparison to MP or DDGA, and no significant differences were found among rates of application.

The effect of P source on the available K in soil was significant, but the rate factor was not significant (see Table 5.3). The difference in soil K was small among all treatments, and was only significantly higher in soil treated with DDGA applied at the high rate.

Both forms of soil residual inorganic N (NH_4^+ -N, NO_3^- -N) were significantly affected by P fertilizer type and rate, and their interaction was significant (see Table 5.3). The dominant inorganic N form remaining in soil after crop harvest was NH_4^+ -N. The residual soil content of

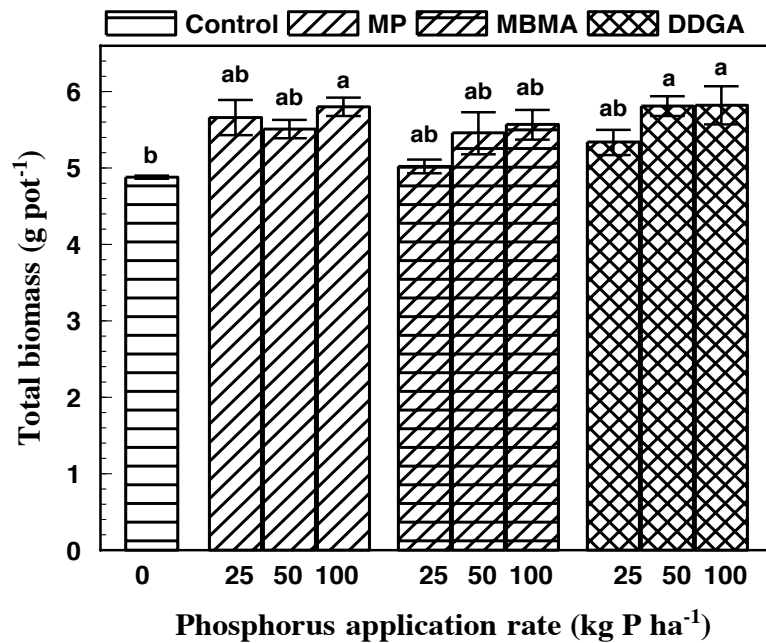


Fig 5.1 Total biomass of canola grown for five weeks in soil amended with mineral P (MP), meat and bone meal ash (MBMA) and dried distillers' grains ash (DDGA), all of which were applied at three rates of P: 25, 50 and 100 kg P ha⁻¹ in addition to a 0-P control. Bars sharing the same letter among treatments are not significantly different according to Student-Newman-Keuls (SNK) test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$).

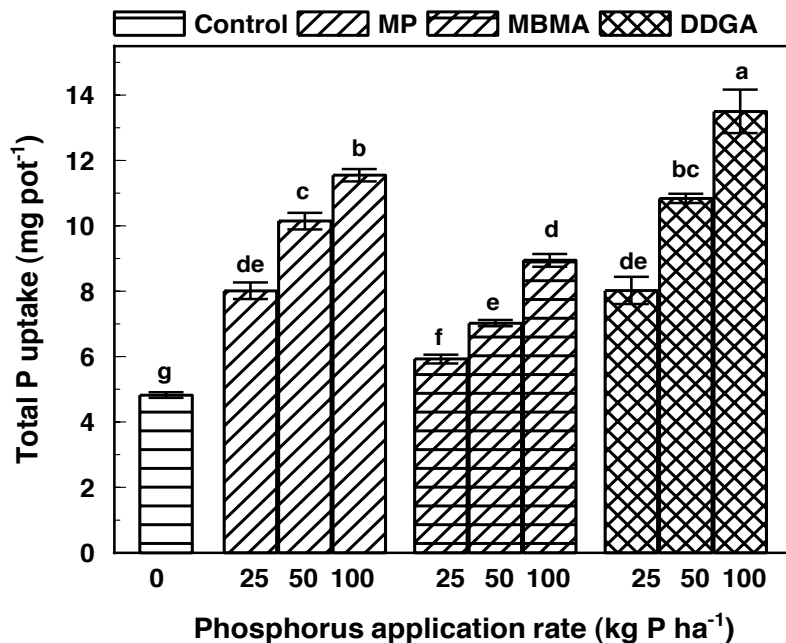


Fig 5.2 Total plant P uptake in soil amended with mineral P (MP), meat and bone meal ash (MBMA) and dried distillers' grains ash (DDGA), all of which were applied at three rates of P: 25, 50 and 100 kg P ha⁻¹ in addition to a 0-P control. Bars sharing the same letter among treatments are not significantly different according to Student-Newman-Keuls (SNK) test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$)

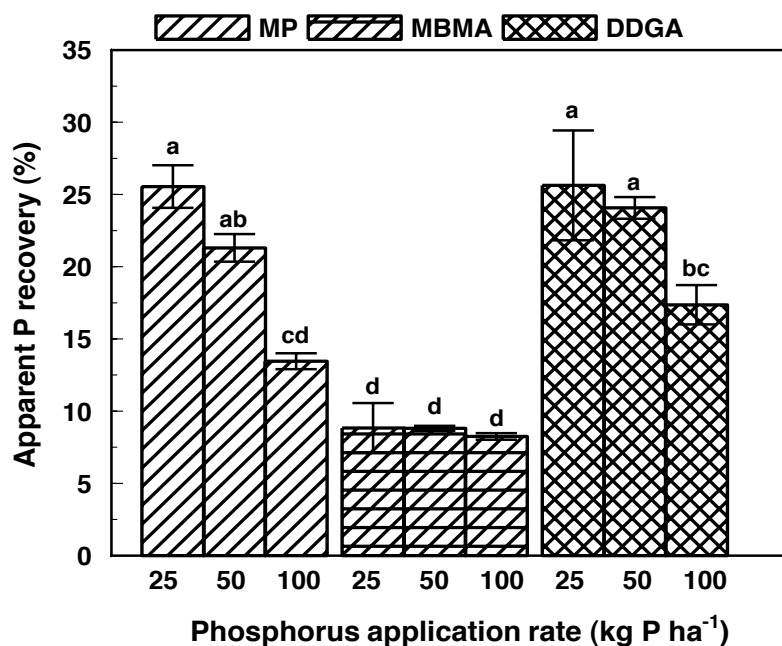


Fig 5.3 Apparent phosphorus recovery by canola plant grown for five weeks in soil amended with mineral P (MP), meat and bone meal ash (MBMA) and dried distillers' grains ash (DDGA), all of which were applied at three rates of P: 25, 50 and 100 kg P ha⁻¹ in addition to a 0-P control. Bars sharing the same letter among treatments are not significantly different according to Student-Newman-Keuls (SNK) test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$)

Table 5.3. Selected soil properties determined after crop removal (mean \pm standard error).

Treatment		P	K	NO ₃ ⁻ -N	NH ₄ ⁺ -N	OC	pH	EC
P Source	Rate	-----mg kg ⁻¹ -----				mg g ⁻¹		dS m ⁻¹
Control	0	7.8 \pm 0.2e [§]	400 \pm 5b	3.2 \pm 0.6bc	6.9 \pm 0.2b	15.7 \pm 0.1	8.40 \pm 0.07a	0.18 \pm 0.00ab
MP	L	9.7 \pm 0.3d	385 \pm 5b	3.4 \pm 0.2bc	6.1 \pm 0.1b	16.2 \pm 0.3	8.10 \pm 0.04b	0.18 \pm 0.00ab
	M	13.4 \pm 0.4c	380 \pm 9b	4.5 \pm 0.3b	7.2 \pm 0.4b	16.3 \pm 0.2	7.93 \pm 0.02c	0.18 \pm 0.00ab
	H	19.2 \pm 0.5b	381 \pm 6b	3.3 \pm 0.2bc	9.1 \pm 0.6b	16.3 \pm 0.3	7.80 \pm 0.01d	0.18 \pm 0.00ab
MBMA	L	9.1 \pm 0.4d	395 \pm 10b	3.1 \pm 0.3bc	9.4 \pm 0.3a	16.6 \pm 0.2	7.74 \pm 0.01d	0.18 \pm 0.01b
	M	10.0 \pm 0.3d	411 \pm 9ab	3.8 \pm 0.3bc	10.0 \pm 0.6a	16.7 \pm 0.2	7.72 \pm 0.01d	0.20 \pm 0.01ab
	H	10.1 \pm 0.4d	390 \pm 4b	2.6 \pm 0.3c	6.9 \pm 0.4b	16.1 \pm 0.5	7.72 \pm 0.01d	0.18 \pm 0.01ab
DDGA	L	10.1 \pm 0.5d	397 \pm 3b	3.5 \pm 0.7bc	6.8 \pm 0.3b	16.5 \pm 0.5	7.64 \pm 0.02e	0.18 \pm 0.00b
	M	12.6 \pm 0.2c	397 \pm 5b	4.3 \pm 0.4bc	8.6 \pm 0.1a	16.6 \pm 0.4	7.60 \pm 0.01e	0.18 \pm 0.00b
	H	22.7 \pm 0.4a	429 \pm 7a	6.4 \pm 0.7a	9.5 \pm 0.4a	16.6 \pm 0.5	7.52 \pm 0.01e	0.21 \pm 0.01a
ANOVA		<i>P</i> value						
P Source		0.0001	0.0004	0.001	0.001	0.6444	0.0001	0.5889
Rate		0.0001	0.408	0.012	0.001	0.7904	0.0001	0.0735
P Source \times Rate		0.0001	0.007	0.001	0.0001	0.8831	0.0001	0.0094

§ Means within a column sharing the same letter are not significantly different at $P \leq 0.05$

NO_3^- -N was all quite low, but was significantly higher in the DDGA treatment when applied at the high rate than all other treatments (see Table 5.3).

The response of soil organic C to treatments application was not significantly affected by P source or rate of application (see Table 5.3). However, phosphorus source, rate and their interaction were significant for soil pH. Addition of P treatments slightly but significantly reduced soil pH (Table 5.3). The greatest reduction in soil pH level was obtained with DDGA treatments. Similarly, there was a slight increase in soil EC, and this change was not significantly affected by P source, but it was affected by rate of P application (see Table 5.3). However, the multiple comparison test showed that none of the treatments were significantly different from the control.

5.6 Discussion

5.6.1 Ash characteristics

The chemical composition differences observed between the two ashes are mainly attributed to the different characteristics of the gasified raw materials (DDG and MBM), as both feedstocks used for gasification were subjected to the same gasification conditions (e.g. temperature, residence time). The DDGA had a greater amount of essential nutrient than reported for most of the biomass ashes reviewed by Tan and Lagerkvist (2011), probably due to the higher nutrient present in the original material of this specific type of DDG. It also showed higher content of P, Mg and K, but less Ca than chicken litter ash, wood ash and sewage sludge ash (Franz et al., 2008; Yusiharni et al., 2007).

The MBMA was also rich in P, but contrary to DDGA, it had much higher concentration of Ca. The MBMA used in this study had relatively similar composition compared to other MBMA reported in other studies (Deydier et al., 2005; Coutand et al., 2008). The Ca and P in MBMA constitute about 42% of the total ash weight. This was anticipated, since a significant part of non-organic fraction of MBM is bone, the mineral portion of which consists of calcium phosphates (Countand et al., 2008). The content of heavy metal in MBMA was consistent with other types of MBMA characterized in previous studies (Deydier et al., 2005; Countand et al., 2008), and less than in many other ash types, such as sewage sludge ash (Franz, 2008) and municipal solid waste ash (Rosen et al., 1994; Ferreira et al., 2003) which may not be suitable for

land application due to their high content of heavy metal. Both ashes used in the present study had low heavy metal content, and were similar or less than that found in natural rock phosphate or soil (Countand et al., 2008).

5.6.2 Crop response

Crop response to addition of DDGA was comparable to the MP treatment, with the MBMA being less effective, which may be attributed to reduced P availability as a result of low solubility of calcium phosphate present in the ash or formed in soil. The effect of P fertilization on crop biomass yield was found to be less pronounced and lower than for crop P uptake. Similarly, Schiemenz and Eichler-Löbermann (2010) deemed that ashes derived from rape meal, straw and cereal were adequate P sources comparable to highly soluble commercial P fertilizer, as demonstrated by their significant impact on crop yield and P uptake. In a two-yr field study in Minnesota, Pagliari et al. (2009) reported that alfalfa yield was similar in turkey manure ash amendment to that of mineral P fertilizer and both were higher than that of the control, when both P sources were applied at the same rate of P. These authors also found that P uptake was significantly increased with ash application. It was also noted under greenhouse conditions that poultry litter ash was as effective for wheat as mineral P (potassium phosphate) and provided greater wheat tissue P concentrations (Codling et al., 2002). In contrast, other studies found that P uptake by corn, alfalfa and Swiss chard was greater in fertilizer treatments compared to ashes derived from wood and municipal waste incineration (Erich and Ohno, 1992; Rosen et al., 1994). These inconsistent effects of ash on crop yield and P uptake may be explained by differences in availability of the P that depends on ash type and composition. This explains the difference in response of crop variables to both ashes used in the current study. The DDGA was a more effective source of P than that of MBMA, as shown in crop P uptake and apparent P recovery. This can be explained by the chemical composition of MBMA, which contains high amount of Ca and that can react with P to form Ca-P compounds both in the ash and as reaction products in the soil. Calcium phosphate in the form of apatite, a primary mineral P (Tiessen et al., 1984), is generally considered to have low solubility and therefore low availability for plant uptake (McKenzie et al., 1992). Therefore, the precipitation of poorly soluble Ca phosphates, mainly hydroxyapatite (Lindsay et al., 1989), could contribute considerably to the lack of recovery of applied P with MBMA, as seen in the present study. Identification of the specific P minerals

present in the ash and in the soil following application is needed. The decreased P recovery with increasing the rate of P application observed in this study in the MP and DDGA treatments was also observed by other researchers (e.g. Eghball and Sander, 1989; Miller et al., 2009).

5.6.3 Soil response

The soil content of extractable available P after crop removal, as influenced by P treatments, was consistent with effects on crop P uptake and apparent P recovery. Schiemenz and Eichler-Löbermann (2010) found higher readily plant available P forms in soil treated with biomass ashes, and Pagliari et al. (2009) found turkey manure ash also increased available P and K in soil. Poultry litter ash was noted to increase soil content of available P higher than mineral P and control, and this was attributed to the slow release of P from the initially insoluble phosphate compound in the litter ash (Codling et al., 2002).

With the exception of MBMA treatments, soil available P content increased with increasing P rate of application, which is consistent with crop P recovery where apparent P recovery decreased with increasing the rate of application. This suggests that application of ash at higher rates may pose environmental concern through accumulation of high levels of soluble P in soil. The higher soil content of K in soil amended with DDGA at the high application rate is a result of higher K content of this type of ash application.

Due to its low content of N, ash is not expected to have a large influence on soil N content following its application. However, the observed small increase in soil content of inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) after crop removal in soil amended with ashes, especially DDGA applied at medium and high rates, could be another positive effect of ash application. One possible explanation for this impact is that ash addition may have caused an indirect effect through stimulation of the microbial activity and thereby N mineralization by amelioration of soil chemical and physical characteristics (Demeyer et al., 2001). Otherwise, the mechanism responsible for this change in soil inorganic N content following ash application remains unknown.

The ash application showed an unexpected effect on soil pH in which it caused a minor but significant decrease in soil pH; especially DDGA treatment applied at the high rate which showed approximately 10% decrease in soil pH compared to the control. Previous studies have shown an inconsistent effect of ash addition on soil pH, and its effect was mostly governed by

ash feedstock type, and amount of ash added (Eichler-Löbermann and Schnug, 2006; Schiemenz and Eichler-Löbermann, 2010). For instance, straw ash addition increased soil pH whereas rape meal ash or cereal ash did not, and this was related to the liming effect of straw ash that was applied at a larger rate because of its low content of P. Some other type of ashes, such as chicken litter ash, wood ash and poultry litter ash were found to increase soil pH (Codling et al., 2002; Yusiharni et al., 2007).

The influence of ash application on total soluble salts (EC) in soil has been shown to be variable according to the feedstock incinerated and the rate of ash application (Codling et al., 2002; Pagliari et al. 2009). The low rate of ash applied in the current study is consistent with the lack of effect on EC. Much higher rates would likely be required to produce significant measureable increases in soil salinity.

5.7 Conclusion

The DDGA was as effective as mineral P fertilizer in the form of mono-calcium phosphate. In contrast, the MBMA was less effective, probably related to the high content of Ca which combines with P to form calcium phosphates that are relatively insoluble and of low availability for plant uptake. The content of available P left in soil after crop harvest in the DDGA treatments was consistent with the crop response to ash addition, reflecting the higher solubility of P applied with DDGA. This suggests that application of this type of ash at high rates repeatedly could result in accumulation of potentially mobile P and pose environmental concern. Future work is recommended to assess the specific P forms present in the ash and formed within the soil following application.

The positive results obtained from this study should stimulate further research on utilization of these ashes as a source of P for different crops in different soil types, especially repeated application under field conditions.

6. PHOSPHORUS SPECIATION IN GASIFIED MBM AND DDG ASH AMENDED SOIL AS ASSESSED USING A SEQUENTIAL FRACTIONATION PROCEDURE

6.1 Preface

Ash phosphorus may reside in soil in different forms due to dissolution or reaction with other compounds following its application to soil. Thus, fractionation of P into different forms of varying solubility and plant availability in soil after ash application will help understand the possible fate of the P and thereby assist in proper management. The results obtained from the study in Chapter 5 showed that meat & bone meal ash (MBMA) derived soil P was not as plant available as dried distillers' grains ash (DDGA) P. This raised questions about what specific P forms may arise in the soil from the two different feedstocks, despite the fact that both ashes were produced using the same processing conditions. Therefore, a further investigation, reported on in this chapter, was conducted to examine the P species residing in the soil after crop harvest. This was carried out using the sequential chemical P extraction procedure as first outlined by Hedley et al. (1982). This technique uses different chemical extractants of increasing strength to separate P in soils into various fractions of decreasing bioavailability, such as readily exchangeable, Ca-associated, Al oxide and Fe oxide-associated P. This chapter is in preparation for submission to a refereed journal.

6.2 Abstract

Gasification of organic materials produces a large amount of P rich ash that can be utilized as a P fertilizer. However, the P present in ash may reside in soil in different forms after application as a result of varying proportions of other constituents of the ash like calcium and interactions with the soil. Therefore, the objective of the current study was to identify P fractions residing in the soil after crop harvest in soil fertilized with ashes derived from gasified organic materials. Under controlled environment conditions, a Brown Chernozemic soil received three rates (25, 50 or 100 kg P ha⁻¹) of meat and bone meal ash (MBMA) and dried distillers' grains ash (DDGA) in comparison to a mineral (mono-calcium phosphate) fertilizer (MP) applied at the same rates, in addition to non-P treated soil (control). Soil P was fractionated into different forms using the sequential chemical extraction procedure. Ash addition had significant impacts on the P forms in the soil, and the content of each extractable form varied according to P source type. The largest amounts of the most labile forms of P (resin-Pi, NaHCO₃-Pi) were extracted from MP amended soil, followed by DDGA and MBMA treated soil. The moderately available forms of P (NaOH-Pi, NaOH-Po) were found to be the lowest in soil treated with MBMA. Averaged over the three rates of application, the more recalcitrant and resistant P forms (HCl-Pi and residual-P) were the greatest in MBMA treated soil, representing 24 and 47% of total P, respectively. This increased content of HCl-Pi and residual-P forms is explained by enhanced formation of calcium phosphate compounds in soil treated with MBMA, as this ash type contains a high amount of calcium (25% by weight). The results of this study demonstrated that the P fractions formed in soil after ash application is to a great extent controlled by the initial composition of ashes, that will vary according the gasified feedstock type

6.3 Introduction

Phosphorus (P) is an essential element for plant nutrition, and its supply is critical in maximizing crop production and improving food and feed quality. Unlike N, P cannot be biologically fixed from the atmosphere, and it is mainly mined from phosphate rock, a non-renewable resource used for manufacturing mineral P fertilizer. The global demand for P supply is projected to increase by 50-100% by 2050 to enable high food production for a

growing world population that is predicted to reach about 9 billion by 2050 (EFMA, 2000; Steen, 1998 as cited in Cordell et al., 2009; Godfray et al., 2010). This will put further pressure on the global natural resource of good quality phosphate rock reserves (Cordell et al., 2009). Therefore, a complete reliance on P fertilizer manufactured from phosphate rock may not be a good strategy for sustainable agricultural productivity. This stimulates the need to find other alternative P sources. One option is the utilization of ash generated during bioenergy production from organic materials via gasification process, a thermal breakdown of organic materials under high temperature (800-900 °C) and in presence of oxygen (Ferreira et al., 2009). Application of this technology to meat and bone meal (MBM) and dried distillers' grains (DDG) as a means of recycling nutrients and energy recovery has recently gained interest (Fedorowicz et al., 2007; Tavasoli et al., 2009; Cascarosa et al., 2012). The gasification of MBM and DDG produces ash by-product that was characterized for its high content of P, and has a potential as P fertilizer source (see chapter 5). Utilization of this by-product as a fertilizer can be a good source of recyclable P and will help to balance off-takes by crop removal. This option can therefore reduce the amount of required mineral P fertilizer.

In general, ashes derived from various feedstocks, such as poultry litter, turkey manure and crop residues were shown to have a positive impact on crop yield and soil properties when utilized as P fertilizer source (Codling et al., 2002; Pagliari et al. 2009; Schiemenz and Eichler-Löbermann, 2010). However, the effects of ash on crop production and soil P availability may vary depending on feedstock as shown in the previous chapter, and gasification conditions as well as tested crop and soil properties.

A variety of robust and adequate methods have been employed to understand phosphorus speciation in waste and waste treated soil. The sequential chemical extraction technique based on Hedley et al. (1982) has widely been applied to characterize different forms of soil P (McKenzie et al., 1992; Guo et al., 2000; Qian et al., 2004; Zhang et al., 2004; Verma et al., 2005; Bachmann and Eichler-Löbermann, 2010). This concept of this technique is to use different chemical extractants of increasing strength to separate P in soils into various fractions of decreasing bioavailability, such as soluble, exchangeable, Ca-associated, Al oxide and Fe oxide-associated P.

However, to the best of our knowledge, there is a lack of documented studies using sequential chemical extraction to understand transformations of P forms in soils receiving ash

application. Identification of P forms also is necessary to help predict the agronomic and environmental impact of ash P forms that may reside in soil after application. Therefore, the objective of this study was to determine chemical speciation of P in soil fertilized with ash under controlled environment conditions using chemical extraction technique.

6.4 Materials and Methods

6.4.1 Soil sample collection and analysis

Bulk soil was manually collected with a shovel from the surface layer (0-20 cm) of a cultivated field (cereal-legume-oilseed rotation) in south-central Saskatchewan, Canada and classified as a Brown Chernozem (U.S. equivalent: Aridic Haploboroll). The composite soil sample was shipped to the soil processing facility at the University of Saskatchewan and mechanically mixed using a stationary mixer to give a homogenized sample. Then, the sample was air-dried and stored until its use. A subsample of the soil was ground to pass a 2-mm sieve and analyzed for its basic characteristics. The soil has organic carbon content of 19 mg C g^{-1} , pH of 7.2, electrical conductivity of 0.19 dS m^{-1} , NO_3^- -N of 9.7 mg kg^{-1} , NH_4^+ -N of 6.1 mg kg^{-1} , and available P and K of 7.3 and 450 mg kg^{-1} , respectively.

6.4.2 Production of ash from gasification of MBM and DDG, procurement and preparation

The ashes used in the current study were by-products generated during gasification of two organic materials, meat and bone meal and dried distillers' grains, that were evaluated locally for their utilization feasibility as feedstock candidates for biogas production. The MBM ash (MBMA) was obtained from bovine MBM cracklings that were provided by Saskatoon Processing Ltd., Saskatoon, SK, Canada. The DDG ash (DDGA) was generated from gasification of DDG that was provided by a wheat-based ethanol production facility near Lanigan, SK, Canada. Both products (MBM, DDG) were first ground in the lab and then fed to a two-stage fixed bed reactor, followed by a gasification process using a fluidized bed gasification pilot system developed by the Fluidization Laboratory of Saskatchewan (FLASKTM) at the University of Saskatchewan, Department of Chemical Engineering to produce syngas from biomasses and other carbonaceous materials (Campbell et al., 2012). Gasification of both materials occurred at atmospheric pressure and a temperature of 650 - 850°C. The gasification

process is described in detail by Campbell et al. (2012). The MBMA and DDGA were collected and ground to pass through a 600 μm sieve to obtain a homogeneous product. The resulting ash was then stored in the lab until its use. Prior to the experiment initiation, a representative sample of each ash type was collected and shipped to a commercial laboratory (ALS Laboratory Group, Saskatoon, SK) for their chemical composition analyses. Basic characteristics of both ashes are provided in Table 6.1.

Table 6.1. Selected basics properties of meat & bone meal ash (MBMA) and dried distillers' grains ash (DDGA), expressed on a dry weight basis.

Parameter (total)	Ash type	
	DDGA	MBMA
	mg g^{-1}	
C	8.7	0.9
N	1.4	2.4
P	187	177
K	149	29
S	8	4
Na	75	66
Ca	79	247
Mg	54	11
Cu	0.2	0.1
Fe	6.2	3.6
Mn	1.7	0.1
Zn	1.2	0.7

6.4.3 Incubation experiment set-up and treatment application

Homogenized field-moist soil (1000 g) was placed into 1-L cylindrical plastic pots and treated with ash fertilizer. Details on experimental design and treatments are provided in Chapter 5 of this dissertation. Briefly, the experimental treatments included three P sources; soluble mineral P fertilizer as mono-calcium phosphate (MP), meat and bone meal ash (MBMA), and dried distillers' grains ash (DDGA) applied at three rates equivalent to 25, 50 and 100 kg P ha⁻¹, referred to as low (L), medium (M), and high (H) rate, respectively. A control treatment that received no P was included. Each treatment received 200 kg N ha⁻¹ as urea, including the control, to supply sufficient amount of N to eliminate N deficiency as a limiting factor for plant growth.

Moreover, as soil used in this experiment shows deficiency in sulfur, and canola crop requirement of sulfur is high (Malhi et al., 2005), each treatment was supplied with a basal application of 40 kg S ha⁻¹ as K₂SO₄ solution, including the control. Each treatment was replicated four times in a completely randomized design. Treated soil in each pot was seeded to Argentine canola (*Brassica napus* L.L. 5030) and incubated in a growth chamber that was set at 22°C d and 13°C night, with an 18-h d length and 6-h night length for a period of five week. After canola plant harvest, soils were removed, air-dried and ground to pass a 2-mm sieve prior to laboratory analysis.

6.4.4 Sequential chemical extraction of P

The P sequential extraction protocol was based on a modified Hedley et al. (1982) procedure as described by Tiessen and Moir (2008). In this method, a 0.5 g sample of air-dried and sieved soil was weighed into a 50-mL centrifuge tube followed by addition of 30 mL of deionized water and two strips of anion-exchange resin membrane. The content was shaken for 16 h on a rotary shaker, and then the resin strips were transferred to a clean 50-mL tube and shaken with 20 mL of 0.5 M HCl for 16 h, followed by a determination of inorganic P (P_i) as described below. The tubes containing soil suspension were centrifuged at 10000 × g for 10 min at 0 °C, and the liquid was discarded. Then, 30 mL of 0.5 M NaHCO₃ (pH 8.5) was added to tubes, shaken and centrifuged as above and the supernatant was filtered through a 0.45-μm filter. The inorganic and total P (P_t) in NaHCO₃ extract was then determined as outlined below. The extraction process was repeated as above with the extractants of 0.1 M NaOH and 1 M HCl, respectively, and P_t and P_i in NaOH extract, and P_i in the HCl extract were determined as described below. The remaining (residual) soil residues were transferred to a 75-mL digestion tube using distilled water, and digested using concentrated H₂SO₄ and 30% H₂O₂ following the method of Thomas et al. (1976), and P concentration in the solution was determined.

Inorganic P recovered from the resin strip and P_i in HCl extracts was determined directly using the method of Murphy and Riley (1962). For the NaHCO₃ and NaOH extracts, a suitable aliquot of each extract was acidified first by adding 0.9 M H₂SO₄ to precipitate organic matter prior to P_i determination using Murphy and Riley method (1962). Total extractable P was determined in NaHCO₃ and NaOH extracts by oxidizing the dissolved organic matter with ammonium persulfate as described by Tiessen and Moir (2008), and then the total P was

determined colorimetrically using Murphy and Riley method (1962). Light absorbance through samples was measured using a Beckman spectrophotometer at a wavelength of 712 nm. Inorganic P was subtracted from total P to determine organic P (P_o) in the respective extract.

The forms of P recovered from the various extractants employed here are interpreted according to our understanding of the action of individual extractants, their sequence, and their relationship to the soil chemical and biological properties (Tiessen and Moir, 2008). Resin-P is reasonably well-defined as labile inorganic P that is directly exchangeable and bioavailable. The NaHCO_3 -P is the labile P_i and P_o sorbed to soil mineral surfaces in addition to a small portion of microbial P. The NaOH-P represents P_i and P_o that are strongly chemisorbed to aluminum- and iron-oxide minerals. The HCl- P_i is defined as insoluble apatite-type minerals (Ca-bound P_i). The acid-digested P is the highly insoluble P_i and recalcitrant and stable P_o .

6.4.4 Statistical analyses

The experiment consisted of three amendment types (factor) with three rates of application in addition to a control. Thus, it was a completely randomized design with a complete factorial arrangement. Prior to statistical analysis, the data were checked for normality using Shapiro-Wilk test. This showed that data were normally distributed, and accordingly statistical analysis was conducted on the raw data. Two-way ANOVA procedure was carried out to study the effects of P sources (MP, MBMA, and DDGA), rate (low, medium, and high), and their interaction on P forms recovered by each extractant. Treatment effects were deemed significant at $P < 0.05$ and they were considered a trend at $0.05 < P < 0.10$. Treatment means were separated at $P \leq 0.05$ using Student-Newman-Keuls (SNK) test.

6.5 Results

The concentration of inorganic P recovered from resin membrane strip, which is defined as freely exchangeable P_i , was significantly affected by P source, rate of P application and their interaction (Table 6.2). The high rate of MP and DDGA treatments had the highest content of resin- P_i , and both treatments were significantly higher than the control. Similarly, P source, rate of application and their interaction had a strong impact on concentration of NaHCO_3 - P_i , showing a relatively similar pattern of treatment effect to that observed in resin fraction. The amount of

organic P in NaHCO_3 fraction was significantly influenced by P source and its interaction with rate of application (Table 6.2). The greatest content of $\text{NaHCO}_3\text{-Po}$ was observed with DDGA when applied at the high or medium rate, and this was significantly higher than the control and MP when applied at any rate (Table 6.2), but did not differ from that in MBMA treatments. When averaged over the three rates of application, the organic P concentration in NaHCO_3 fraction was the highest in DDGA treatment, followed by MBMA and then MP treatments (Table 6.3). It comprised 6%, 5% and 3% of total P in DDGA, MBMA and MP treatments, respectively. In the MP treatments, the P content in resin and $\text{NaHCO}_3\text{-Pi}$ fractions were the greatest, especially at the highest rate of application when compared to MBMA or DDGA treatments, and this was significantly higher than that in MBMA or DDGA treatments, when averaged over the three rates of application (Table 6.3).

The inorganic P content in NaOH fraction was significantly affected by P source, rate and their interaction whereas organic P concentration in this fraction was significantly influenced by P source and its interaction with rate of application (Table 6.2). In this fraction, both inorganic and organic P were higher than the most labile P (resin-Pi, $\text{NaHCO}_3\text{-Pi}$, $\text{NaHCO}_3\text{-Po}$), with the NaOH-Po representing 64% of total P (inorganic + organic) in this fraction, on average. The soil content of inorganic and organic P in NaOH fraction varied amongst the various treatments, with the greatest amount observed with the control (Table 6.2).

Phosphorus source, rate and their interaction had a strong significant impact on inorganic P content in HCl fraction (Table 6.2). On average, the majority of the total P in this soil is present in HCl-Pi pool, with the MBMA applied at the high rate having a large proportion (61% of the total P in HCl-Pi pool), which was significantly higher than all other treatments. When averaged across the three rates of application, MBMA treatment had a significantly higher concentration of HCl-Pi than DDGA or MP treatments (Table 6.3). This fraction comprised 47%, 36% and 22% of the total P in MBMA, DDGA and MP treatments, respectively.

The residual P fraction was significantly influenced by P source, but not by rate or its interaction with P source (Table 6.2). The greatest amount of residual P was observed with MBMA when applied at the high rate, making up approximately 25% of the total P present in this treatment (Table 6.2). This treatment was significantly higher than all the other treatments.

Table 6.2. Effects of meat & bone meal ash (MBMA) and dried distillers' grains ash (DDGA) application on sequentially extracted P fractions in soil (mean \pm SE)

Treatment		Resin P _i	NaHCO ₃ P _i	NaHCO ₃ P _o	NaOH P _i	NaOH P _o	HCl P _i	Residual P	Total P
P source	Rate kg ha ⁻¹	-----mg P kg ⁻¹ soil-----							
Control	0	7 \pm 4 de [¶]	12 \pm 5 c	20 \pm 4 bc	52 \pm 1 abc	124 \pm 11 a	115 \pm 10 de	105 \pm 8 b	471 \pm 4 cd
MP	25	15 \pm 5 cd	32 \pm 3 ab	15 \pm 1 c	56 \pm 2 abc	116 \pm 5 a	70 \pm 18 e	103 \pm 6 bc	467 \pm 7 cd
	50	22 \pm 3 bc	24 \pm 2 abc	17 \pm 2 c	52 \pm 3 abc	94 \pm 7 bc	119 \pm 19 de	105 \pm 4 bc	471 \pm 9 cd
	100	54 \pm 4 a	32 \pm 4 ab	16 \pm 1 c	59 \pm 2 ab	100 \pm 3 b	132 \pm 17 cd	96 \pm 8 bcd	510 \pm 7 a
MBMA	25	9 \pm 2 de	9 \pm 3 c	27 \pm 3 ab	39 \pm 2 bcd	69 \pm 4 d	198 \pm 23 b	107 \pm 1 b	466 \pm 7 cd
	50	7 \pm 2 de	13 \pm 3 c	25 \pm 1 abc	39 \pm 2 bcd	76 \pm 5 cd	179 \pm 19 bc	109 \pm 4 b	483 \pm 9 bd
	100	8 \pm 1 de	20 \pm 4 bc	23 \pm 1 abc	38 \pm 2 cd	73 \pm 3 cd	313 \pm 18 a	129 \pm 7 a	513 \pm 15 a
DDGA	25	6 \pm 1 e	13 \pm 1 c	23 \pm 1 abc	32 \pm 1 d	65 \pm 4 d	158 \pm 17 bcd	90 \pm 2 cd	459 \pm 7 d
	50	10 \pm 1 de	18 \pm 2 bc	29 \pm 3 ab	48 \pm 10 abcd	75 \pm 3 cd	161 \pm 7 bcd	85 \pm 1 d	477 \pm 5 cd
	100	25 \pm 1 b	35 \pm 5 a	31 \pm 3 a	62 \pm 9 a	76 \pm 4 cd	199 \pm 28 b	93 \pm 8 bcd	505 \pm 5ab
<i>P > F</i>									
Source of variation									
P source		***	***	***	***	***	***	**	ns
Rate		***	***	ns	*	ns	***	ns	***
P source \times Rate		***	*	*	*	**	*	ns	ns

¶ For a P fraction in a column, values followed by the same letter are not significantly different ($P < 0.05$).

Table 6.3. Sequentially extracted P forms in soil treated with mineral P (MP), meat & bone meal ash (MBMA) or dried distillers' grains ash (DDGA), averaged over rates of P application).

P Source	Resin P _i	NaHCO ₃ P _i	NaHCO ₃ P _o	NaOH P _i	NaOH P _o	HCl P _i	Residual P
-----mg P kg ⁻¹ soil-----							
MP	30 a [¶]	29 a	16 b	56 a	104 a	107 c	102 b
MBMA	8 b	15 c	25 a	39 c	73 b	230 a	114 a
DDGA	13 b	22 b	28 a	47 b	72 b	173 b	90 c

[¶] For a P fraction in a column, values followed by the same letter are not significantly different ($P < 0.05$).

The DDGA applied at the medium rate treatment provided the lowest content of residual P form, which significantly differed from all other treatments including the control (Table 6.2). When averaged over the three rates of application, the MBMA showed the highest amount of residual P, which was significantly higher than MP and DDGA treatments (Table 6.3). The total P determined at the end of the experiment was not significantly affected by P source, but was greatly influenced by the rate of application (Table 6.2). Application of P at the high rate of 100 kg P ha⁻¹ resulted in higher amount of total P in soil treated with any P source, and this was significantly higher than all other treatments (Table 6.2).

6.6 Discussion

The labile P fractions of resin-P_i and NaHCO₃-P (inorganic and organic) represented together only a small proportion of the total P, ranging from 8%, in unamended soil, to 15% in MP treatments, averaged across the three rates of application. This is relatively consistent with previous studies conducted with grassland or cultivated Brown Chernozem soil in which the summed P fraction recovered by resin and NaHCO₃ did not exceed 10% of the total P (Tiessen et al., 1983; Schoenau et al., 1989). The greatest amount of P in resin and NaHCO₃-P_i fractions observed in the MP treatments is not surprising, given that the MP is comprised of highly water soluble Ca(H₂PO₄)₂. In general, the labile P was higher in DDGA treatments in comparison to that in MBMA treatments, and this is in line with the higher P uptake by canola observed in DDGA-amended soil (see Chapter 5), indicating the high solubility of P in this type of ash. A similar pot study that examined P fractions in soil fertilized with poultry litter ash found that resin-P_i content in ash treated soil was higher than the control and comparable to that in soil

treated with mineral P, implying the high release of P from this type of ash (Bachmann and Eichler-Löbermann, 2010). Ash produced from gasified alfalfa stems increased soil extractable P (Mozaffari et al., 2000a). Soil treated with poultry litter ash was also found to have a greater content of soluble P (Codling et al., 2002). In a pot experiment, addition of ash generated from straw gasification showed a significant increase in soil content of soluble P whereas ash generated from citrus peel fiber gasification did not (Müller-Stöver et al., 2012). It seems that the level of P solubility in soil following ash application can be related to the P solubility in the ashes, which is variable according to feedstock and combustion/gasification processes, as demonstrated in the current study. For instance, agricultural residue-derived ashes appear to exhibit greater P solubility than that in wood biomass-derived ashes (e.g. Eichler-Löbermann et al., 2008; Mozaffari et al., 2000b; Clarholm, 1994; Patterson et al., 2004) whereas ash generated from meat and bone meal is dominated by calcium phosphates (e.g. apatite), which are recalcitrant form of P and of low plant-availability (Deydier et al., 2003; Coutand et al., 2008). This can explain the different effects of both ashes in the current study on the level of P solubility in soil.

The NaOH-extractable P fraction (P_i and P_o) is considered as moderately labile P (Hedley et al., 1982) and assumed to have relatively low availability to plants (Hedley et al., 1982; Cross and Schlesinger, 1995). Generally, the percentage of P in NaOH fraction (P_i and P_o) observed in this study is within the range of that found in different soil orders worldwide as reviewed by Cross and Schlesinger (1995). The addition of either ash did not enrich the NaOH-P pool when compared to the control, probably related to the presence of large concentration of the ash P in Ca-bound phosphate, especially with MBMA, with the slow release of P from initially insoluble P compounds in both ashes, leading to lack of fixation of inorganic P as Fe and Al phosphates. The reduced contribution of ash addition to this pool might also be attributed to low initial content of Fe and Al compounds added with the ashes, leading to insignificant formation of moderately soluble Al- and Fe-phosphates that can be extracted with NaOH extractant. This is in agreement with previous work, which reported that poultry litter ash addition did not significantly increase NaOH-P fraction when compared to the control (Bachmann and Eichler-Löbermann, 2010). According to these authors, the P in their specific ash type might not be converted quickly to enrich the NaOH-P pool, as the P in the poultry litter ash was previously found to be mainly Ca-phosphates (Codling, 2006). It was also reported in a recent study that

bone char had no significant impact on soil concentration of inorganic and organic forms of P extracted with NaOH, and this was attributed to the direct effect of Ca-associated P where the char contains 80% of H₂SO₄-extractable P (Siebers et al., 2013). However, it was documented in other studies that mineral fertilizer application resulted in an increase of NaOH-P fraction in soil (He et al., 2004; Bachmann and Eichler-Löbermann, 2010).

Phosphorus extracted with diluted HCl constitutes stable Ca-bound P in soils that is considered to be very low of availability for plant uptake (Williams et al., 1980; McKenzie et al., 1992). Calcium is the dominant cation in MBMA (25%) and was anticipated to react with phosphate in ash and in soil after application, leading to a preponderance of calcium phosphate compounds (e.g. apatite). It was also demonstrated earlier that calcium phosphates compounds were found to be major constituents of ash derived from combusted meat and bone meal (Deydier et al., 2003; Coutand et al., 2008). This can explain the high concentration of P in HCl-P_i fraction in MBMA, explaining the low availability of P in this ash type and therefore reducing its effectiveness as a P source for crop nutrition, as in Chapter 5. In contrast, the lower content of HCl-P_i in DDGA, compared to MBMA, may be attributed to its lower content of Ca (8%), contributing less to formation of Ca-bound P in soil. This is reflected in the high concentration of P in the labile fractions (resin-P_i, NaHCO₃-P_i, NaHCO₃-P₁) in DDGA, compared to the control or MBMA. Furthermore, P in DDGA was very accessible by the canola crop, similar to that in MP. However, it was reported in another study that poultry litter ash addition did not influence soil content of H₂SO₄-P fraction in comparison to mineral P or control treatment after crop harvest, indicating the high solubility of P in this particular ash or of its possibly rapid transformation into other forms of P (Bachmann and Eichler-Löbermann, 2010).

The residual P fraction is regarded as the most recalcitrant fraction with very low solubility and bioavailability (Cross and Schlesinger, 1995). It is considered to be a mixture of insoluble (occluded) inorganic P adsorbed onto sesquioxides and nonextractable stable organic P (Dedley et al., 1982; Tiessen and Moir, 1993). On average, this fraction accounted for about 24% of the total P in soil treated with MBMA. The greater amount of this fraction in MBMA indicates that a higher Ca in this type of ash together with the high pH of the tested soil may have favoured formation of more stable forms of P in treated soil. Formation of this resistant fraction of P may have limited the availability of P to crop grown in soil treated with MBMA as observed in the canola P uptake covered in Chapter 5 of this dissertation.

6.7 Conclusion

Both ashes used in the current study had contents of total P around 18% P (41% P_2O_5 equivalent), making them attractive as high analysis recycled P sources for agriculture. However, this P was found to reside in different forms in soil following application. The labile P fractions: resin- P_i and $NaHCO_3$ - P_i in soil were in the order of MP > DDGA > MBMA, and it increased with increasing rate of P application. The moderately labile P forms ($NaOH$ - P_i , $NaOH$ - P_o) in all P-treated soils did not significantly differ from the control. The soil content of P in HCl - P_i and residual-P pools was found in greater amount in MBMA treatment, comprising 47% and 24% of total P in HCl - P_i and residual-P fraction, respectively. This is mainly due to the greater content of calcium (25%) present in this type of ash that led to the formation of Ca-P compounds in soil. This study clearly showed that the P solubility and availability varies according the type of ash applied. Future studies may consider using spectroscopic techniques like XANES to specifically identify P minerals in the ash and in amended soils.

7. FIELD EVALUATION OF FERTILIZER POTENTIAL OF THIN STILLAGE

7.1 Preface

Thin stillage (TS) is a major by-product of bioethanol production industry. Positive effects of TS application on soil biological and chemical attributes under controlled environment conditions were observed and are described in Chapters 3 and 4. This warranted a further investigation into the suitability of this by-product for being utilized as an organic fertilizer in prairie soil under field conditions in a field trial. In Chapter 3, the TS addition greatly increased nutrient supply rates, especially $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ under optimum conditions and this needs to be verified on a larger scale, over a longer period and under conditions in which the material is applied using field scale equipment. Advanced equipment for land application of liquid manure is readily available and was utilized for direct injection of thin stillage into soil in this study. In this two-yr field study, the response of crop yield, nutrient uptake and recovery to broadcasted and incorporated or injected TS was assessed.

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7.2 Abstract

Accumulation of thin stillage (TS), a by-product resulting from ethanol production, has led to a need to explore all possible means of its utilization. The objective of this research was to evaluate the effectiveness of TS derived from wheat (*Triticum aestivum* L.) based ethanol production as a fertilizer. The experiment was conducted over a two-yr period in east-central Saskatchewan, Canada. Treatments included three rates of TS: 50, 100 and 200 kg N ha⁻¹ using two methods of application: 1) broadcast and incorporation and 2) injection. For comparison, conventional fertilizer urea (46-0-0) was applied at the same rates of N as the TS. Responses of crop yield (wheat and canola (*Brassica napus* L), N and P uptake, and apparent N recovery were measured over two growing seasons on a Black Chernozemic soil. For both seasons, at equivalent N rate the TS produced similar or greater crop yield and nutrient recovery compared to urea fertilizer, especially when injected. This is attributed to the effect of other plant nutrients, such as P and S in TS, and its relatively high plant available NH₄⁺-N content. The injection of TS appears to be a more effective application method compared to broadcasting, likely through reducing volatile N loss, and placing nutrient closer to the growing crop roots when injected in bands in soil. The TS did not show any adverse effect on measured crop parameters even at the high rate of application. The results of this study suggest that land application of TS can be an effective solution for TS management that recycles nutrients in the feedstock grain used for the ethanol production back into the soil.

7.3 Introduction

Thin stillage (TS) is the aqueous by-product generated from the distillation of ethanol following fermentation of starch or sugar crops (Wilkie et al., 2000) during the ethanol production process. The fermentation and distillation processes of the feedstocks generate the whole stillage, which contains solids from the grain along with added yeast and liquid from the water added during the process. The whole stillage is then centrifuged to separate the liquid component called thin stillage, and the solid component called wet distillers' grains (WDG). The TS is then further processed by evaporation to produce syrup that can be blended with WDG resulting in WDG with solubles (Bonnardeaux, 2007). High volumes of TS are produced during

ethanol production, as each litre of ethanol produced results in approximately 20 L of associated TS (van Haandel and Catunda, 1994). Thus, increases in ethanol production will require effective solutions for TS utilization (Wilkie et al., 2000). Although the evaporation process helps to concentrate TS constituents into a lesser volume, it can have a negative impact on the energy balance of ethanol production (Faust et al., 1983).

Although it is not a common practice, one of the alternative uses of TS is as a partial or complete drinking water replacement for cattle (Mustafa et al., 2000). However, this method of utilization may not accommodate the continual rise in TS production, as the rapid growth in ethanol manufacturing is anticipated to create a surplus of ethanol by-products (Rausch and Belyea, 2006). Alternative utilization approaches need to be considered. One potential use is the direct land application as a fertilizer. This option may create another outlet for utilization of ethanol by-products, and can help to recycle the plant nutrients that are used in producing the original feedstocks. Since TS contains essential plant nutrients that can promote crop production and soluble organic matter that can stimulate soil biological activity (see Chapters 3 and 4 of this dissertation), its direct application to agricultural soil might be a practical alternative use. Equipment for land application of liquid manure is readily available and anticipated to work well for direct injection of TS into soil.

The chemical characteristics of TS are variable, and differ according to feedstock type and the treatments used in the bioenergy production plant. For example, TS derived from fermentation and distillation of corn or wheat, two most popular crops for ethanol production in the U.S., Canada and Europe (Mustafa et al., 2000; Cardona and Sánchez, 2007), is different in its chemical properties from distillery wastewater (vinasse) generated from sugar cane or molasses (España-Gamboa et al., 2011).

The fertilizer value of vinasse stillage produced from molasses, a by-product of the sugar industry, has previously been studied (Gemtos et al., 1999; Singh et al., 2003; Resende et al., 2006; Hati et al., 2007). However, the fertilizer potential of TS derived from starch crops has received little attention. Field studies examining effects of TS as a fertilizer under field conditions using advanced application techniques are needed. Therefore, the objective of the current study was to assess the fertilizer potential of TS derived from wheat-based ethanol production by examining the response of crop yield, and nutrient uptake and recovery to TS application.

7.4 Materials and Methods

7.4.1 Experimental site

The field experiment was initiated in the fall of 2008 near the town of Dixon located in east-central Saskatchewan, Canada. The predominant soil at the site is classified as a Black Chernozem (Cudworth Association) of clay-loam texture. The average particle-size distribution in the 0-15 cm depth was 30% sand, 23% silt and 47% clay, determined using pipette method (Gee and Bauder, 1986). The study site has a nearly level topography and is considered fertile agricultural land in Saskatchewan (Stumborg et al., 2007). The field was cropped to barley (*Hordeum vulgare* L.) in the year prior to the current study. The basic characteristics of the field soil are provided in Table 7.1. The average long-term annual precipitation and temperature for this area is 373 mm and 0.7 °C respectively (Stumborg et al., 2007). Monthly cumulative rainfall and mean air temperature over the two growing seasons and the 30-yr average are summarized in Fig. 7.1. The climate data were retrieved from a weather station located at Humboldt approximately 5 km from the experimental site (Environment Canada, 2012).

7.4.2 Experimental design

The field experimental setup used a 3×3 factorial design and consisted of three main factors: injected TS (INJ-TS), broadcasted and incorporated TS (BR-TS) and banded urea and three levels of application for each factor: low (L), medium (M) and high (H). An unfertilized, unamended control was included for comparison. The three levels of TS application were equivalent to 16800, 33600 and 67200 L ha⁻¹, providing approximately 50, 100 and 200 kg available N ha⁻¹, respectively, based on an assumption that about 60% of the total N in TS would be available during the course of the year (Qian et al., 2011). The actual amounts of N applied as thin stillage are found in Table 2. The three levels of commercial fertilizer urea (46-0-0) N applied for comparison were 50, 100 and 200 kg N ha⁻¹. Treatments were arranged in a randomized complete block design with four replications. Each treatment plot had dimensions of 3 m width \times 9 m length. Plots received the same treatments in both 2009 and 2010.

Table 7.1 Selected soil properties at the start of the field study in fall 2008 in samples collected from control plots at 0-15 cm depth.

Property	Value [§]
NO ₃ ⁻ -N (mg kg ⁻¹)	7.0 ± 0.3
NH ₄ ⁺ -N (mg kg ⁻¹)	4.3 ± 0.3
Available P (mg kg ⁻¹)	5.1 ± 1.0
Available K (mg kg ⁻¹)	275 ± 23
Organic C (mg g ⁻¹)	28 ± 2.0
pH	8.0 ± 0.1
Electrical Conductivity (dS m ⁻¹)	1.5 ± 0.8
Sand (%)	29.7 ± 0.5
Silt (%)	23.6 ± 3.0
Clay (%)	46.7 ± 3.5

§ Values presented are means followed by standard error.

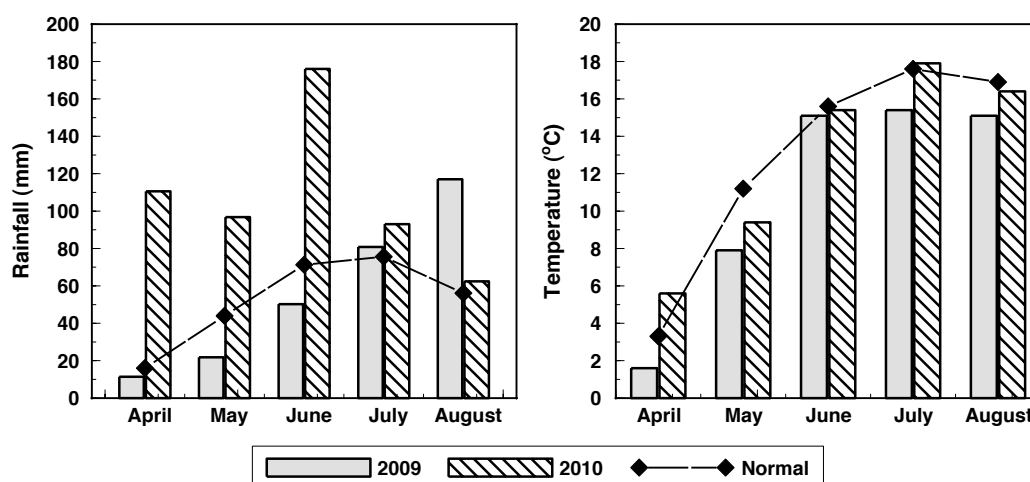


Fig. 7.1. Monthly total rainfall and mean air temperature at Humboldt for the 5-mo growing season for both years of the study (2009, 2010). The 30-yr rainfall and temperature averages (normal) are also included

Table 7.2. Thin stillage nutrients applied at the rates of application (L, M and H) for both years of the study.

Treatment		Total N	Total P	NH ₄ ⁺ -N
Rate	Yr	-----kg ha ⁻¹ -----		
L	2009	79	15	15
M		158	30	30
H		316	60	67
L	2010	95	19	17
M		190	37	34
H		380	75	60

7.4.3 Thin stillage procurement and treatment application

The TS was obtained from Pound-Maker Agventures ethanol plant located at Lanigan, Saskatchewan. It is a by-product of ethanol production using wheat grain as feedstock. The TS materials were collected, delivered to the site and applied by the Prairie Agricultural Machinery Institute (PAMI, Humboldt, SK). Treatments were applied to the field in the preceding fall (1st wk of October) of each growing season. For the injection method of application, TS was applied using the PAMI liquid slurry injector truck. The TS was agitated as it was pumped into the PAMI injector truck. The injector truck is equipped with modified Bourgault low disturbance injector disc coulters spaced 30 cm apart. The TS was applied in bands behind the coulter at an average depth of 8-10 cm. For the broadcast and incorporation method, the injectors were lifted above the soil surface to get TS applied on the soil surface in a band, followed by immediate incorporation with a chisel plow cultivator using one pass with 30 cm sweeps on a 20 cm row spacing, followed by harrowing. During TS application to soil and for both years, several samples were collected at the injector opening, mixed to yield a homogenous representative sample and stored in the freezer (-20 °C) until analysis for chemical composition. The analysis of TS was conducted at a commercial laboratory (ALS Laboratory Group, Saskatoon, SK). Basic characteristics of TS applied for each year are provided in Table 7.3. Commercial granular urea fertilizer (46-0-0) was banded into the soil using PAMI's plot drill at an 8 cm depth with knives on a 20 cm row spacing.

The field was seeded to Lillian hard red spring wheat on May 9th of 2009 at a rate of 128 kg ha⁻¹ and to BrettYoung 719 Roundup Ready canola (*Brassica napus* L.) on May 19th of 2010 at a rate of 6 kg ha⁻¹. In-crop weed control was achieved by using appropriate systemic herbicides.

Table 7.3. Basic characteristics of thin stillage (TS) applied in 2008 and 2009. All contents are expressed on a fresh wet weight basis.

Property	Year	
	2009	2010
Total N (mg g ⁻¹)	4.7	5.7
NH ₄ ⁺ -N (mg g ⁻¹)	0.90	0.10
Total P (mg g ⁻¹)	0.90	0.11
Total K (mg g ⁻¹)	1.1	1.2
Total S (mg g ⁻¹)	0.6	0.7
Na (mg g ⁻¹)	0.4	0.3
Ca (mg g ⁻¹)	0.2	ND
Mg (mg g ⁻¹)	0.4	ND
pH	3.8	4.10
Moisture (%)	92.5	91.6

7.4.4 Plant harvest and analysis

In both years, the crop was harvested when it reached physiological maturity in September. Duplicate 1-m² plant samples per plot were cut manually at 5 cm above the soil surface. The samples collected were dried by forced air at 45 °C, the total biomass weighed, and mechanically threshed using a stationary thresher followed by weighing to determine yield. Straw samples were ground to < 2 mm in a WileyTM mill and grain samples were finely ground with a CycloneTM mill. Total N and P were measured by digesting the ground grain and straw samples in sulfuric acid-peroxide (H₂SO₄-H₂O₂) using a temperature-controlled digestion block (Thomas et al., 1967), followed by automated colorimetry for determining P and the NH₄⁺-N using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1978). Total N and P uptake were then calculated from plant N and P contents and total dry matter yield. Apparent N recovery (ANR) and apparent P recovery (APR) were calculated as follows:

$$\text{ANR or APR} = \frac{TNUTP - TNUC}{\text{total } N \text{ or } P \text{ applied}} \times 100$$

where TNUTP denotes total N or P uptake for a given treatment plot, TNUC is the total N or P uptake in control plot and total N or P applied is the amount of N or P applied in the amendment treatment for the crop year.

7.4.5 Statistical analyses

Prior to conducting the statistical analysis, raw data were checked for normality and homogeneity of variance using histograms and Shapiro-Wilk tests. Data that were not normally distributed and showed lack of variance homogeneity were transformed by performing an appropriate mathematical operation. Two statistical procedures were conducted. First, a mixed effects model was used to determine the effect of the main treatments factors that included fertilizer type (INJ-TS, BR-TS, Urea) and rate of application (L, M, H) and all interactions on crop dependant variables. Fertilizer type and rate of application were treated as fixed effects whereas block was treated as a random effect in the model. In this analysis, the control treatment was omitted because there was only one level of this treatment. The second procedure of analysis was a one-way ANOVA that was used to determine the effect of all 10 individual treatments including the control on crop dependant variables. This one-way ANOVA procedure permits comparison of all 10 treatments means, which were separated using LSD test. Treatment effects were considered significant at a probability level of $P \leq 0.05$ at which means were also separated, and they were considered a trend at $P < 0.10$. The analyses were conducted on each year's data separately due to differences in the crop type. Statistical analysis was conducted using the statistical analysis system (SAS) software, version 9.2 (SAS Institute, Cary, NC).

7.5 Results

7.5.1 Climatic conditions

The weather data for the two growing seasons are shown in Fig. 7.1. In the 2009 growing season (April to August), total rainfall (281 mm) was slightly above the long-term average (268 mm). The total amount of the rainfall received during the month of May when the crop was seeded and the month of June was less than the long-term average (Fig. 7.1). Unusually high

rainfall was received during the 2010 growing season, in which the total rainfall during the 2010 growing season was about 539 mm. This is nearly double the amount of rainfall that was received in 2009 growing season and more than double the long-term average. The total rainfall received during the first three months of the 2010 growing season was approximately 66% higher than the long-term average. The excess moisture that occurred in the 2010 growing season was anticipated to adversely affect crop performance. The monthly mean air temperature for the two growing seasons did not differ much from the long-term average (Fig. 7.1).

7.5.2 Thin stillage characteristics

The chemical analysis of TS applied in the fall of 2008 or 2009 showed that the chemical composition of TS applied in both years was relatively similar, consistent with its origin from the same production facility (Table 7.3). According to its basic chemical analysis, the TS by-product used in this experiment contains a significant amount of essential plant nutrient, especially N, P, K and S. On a wet basis, the TS contains approximately 0.52%, 0.10%, 0.12% and 0.10% of total N, P, K and S, respectively, when averaged across the two-yr. More importantly, the TS showed a relatively high content of $\text{NH}_4^+\text{-N}$, which represents about 20% of the total N contained in TS. Nitrate-N was not detected in the TS. The TS is considered acidic material, as shown by its low pH value of 4.0. Most of the wet weight of the TS was comprised of water, which represents approximately 92% of its weight.

7.5.3 Crop yield response

The statistical analysis showed that wheat yield response to fertilizer type and its rate of application was highly significant (Table 7.4) while the interaction between these factors (fertilizer type and rate) tended to be significant ($P = 0.10$) in the 2009 season. The effect of treatments on crop yield was very evident when compared to the control (Fig. 7.2), and this effect increased with increasing the fertilizer rate of application. Crop yields from all amended treatments were significantly higher than the control treatment (Fig. 7.2). The greatest yield response was observed with INJ-TS when applied at the medium and high rate and BR-TS when applied at the high rate. When averaged across the three rates of application, the INJ-TS

provided a significantly higher yield than the BR-TS or urea whereas BR-TS and urea amendments produced similar crop yield.

The significant impact of treatments on crop yield of canola was also seen in the 2010 season, but the overall yield response compared to the control was less pronounced than that observed in the season of 2009. The yield response to fertilizer type and rate of application was observed to be highly significant (Table 7.4) whereas their interaction was not. With the exception of BR-TS and urea treatments when applied at the low rate, all treatments provided higher yield than the control (Fig. 7.2), and the yield showed an increase trend with the rate of application. As in 2009, the most pronounced effect on canola yield was observed with INJ-TS treatments (Fig. 7.2), while the BR-TS and urea treatments mostly followed a similar pattern in their effect on crop yield. The yield obtained from INJ-TS treatments was significantly higher than that from BR-TS or urea treatments, when averaged across the three rates of application.

7.5.4 Nutrient uptake response

Fertilizer type and rate of application had a significant influence on total plant N uptake in both growing seasons; however, the interaction between the two factors was significant only in the 2010 growing season (Table 7.4). For the 2009 growing season, all treatments showed a significantly greater N uptake than the control treatment (Fig. 7.3), and N uptake increased with the rate of fertilizer application. For the 2010 growing season, the treatment effect followed a similar pattern as in the first growing season, but was more pronounced (Fig. 7.3). For both growing seasons, N uptake was significantly higher in INJ-TS treatments in comparison to BR-TS or urea treatments, when averaged across the three rates of application.

Total plant P uptake was significantly affected by fertilizer type, rate and their interaction in both growing seasons (Table 7.4). For the 2009 growing season, all treatments were significantly higher than the control (Fig. 7.4), and INJ-TS and BR-TS treatments were more effective than the urea treatments. The impact of treatments on P uptake was more evident in the 2010 growing season, especially with INJ-TS and BR-TS treatments whereas urea treatments applied at any rate did not significantly differ from the control (Fig. 7.4). In this season, the INJ-TS treatments showed the greatest impact on P uptake, followed by BR-TS, while urea treatments were the least.

Table 7.4. Significance levels of treatment factors of fertilizer, rate of application and interactions for analysis of crop yield, N and P uptake, and apparent N and P recoveries (ANR, APR) for 2009 and 2010 growing seasons.

	2009					2010				
	Yield	N uptake	P uptake	ANR	APR	Yield	N uptake	P uptake	ANR	APR
	<i>P</i> value									
Fertilizer (F)	0.0007	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	<0.0001	<0.0001	0.0048	0.0006
Rate (R)	<0.0001	<0.0001	0.0003	0.0002	0.0019	0.0001	0.0004	<0.0001	0.141	0.3416
Interaction										
F × R	0.097	0.5876	0.0313	0.0900	0.0173	0.7087	0.0813	0.0064	0.4387	0.4155

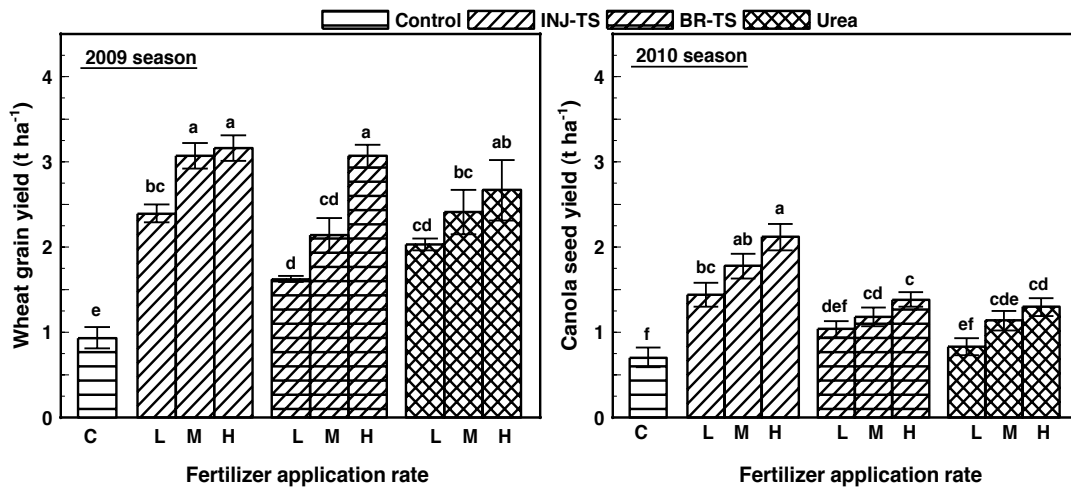


Fig. 7.2. Wheat and canola yield responses to experimental treatments during the 2009 (wheat) and 2010 (canola) seasons at Dixon, SK. The experimental treatments included injected thin stillage (INJ-TS), broadcast and incorporated thin stillage (BR-TS) and urea, all of which are applied at three rates: low (L), medium (M) and high (H) in addition to a control. For a season, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.05$). Errors bars represent standard error of mean ($n = 4$)

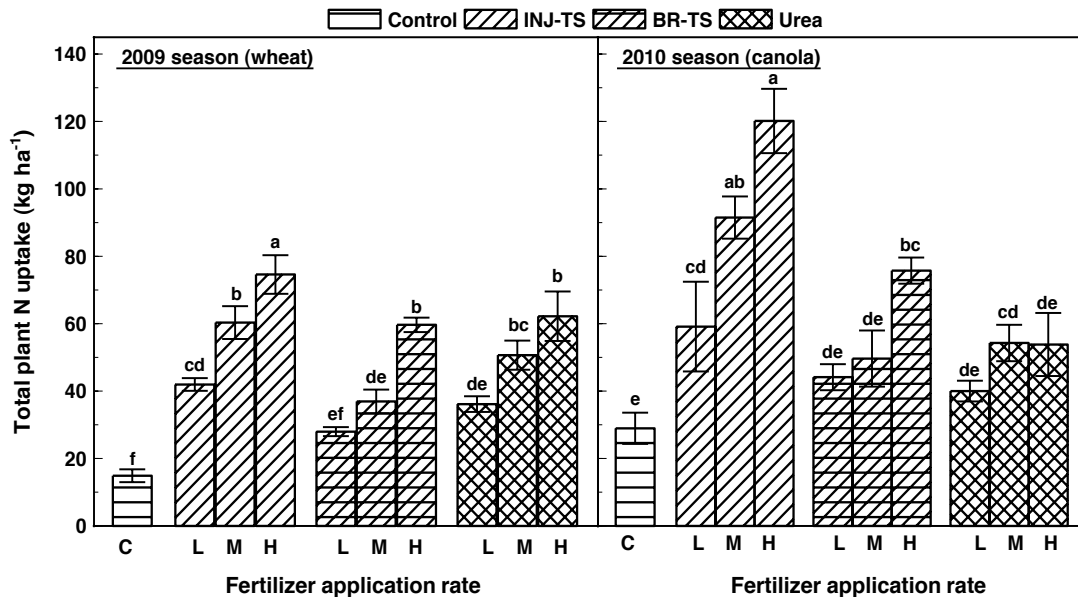


Fig. 7.3. Total plant N uptake responses to experimental treatments during 2009 and 2010 growing seasons at Dixon, SK. The experimental treatments included injected thin stillage (INJ-TS), broadcast and incorporated thin stillage (BR-TS) and urea, all of which are applied at three rates: low (L), medium (M) and high (H) in addition to a control. For a season, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$)

7.5.5 Apparent N and P recovery

Fertilizer type, rate and their interaction had a significant impact on apparent N recovery (ANR) in the 2009 growing season while the fertilizer type was the only factor that showed a significant effect on ANR in the 2010 growing season (Table 7.4). For both seasons, the ANR tended to decrease with increasing rate of application in INJ-TS and urea treatments (Fig. 7.5). The ANR was the lowest in soil treated with BR-TS, with no significant differences among the rate of its application in both years. In the 2009 growing season, the INJ-TS and urea treatments provided ANR significantly higher than that in BR-TS treatments whereas the INJ-TS and urea treatments did not significantly differ from each other, when averaged across the three rates of application. However, in the 2010 growing season, the INJ-TS treatments showed a significantly higher ANR than BR-TS and urea treatments, when averaged across the three rates of application.

The impact of fertilizer type, rate and their interaction on apparent P recovery (APR) was significant in the 2009 growing season (Table 7.4). Fertilizer type had also a significant effect on APR in the 2010 growing season, but rate and interaction between fertilizer type and rate had no effect (Table 7.4). The APR tended to decrease with increasing the rate of application in INJ-TS treatments in both seasons (Fig. 7.6). No significant differences were found among the rates of BR-TS application (Fig. 7.6). The APR was significantly higher in INJ-TS treatments than that in BR-TS treatments, when averaged across the three rates of application.

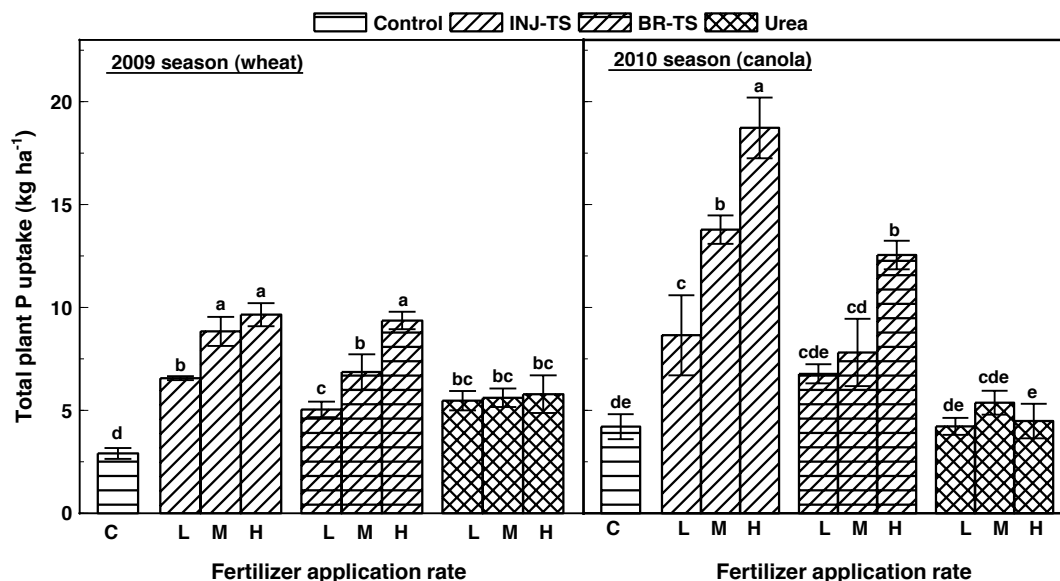


Fig. 7.4. Total plant P uptake responses to experimental treatments during the 2009 and 2010 growing seasons at Dixon, SK. The experimental treatments included injected thin stillage (INJ-TS), broadcast and incorporated thin stillage (BR-TS) and urea, all of which are applied at three rates: low (L), medium (M) and high (H) in addition to a control. For a season, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$)

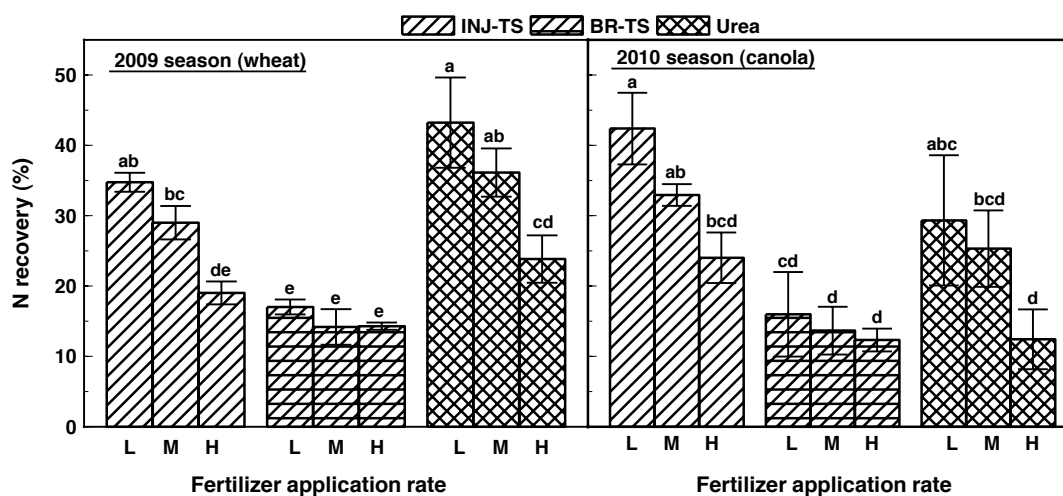


Fig. 7.5. Apparent N recovery (ANR) responses to experimental treatments during the 2009 and 2010 growing seasons at Dixon, SK. The experimental treatments included injected thin stillage (INJ-TS), broadcast and incorporated thin stillage (BR-TS) and urea, all of which are applied at three rates: low (L), medium (M) and high (H) in addition to a control. For a season, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$)

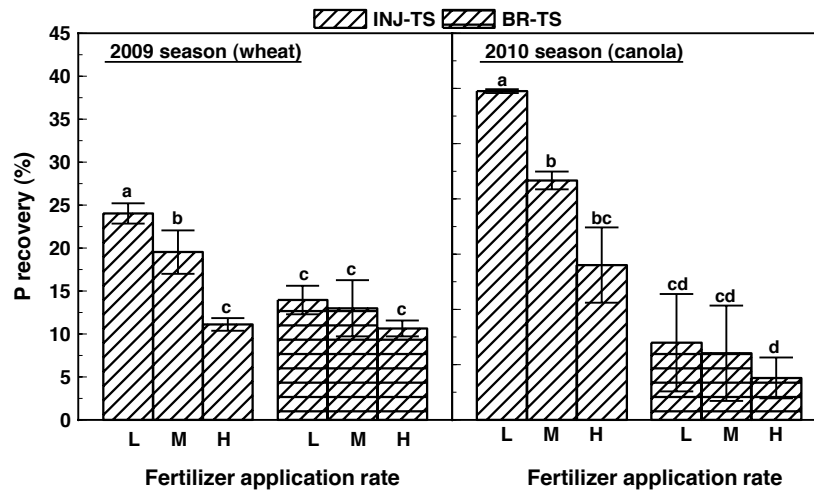


Fig. 7.6. Apparent P recovery (APR) responses to experimental treatments during the 2009 and 2010 growing seasons at Dixon, SK. The experimental treatments included injected thin stillage (INJ-TS) and broadcast and incorporated thin stillage (BR-TS), all of which are applied at three rates: low (L), medium (M) and high (H) in addition to a control. For a season, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.05$). Errors bars represent standard error of the mean ($n = 4$)

7.6 Discussion

Fertilization of soil with TS clearly stimulated crop growth, resulting in yield that was similar or better than that in plots treated with urea fertilizer at similar rate of added N in both growing seasons. This is supported by results of work in the growth chamber reported on in Chapter 3 where TS addition contributed a significant amount of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and P over a 10-d incubation study, indicating the rapid decomposition of TS organic matter in soil and associated release of available nutrient for plant uptake. Moreover, the positive effect of TS application on crop yield in the field study can also be explained by the presence of other nutrients in TS, such as P and S that contribute to plant nutrition and yield response. Increasing rate of fertilizer application, especially TS, contributed to higher yield and N and P uptake in both growing seasons. The $\text{NH}_4^+\text{-N}$ represents approximately 20% of total N in TS and is considered to be available for plant uptake. This portion of $\text{NH}_4^+\text{-N}$ along with mineralization of organic N would enhance the fertilizing value of TS by-product, as previously demonstrated with liquid manure, whose fertilizer value was mainly based on its $\text{NH}_4^+\text{-N}$ content (Bechini and Marino, 2009).

Overall treatment effects on crop yield were less pronounced in the second year (2010) with canola. This may be attributed to the excess moisture experienced in the growing season of 2010 that was expected to negatively influence the crop growth and consequently limit the treatment effects. However, in contrast to the first year, the nutrient uptake (N, P) and recovery, especially P, were clearly greater in the second year even at the low rate of TS application. This is a possible consequence of additional nutrient supply resulting from the mineralization of residual organic nutrient in the TS applied for the previous year. This was also confirmed by other studies conducted with different organic fertilizers, such as slurry or solid animal manures (Pratt et al., 1973; Paul and Beauchamp, 1993; Eghabll and Power, 1999; Mooleki et al., 2004), paper mill sludges (N'Dayegamiye et al., 2003; N'Dayegamiye, 2006) and TS derived from sorghum grain feedstock (Jenkins et al., 1987). The other reason is the generally higher nutrient demand and uptake potential of canola versus wheat (Malhi et al., 2008).

Both ANR and APR markedly decreased with increasing rate of application in both growing seasons, especially with injected TS and urea treatments. The ANR was reported in other studies to decline with increasing rate of different types of organic fertilizers or

amendments (e.g., N'Dayegamiye et al., 2003; Mooleki et al., 2004; N'Dayegamiye, 2006). Mooleki et al. (2004) related the decrease in ANR with increasing rate in soil fertilized with liquid swine manure, which has, to some extent, a similar nature to TS, to several reasons. The first and most obvious reason is the finite capacity of the plant to assimilate the added N and produce biomass and protein. Suppression of organic N mineralization could result from the abundance of $\text{NH}_4^+\text{-N}$. Another reason could be higher N losses associated with high application rates of slurry, especially when broadcasted (Qian and Schoenau, 2000). In addition, application of TS, particularly at the high rates, may cause N losses through promoting conditions that are favorable for denitrification processes in which the TS provides carbon, which stimulates microbial activity and depletes soil oxygen supply. This would be accompanied by the filling of soil pores by the water in the TS effluent band, enhancing denitrification. This has been reported for injected animal slurries as well (Paul and Zebarth, 1997; Mooleki et al., 2004). Similar to ANR in urea treatments in the current study, it was also found that increasing urea rate linearly decreased ANR by corn crop (Gagnon et al., 2012). However, Paul and Beauchamp (1993) did not find significant differences in ANR among the rates of liquid dairy cattle manure, solid cattle manure and composted cattle manure when applied at 100, 200 and 300 kg total N ha^{-1} by broadcast and incorporation method. This is in agreement with the current study in which there were no significant differences observed among the rates of the broadcasted and incorporated TS treatments. This may be due to low efficiency of broadcast method. The higher APR with the low rate of TS application, especially when injected, is consistent with the previous studies conducted with animal manures that found P use efficiency was usually greater with a low rate of manure application (Eghball and Sander, 1989; Miller et al., 2009).

The injection method of TS application was clearly more effective than the broadcast and incorporation method. The greater yield and nutrient uptake and recoveries observed with the injection TS treatments may be in part attributed to conserving TS $\text{NH}_4^+\text{-N}$ by reducing NH_3 volatilization loss, and may also relate to placement of TS nutrient closer to the growing crop roots. It was also reported by Huijsmans et al. (2003) that band injection is an effective technique for organic material application, to reduce NH_3 volatilization and improve N recovery. Similarly, Mooleki et al. (2004) revealed that the band injection method of swine slurry was efficient and superior to broadcast/incorporated in providing higher yield, plant N concentration and N use efficiency.

7.7 Conclusion

The TS derived from wheat grain feedstock greatly promoted crop growth, showing significant potential fertilizer value. The TS supplied nutrients that were sufficient for wheat and canola production since the nutrient removal and recoveries were higher than or similar to the urea commercial fertilizer. However, the benefit of TS as a fertilizer depends on the rate and method of application. Injected TS was superior to broadcast and incorporated, similar to other fertilizer materials. The TS was effectively applied in the field using equipment developed for injecting liquid swine manure slurries. If injection method is to be adopted for TS application, the low rate of application generally appears to be appropriate and sufficient, especially under repeated application. The results of this study suggest that fertilization with injected TS using liquid manure applicators can be an effective solution for TS management. Recycling nutrients contained in TS by application as a fertilizer to soil will help compensate for the nutrients that are removed from the soil when ethanol feedstocks are grown and thereby lead to a more sustainable ethanol production system. Further research should investigate changes in chemical and biochemical soil properties following repeated application of TS.

8. UTILIZATION OF THIN STILLAGE FROM WHEAT-BASED ETHANOL PRODUCTION IN AGRICULTURAL SOIL FERTILIZATION: ITS IMPACT ON SOIL CHEMICAL AND BIOLOGICAL PROPERTIES IN THE FIELD

8.1 Preface

In Chapter 7, thin stillage (TS) was shown to be an effective nutrient source for field production of wheat and canola, especially when injected. However, the acidic nature ($\text{pH} = 4.0$) of TS may alter soil pH, affecting pH dependant parameters such as trace metal availability, microbial activity and also nutrient solubility. Residual nutrient accumulation, especially available P and NO_3^- -N, in the soil profile in the field is also of environmental concern, especially if rate of nutrient application exceeds crop requirements for many years. Therefore, the objective of the current study reported in this chapter was to assess some selected soil chemical and biological properties in the Black Chernozemic soil after two field seasons of TS application.

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8.2 Abstract

Rapid growth in the bioenergy industry has resulted in a large volume of its associated by-product of thin stillage (TS). Appropriate uses of TS need to be explored, including its application to soil. The aim of the current study was to assess the effects of direct application of TS over two growing seasons on soil residual available N and P content along with some selected soil chemical (organic C, pH, electrical conductivity, metals content) and biological (dehydrogenase activity and microbial biomass) properties after crop harvest. The experimental treatments included injected TS, broadcasted and incorporated TS, and urea fertilizer with three application rates equivalent to 50, 100, and 200 kg available N ha⁻¹ in addition to a control. In the second year, TS increased NO₃⁻-N content after crop harvest even at the lower soil depths, especially with injected TS at high rate. Soil available P content significantly increased in both years, but was greater in the second year. This reflects the high plant availability of N and P in TS and a persistence of release of nutrient through TS organic matter decomposition. In selected treatments, microbial biomass was higher in TS than urea treatments, but not significantly different from the control. The other measured soil parameters remained unchanged for both years. This study indicates that continuous application of TS may contribute to NO₃⁻-N and P accumulation in soil beyond the year of application via carryover of unused inorganic forms and also possibly through increased microbial mineralization.

8.3 Introduction

Biochemical conversion of starch or sugar crops to produce ethanol also results in generation of TS, a major by-product associated with this process. This by-product is a consequence of the fermentation and distillation processes involved in ethanol generation from renewable sources (Mustafa et al., 2000). The fermentation and distillation process of the feedstock results in a product called whole stillage, which contains solids from the used feedstock plus added yeast and liquid from the water added during the process. This whole stillage is then centrifuged to separate the liquid components, called TS, and the solid components termed WDG. The TS may then be further processed by evaporation to produce syrup which can be blended with WDG resulting in WDG with solubles (Bonnardeaux, 2007).

The pressing demand for sustainable energy from renewable sources has resulted in expansion of ethanol production worldwide, and coincidentally the associated by-product of TS. It has been estimated that each litre of ethanol produced is associated with about 20 L of TS generated (van Haandel and Catunda, 1994). This high volume of TS requires a proper method of utilization that is economically sound and environmentally benign. The evaporation process can help to concentrate TS constituents into a lesser volume; however, this process can have an undesirable impact on the energy balance of ethanol production (Faust et al., 1983). One possible uses of TS is to use it to partially or completely replace drinking water for cattle (Mustafa et al., 2000). However; this method is not a commonly adopted practice and may not be able to accommodate the continual rise in TS accumulation, as the rapid growth in ethanol manufacturing is expected to create a surplus of ethanol by-products (Rausch and Belyea, 2006). Therefore, other alternative utilization avenues need to be sought, including direct application to soil as organic amendment. This possible option can benefit plant and soil by recycling the plant nutrients and carbon that are present in TS. A broad range of organic material (e.g. animal manures, biosolids, composts) application to agricultural soils have already been extensively evaluated, with results showing positive impacts of additions of these materials on various soil properties that far outweighed the negative impacts (Edmeades, 2003; Hargreaves et al., 2008; Diacono and Montemurro, 2010; Quilty and Cattle, 2011). However, effects of bioenergy by-products, such as TS on soil attributes are not well documented.

A few studies have evaluated the effect that application of vinasse stillage produced from molasses, a by-product of the sugar industry, has on soil properties (Gemtos et al., 1999; Singh et al., 2003; Resende et al., 2006; Tejada and Gonzalez, 2005; Hati et al, 2007; Tejada et al., 2007). However, the chemical characteristics of TS are different from vinasse and variable, and differ according to feedstock type and the treatments used in the bioenergy production plant. For instance, TS derived from biochemical conversion of a starch crop, such as corn or wheat (the two most popular crops for ethanol production in North America and Europe) (Mustafa et al., 2000; Cardona and Sánchez, 2007), is different in its chemical properties from distillery wastewater (vinasse) produced from sugar cane or molasses (España-Gamboa et al., 2011). Field studies examining effects of repeated application of TS using advanced application techniques on soil attributes are lacking. Fertilization with TS may contribute to build-up of residual available N and P in the soil as a result of unused plant available forms added in excess of crop demand, and also as a result of continued mineralization of organic forms of these nutrients into inorganic

forms. Excess nutrient may pose an environmental risk, especially if rate of nutrient application exceed crop requirements for many years. In addition, the acidic nature of TS and its high content of salts may adversely affect soil chemical and biological properties. These possible effects need to be clarified. Therefore, the objective of the current study was to assess changes in some selected soil chemical and biological properties in a Black Chernozemic fertilized for two years in the field with TS derived from wheat-based ethanol production.

8.4 Materials and Methods

8.4.1 Site description

The experimental site was located near the town of Dixon in east-central Saskatchewan, Canada. It is located within a productive agricultural region in Saskatchewan (Stumborg et al., 2007). The predominant soil at the site is classified as a Black Chernozem (Cudworth Association) of clay-loam texture. The average particle-size distribution in the 0-15 cm depth was 30% sand, 23% silt and 47% clay, determined using pipette method (Gee and Bauder, 1986). The study site has a nearly level topography. Prior to the current study, the field was cropped to barley (*Hordeum vulgare* L.). The basic characteristics of the field soil are shown in Table 8.1. The average long-term annual precipitation and temperature for this area is 373 mm and 0.7 °C respectively (Stumborg et al., 2007). Monthly cumulative rainfall and mean air temperature over the two growing seasons and the 30-yr average are summarized in Fig. 8.1. The climate data were retrieved from a weather station located at Humboldt approximately 5 km from the experimental site (Environment Canada, 2012).

8.4.2 Experimental design

The field experiment was established in fall 2008. The experimental setup was a randomized complete block design with 10 treatments repeated four times. The experimental treatments included: injected TS (INJTS), broadcasted and incorporated TS (BRTS) and banded urea (UR) for comparative purposes, each of which was applied at three rates: low (L), medium

Table 8.1. Selected soil properties at the start of the field study in fall 2008 in samples collected from control plots at three depths.

Property	Soil depth (cm) [§]		
	0-15	15-30	30-60
NO ₃ ⁻ -N (mg kg ⁻¹)	7.0 ± 0.3	4.8 ± 0.2	5.1 ± 0.3
NH ₄ ⁺ -N (mg kg ⁻¹)	4.3 ± 0.3	5.0 ± 0.1	5.0 ± 0.5
Avail. P (mg kg ⁻¹)	5.1 ± 1.0	1.9 ± 0.0	2.0 ± 0.1
Avail. K (mg kg ⁻¹)	275 ± 23	128 ± 15	115 ± 3
Organic C (mg g ⁻¹)	28 ± 2.0	13 ± 2.0	7.0 ± 1.0
pH	8.0 ± 0.1	7.8 ± 0.1	7.9 ± 0.03
EC (dS m ⁻¹)	1.5 ± 0.8	3.7 ± 0.7	4.8 ± 0.2
Sand (%)	29.7 ± 0.5	19.4 ± 3.3	22.8 ± 3.3
Silt (%)	23.6 ± 3.0	26.3 ± 1.8	22.4 ± 2.1
Clay (%)	46.7 ± 3.5	54.2 ± 5.1	54.8 ± 1.2

§ values presented are means followed by standard error

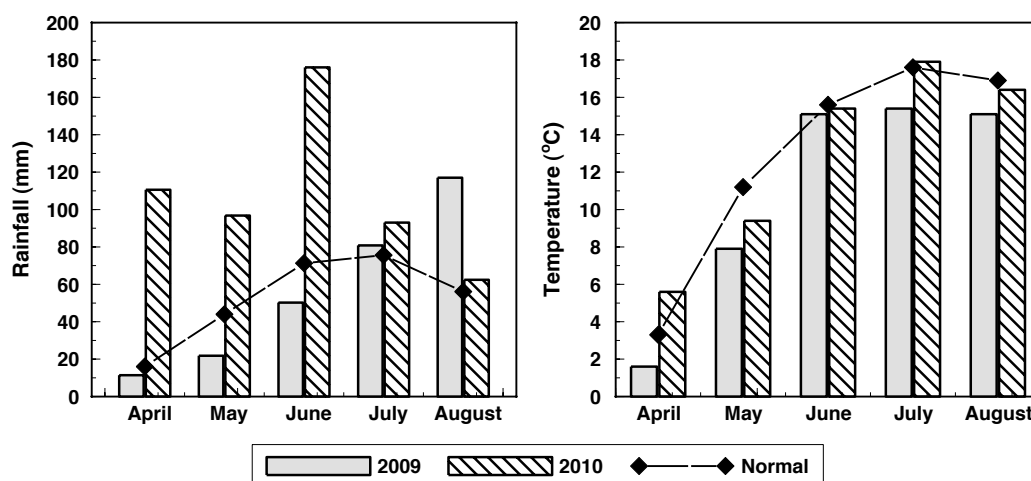


Fig. 8.1. Monthly total precipitation and mean air temperatures at the experimental site for the entire growing season for the two years of the study (2009, 2010). The 30-yr rainfall and temperature averages (normal) are also included.

(M) and high (H). A control (unfertilized, unamended) was included for comparison. The three rates of TS application were: 16800, 33600 and 67200 L ha⁻¹, intended to provide approximately 50, 100 and 200 kg available N ha⁻¹, respectively, based on an assumption that about 60% of the total N in TS would be available during the course of the year (Qian et al., 2011). The three rates of conventional fertilizer urea (46-0-0) applied for comparison were 50, 100 and 200 kg N ha⁻¹. Plot dimensions were 3 by 9 m in blocks that were spaced apart by 27 m. Plots received the same treatments in both 2009 and 2010.

8.4.3 Treatment application

The TS utilized in this study is a by-product of bio-ethanol production from wheat grain feedstock. It was provided by Pound-Maker Agventures ethanol plant located at Lanigan, Saskatchewan. The collection, delivery and application of the TS were carried out by the Prairie Agricultural Machinery Institute (PAMI, Humboldt, SK). Application of treatments to the field took place in the preceding fall (1st wk of October) of each growing season. For the injection method of application, TS was applied using the PAMI liquid slurry injector truck. The TS was agitated as it was pumped into the PAMI injector truck. The injector truck is equipped with modified Bourgault low disturbance injector disc coulters spaced 30 cm apart. The TS was applied in bands behind the coulters at an average depth of 8-10 cm. For the broadcast and incorporation method, the injectors were lifted above the soil surface to get TS applied on the soil surface in a band, followed by immediate incorporation with a chisel plow cultivator using one pass with 30 cm sweeps on a 20 cm row spacing, followed by harrowing. During TS application to soil and for both years, several samples were collected at the injector opening, mixed to yield a homogenous representative sample and stored in the freezer (-20 °C) until analysis for chemical composition. The analysis of TS was conducted at a commercial laboratory (ALS Laboratory Group, Saskatoon, SK). Basic characteristics of TS applied for each year are provided in Table 8.2. Commercial granular urea fertilizer (46-0-0) was banded into the soil using PAMI's plot drill at an 8 cm depth with knives on a 20 cm row spacing.

The field was seeded to Lillian variety hard red spring wheat (*T. aestivum*) and BrettYoung 719 Roundup Ready variety canola (*Brassica napus* L.) in the seasons of 2009 and 2010, respectively. Information regarding planting and harvesting details and crop related

parameters measured and their response to treatment application were previously reported in Chapter 7.

Table 8.2. Basic characteristics of thin stillage (TS) applied in 2008 and 2009. All contents are expressed on a fresh wet weight basis.

Property	Year	
	2009	2010
Total N (mg g ⁻¹)	4.7	5.7
NH ₄ ⁺ -N (mg g ⁻¹)	0.90	0.10
Total P (mg g ⁻¹)	0.90	0.11
Total K (mg g ⁻¹)	1.1	1.2
Total S (mg g ⁻¹)	0.6	0.7
Na (mg g ⁻¹)	0.4	0.3
Ca (mg g ⁻¹)	0.2	ND
Mg (mg g ⁻¹)	0.4	ND
pH	3.8	4.10
Moisture (%)	92.5	91.6

8.4.4 Soil sample collection

The first soil sample collection occurred immediately after experimental plot layout in fall of 2008 for initial soil characterization of the study site. For this purpose, soil samples were collected only from the control plots at three soil depth increments (0-15, 15-30 and 30-60 cm). To evaluate treatments effects on residual soil available N and P and some selected soil chemical and biological properties, soil samples were collected immediately after crop harvest at the end of growing seasons before freeze-up (mid October) for both years from all plots. A hydraulic punch truck was used to collect soil cores (three per plot), which were separated into different soil depths increments: 0-15, 15-30 and 30-60 cm in the first year whereas in the second year the samples were collected at 0-15, 15-30, 30-60 and 60-90 cm depths. The triplicate soil samples collected from each depth were mixed thoroughly to produce a single composite sample for each depth per plot. A subsample was taken from the prepared composite soil sample of selected treatments and immediately frozen at -20°C for later use for microbial biomass analysis. The rest of soil samples were air-dried and ground to pass a 2-mm sieve prior to laboratory analysis. Soil samples collected in both years at all depths were analyzed for inorganic N (NH₄⁺-N and

NO₃⁻-N) whereas the analysis of other selected parameters were limited to soil samples collected from the upper soil depth (0-15 cm). Dehydrogenase enzyme activity and microbial biomass were determined only in medium rate of injected TS and urea treatments in addition to the untreated soil.

4.5 Soil analysis

Exchangeable NH₄⁺-N and NO₃⁻-N were extracted by shaking 5 g of soil with 50 mL of 2 *M* KCl for 1 h on rotary shaker, followed by filtration. The NH₄⁺-N and NO₃⁻-N content in the KCl extracts were measured colorimetrically using a Technicon Autoanalyzer II (Keeney and Nelson, 1982). Available P and K were determined by a modified Kelowna method (Qian et al., 1994). Electrical conductivity and pH were measured in 1:1 soil:water suspension. Soil available Cu, Zn and Cd were extracted by ammonium bicarbonate (AB)-diethylenetriaminepentaacetic acid (DTPA) as described by Lipoth and Schoenau (2007). Extracted Cu, Zn and Cd were then determined using atomic absorption spectrometry (Baker and Amacher, 1982). Dehydrogenase activity analysis involved the reduction of 2,3,5-triphenylterazolium chloride (TTC) to triphenyle formazan (TPF) as described by Casida et al. (1964) and slightly modified by Serra-Wittling et al. (1995). Soil content of microbial biomass C (MBC) and microbial biomass N (MBN) was determined by fumigation extraction procedure as outlined by Voroney et al. (2008). The values of nonfumigated samples were subtracted from those obtained from fumigated samples, and MBC and MBN were calculated using K_{EC} factor of 0.45 for MBC (WU et al., 1990; Joergensen, 1996) and K_{EC} factor of 0.54 for the MBN (Joergensen and Mueller, 1996).

8.4.6 Statistical analyses

Normality of raw data and homogeneity of variance were checked prior to data analysis using Shapiro-Wilk and Bartlett tests, respectively. Then, a one-way analysis of variance (ANOVA) was employed to analyze treatment effects on soil variables. Treatments effects were declared statistically significant at a probability level of $P \leq 0.1$ at which means were also separated by Fisher's protected LSD. A probability level of 0.1 rather than 0.05 was selected as the significance level for this study owing to the inherently high degree of variability in soil and biological properties encountered in liquid fertilized fields (Stumborg and Schoenau, 2008).

8.5 Results

8.5.1 Effects on residual available N and P

Residual inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) contents in soil collected from different depths after harvest in the first year are shown in Table 8.3. The inorganic N content at all depths was dominated by $\text{NH}_4^+\text{-N}$, which was not significantly affected by treatment application at any depth. Concentrations of residual $\text{NO}_3^-\text{-N}$ were low ($< 7 \text{ mg kg}^{-1}$) and $\text{NO}_3^-\text{-N}$ concentration was only affected by treatment at the depth of 15-30 cm. At this depth, urea applied at the high rate had the highest content of residual $\text{NO}_3^-\text{-N}$, but was not significantly different from the control (Table 8.3). In contrast to the fall of 2009, the effects of two years of treatments made on residual $\text{NO}_3^-\text{-N}$ as measured in the fall of 2010 were more pronounced, with higher levels of residual $\text{NO}_3^-\text{-N}$ observed in the year 2010 (Table 8.4). In fall of 2010, treatment had a significant impact on soil $\text{NO}_3\text{-N}$ content in all soil depths measured after harvest. In the surface soil (0-15 cm), the residual $\text{NO}_3^-\text{-N}$ concentration was greatly affected by treatment application, with injected TS treatment having the highest $\text{NO}_3^-\text{-N}$ content compared to the other treatments, when averaged across the three rates of application. With the exception of BRTS-L and UR-L treatments, all the treatments had significantly higher amounts of soil $\text{NO}_3\text{-N}$ than the control. The medium and high rates of injected TS and urea plus the high rate of broadcasted and incorporated TS treatments contained the greatest concentrations of $\text{NO}_3^-\text{-N}$ ($> 10 \text{ mg kg}^{-1}$) and these treatments did not differ significantly from each other. Treatment effects on $\text{NO}_3^-\text{-N}$ content persisted to the second soil depth increment (15-30 cm) in which most of the treatments had significantly higher concentration of $\text{NO}_3^-\text{-N}$ than that in the control (Table 8.4). At this depth, the $\text{NO}_3\text{-N}$ content in injected TS at any rate was significantly higher than that in the control, with the INJTS-H treatment showing the greatest amount of $\text{NO}_3^-\text{-N}$.

When averaged over the three rates of application, injected TS had the highest content of $\text{NO}_3^-\text{-N}$ followed by urea whereas the broadcasted and incorporated TS treatment had the least. Greater losses of N as a result of volatilization and immobilization may be occurring with broadcast and incorporate application compared to injection. Moreover, the significant impact of treatments continued to the lower soil layer (30-60 cm). At this depth, the high rate of injected TS and urea treatments had the highest $\text{NO}_3^-\text{-N}$ content, and both were significantly higher than

Table 8.3. Soil content of residual inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) at three soil depth increments determined in soils collected in the fall of the first year (2009) of the two-yr field trial (mean \pm standard error).

Treatment [¶]	Depth (cm)					
	0-15		15-30		30-60	
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$
	-----mg kg ⁻¹ -----					
Control	10.4 \pm 0.9 [§]	4.5 \pm 0.3	10.5 \pm 2.3	3.7 \pm 0.5 ab	20.1 \pm 6.4	2.7 \pm 0.7
INJTS-L	11.7 \pm 1.9	5.2 \pm 0.2	19.2 \pm 2.0	2.7 \pm 0.2 bc	15.0 \pm 3.8	3.4 \pm 1.4
INJTS-M	10.2 \pm 1.3	6.5 \pm 0.5	14.6 \pm 4.2	3.0 \pm 0.5 bc	8.2 \pm 4.3	3.9 \pm 1.3
INJTS-H	8.0 \pm 1.1	5.4 \pm 1.3	15.1 \pm 3.8	3.6 \pm 1.3 bc	11.2 \pm 2.0	2.7 \pm 0.7
BRTS-L	9.6 \pm 2.1	6.0 \pm 0.6	18.3 \pm 2.9	2.4 \pm 0.2 c	16.2 \pm 4.8	2.3 \pm 0.1
BRTS-M	8.5 \pm 2.0	5.2 \pm 0.7	12.1 \pm 2.0	2.7 \pm 0.2 bc	6.1 \pm 0.5	2.5 \pm 0.4
BRTS-H	8.7 \pm 0.7	5.3 \pm 0.6	16.3 \pm 4.5	2.3 \pm 0.1 c	9.1 \pm 2.5	1.8 \pm 0.1
UR-L	11.3 \pm 1.5	5.8 \pm 0.7	18.9 \pm 4.9	2.4 \pm 0.1 c	17.3 \pm 2.2	2.4 \pm 0.4
UR-M	8.7 \pm 1.6	5.9 \pm 0.8	11.2 \pm 2.5	3.2 \pm 0.4 bc	8.8 \pm 2.0	4.8 \pm 1.7
UR-H	8.1 \pm 0.8	6.3 \pm 0.9	11.3 \pm 3.0	4.5 \pm 0.4 a	10.1 \pm 3.3	4.1 \pm 0.6

ANOVA

Treatment	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>0.057</i>	<i>NS</i>	<i>NS</i>
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¶ INJTS denotes injected thin stillage, BRTS denotes broadcasted and incorporated thin stillage, UR denotes urea fertilizer. Each of these treatments are followed by the rate of application: high (H), medium (M) and low (L).). For TS, high rate is 67200 L ha⁻¹, medium rate is 33600 L ha⁻¹, and low rate is 16800 L ha⁻¹

§ Means within a column sharing the same letter are not significantly different at $P < 0.10$.

all other treatments including the control (Table 8.4). However, NO_3^- -N content in broadcasted TS at any rate treatment did not differ from the control. The treatments had also a strong impact on NO_3^- -N content at the deepest soil depth increment (60-90 cm), with the high rate of urea fertilizer treatment having the highest level of NO_3^- -N, followed by the injected TS applied at the high rate (Table 8.4). This suggests greater leaching potential of nitrate derived from the fertilizer urea compared to the TS. At this depth, the broadcast and incorporate TS treatment had lowest NO_3^- -N, when averaged over the three rates of application.

Soil content of residual extractable P was significantly influenced by amendment application in both years, but the treatment effects were more evident after the second year of application (Table 8.5). In both years, post-harvest extractable P content of the soil tended to increase with increasing rate of TS application, and was the greatest in soil when the TS was injected or broadcasted at the high rate (Table 8.5). At equivalent rate of application for a second year, the amount of extractable P in the fall of 2010 was higher than that observed in the fall of 2009. As expected, soil fertilized with urea had the lowest content of available P, and this was significantly lower than the control in the year 2010, reflecting depletion by crop uptake over two years.

8.5.2 Effects on other selected soil chemical properties

Extractable K and organic C determined after crop harvest in both years were not affected by treatment application (Table 8.5). Soil pH measured after crop harvest in both years remained unchanged, and the same was also observed with EC (Table 5). Extractable trace metals (Cu, Zn, Cd) determined in the second year were not also influenced by treatment application (Table 8.5).

8.5.3 Effect on dehydrogenase activity and microbial biomass

Dehydrogenase activity determined in the selected treatments was not significantly influenced by treatment application in both years (Table 8.6). However, in the same selected treatments, MBC and MBN were both affected by treatment application in the year 2009, but not in the year 2010 (Table 8.6). In general, soil content of MBC and MBN was higher in the fall of

2009 than in 2010. Urea addition resulted in reduced MBC and MBN content, that was significantly lower than the INJTS-M and control treatments (Table 8.6).

Table 8.4. Soil content of residual inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) at four soil depth increments determined in the fall of the second year (2010) of the two-yr field trial (mean \pm standard error).

Treatment [¶]	Depth (cm)							
	0-15		15-30		30-60		60-90	
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$
	-----mg kg ⁻¹ -----							
Control	5.8 \pm 1.2	6.4 \pm 0.7 d [§]	7.1 \pm 1.9	4.0 \pm 0.2 d	10.6 \pm 2.8	2.6 \pm 0.2 b	13.0 \pm 3.4	2.0 \pm 0.1 e
INJTS-L	5.6 \pm 0.6	9.5 \pm 1.4 bc	10.3 \pm 5.2	6.5 \pm 1.7 abc	11.4 \pm 2.2	3.0 \pm 0.3 b	8.7 \pm 2.3	2.3 \pm 0.1 de
INJTS-M	6.1 \pm 1.1	16.2 \pm 3.3 a	12.7 \pm 4.3	6.8 \pm 1.1 abc	10.5 \pm 3.3	4.0 \pm 0.6 b	9.2 \pm 3.9	3.5 \pm 0.8 cd
INJTS-H	5.5 \pm 1.4	11.9 \pm 2.0 ab	6.5 \pm 2.1	10.3 \pm 2.9 a	6.9 \pm 2.7	10.3 \pm 3.3 a	6.3 \pm 1.9	7.9 \pm 1.8 b
BRTS-L	7.6 \pm 0.9	7.7 \pm 0.5 cd	13.3 \pm 4.2	5.2 \pm 0.9 bcd	12.0 \pm 2.4	3.1 \pm 0.4 b	11.6 \pm 2.6	2.5 \pm 0.2 de
BRTS-M	6.5 \pm 0.5	8.5 \pm 0.3 c	7.4 \pm 1.3	4.8 \pm 0.2 cd	15.0 \pm 2.6	2.9 \pm 0.1 b	11.8 \pm 2.1	3.2 \pm 0.8 cde
BRTS-H	6.5 \pm 1.2	11.5 \pm 0.7 ab	5.7 \pm 1.0	5.9 \pm 1.2 bcd	15.2 \pm 3.9	3.2 \pm 0.1 b	11.3 \pm 2.4	3.1 \pm 0.1 cd
UR-L	5.6 \pm 0.5	7.5 \pm 0.9 cd	10.0 \pm 2.5	4.9 \pm 1.0 cd	8.3 \pm 1.8	3.6 \pm 0.7 b	8.4 \pm 1.6	3.6 \pm 1.1 cd
UR-M	5.5 \pm 0.6	13.3 \pm 1.1 a	8.1 \pm 2.3	6.2 \pm 0.5 abc	8.8 \pm 3.2	4.2 \pm 0.7 b	6.3 \pm 1.3	4.4 \pm 1.1 c
UR-H	6.4 \pm 0.6	12.1 \pm 1.3 ab	6.0 \pm 0.9	8.2 \pm 1.8 ab	11.0 \pm 3.4	10.1 \pm 2.8 a	9.9 \pm 1.9	12.0 \pm 2.1 a
ANOVA								
Treatment	NS	<0.01	NS	0.066	NS	<0.001	NS	<0.001

¶ INJTS denotes injected thin stillage, BRTS denotes broadcasted and incorporated thin stillage, UR denotes urea fertilizer. Each of these treatments are followed by the rate of application: high (H), medium (M) and low (L). For TS, high rate is 67200 L ha⁻¹, medium rate is 33600 L ha⁻¹, and low rate is 16800 L ha⁻¹.

§ Means within a column sharing the same letter are not significantly different at $P = 0.10$.

Table 8.5. Soil content of residual available P and other selected soil properties at 0-15 cm depth determined in fall of both years of the two-yr field trial (mean \pm standard error).

Treatment [¶]	2009					2010							
	P	K	OC	pH	EC	P	K	Cu	Zn	Cd	OC	pH	EC
	-----mg kg ⁻¹ -----		%		dS m ⁻¹	-----mg kg ⁻¹ -----					mg g ⁻¹		dS m ⁻¹
Control	7.1 bc [§]	295	2.76	7.8	1.31	10.5 bc	281	2.49	2.16	0.28	27	8.0	0.8
INJTS-L	6.3 c	263	2.60	7.8	2.26	9.8 cde	274	1.68	2.28	0.26	30	8.0	0.7
INJTS-M	8.6 bc	313	3.01	7.6	1.49	11.2 bc	274	1.59	1.98	0.32	29	8.0	0.8
INJTS-H	13.7 a	307	3.01	7.6	1.24	15.2 a	280	2.05	2.28	0.25	28	7.9	1.4
BRTS-L	7.7 bc	330	2.92	7.8	1.14	10.4 bcd	316	1.79	2.15	0.37	27	8.1	0.7
BRTS-M	7.8 bc	298	2.95	7.7	1.32	11.9 b	312	2.09	1.97	0.28	26	8.0	0.8
BRTS-H	10.5 ab	320	2.92	7.8	0.82	17.0 a	350	2.04	2.31	0.39	27	8.0	0.7
UR-L	6.5 c	275	3.14	7.8	0.58	8.4 e	270	1.91	1.85	0.26	27	8.0	0.6
UR-M	6.1 c	298	2.97	7.9	0.65	8.1 e	285	1.91	1.84	0.36	27	8.1	0.6
UR-H	6.1 c	226	2.37	7.8	1.50	8.5 de	252	1.56	2.03	0.29	27	8.1	0.7
ANOVA													
Treatment	0.080	NS	NS	NS	NS	<0.001	NS	NS	NS	NS	NS	NS	NS

¶ INJTS denotes injected thin stillage, BRTS denotes broadcasted and incorporated thin stillage, UR denotes urea fertilizer. Each of these treatments are followed by the rate of application: high (H), medium (M) and low (L). For TS, high rate is 67200 L ha⁻¹, medium rate is 33600 L ha⁻¹, and low rate is 16800 L ha⁻¹.

§ Means within a column sharing the same letter are not significantly different at $P = 0.10$.

Table 8.6. Dehydrogenase activity, microbial biomass C and microbial biomass N responses to experimental treatments in soil collected in fall for both years (mean \pm standard error).

Treatment [¶]	Dehydrogenase Activity		Microbial Biomass C		Microbial Biomass N	
	2009	2010	2009	2010	2009	2010
	$\mu\text{g TPF g}^{-1}$		$\mu\text{g g}^{-1}$			
Control	263 \pm 45	386 \pm 28	546 \pm 23 a [§]	259 \pm 23	146 \pm 18 a	31 \pm 5
INJTS-M	222 \pm 39	445 \pm 50	602 \pm 66 a	247 \pm 34	150 \pm 20 a	37 \pm 7
UR-M	191 \pm 38	370 \pm 17	293 \pm 26 b	246 \pm 20	51 \pm 12 b	37 \pm 3
<i>ANOVA</i>						
Treatment	<i>NS</i>	<i>NS</i>	<i>0.002</i>	<i>NS</i>	<i>0.004</i>	<i>NS</i>

[¶] INJTS-M denotes injected thin stillage at medium (M) rate of 33600 L ha⁻¹, UR-M denotes urea fertilizer applied at 100 kg N ha⁻¹, referred to as medium rate (M).

[§] Means within a column sharing the same letter are not significantly different at $P = 0.10$.

8.6 Discussion

The lack of clear effect of treatments on residual NO₃⁻-N content following crop harvest in the first year is a consequence of the overall higher plant N uptake observed in treatment plots in that year (see Chapter 7) as the plants efficiently utilized the nutrient applied in the treatments. The accumulation of NO₃-N in the soil profile observed in this study in the fall of 2010 after two years of treatment application is in line with other studies that reported a potential of NO₃-N buildup in soil treated with distillery effluent (Sweeney and Graetz, 1991; Jenkins et al., 1988). This can be related to the high mineralization rate of applied thin stillage N, as previously documented by Jenkins et al., 1988. In a growth chamber study Qian et al. (2011) estimated that more than half of the TS total N became available for plant uptake over the five wk period. The higher NO₃-N observed in the second year of the current study might be also related to additional contribution from the release of N from TS organic matter applied in the previous year.

Tejada and Gonzalez (2005) found that application of beet vinasse during three consecutive years increased soil NO₃⁻-N concentration, but this increase was limited to the lower rates (3 and 6 t ha⁻¹) whereas higher rates of application decreased NO₃⁻-N in soil. However, in most cases, and as in the current study, increases in soil NO₃⁻-N were associated with increasing rate of application and higher levels were also observed at the high rate of TS application, especially when injected. Soil treated with digestate was also found to contain higher amount of

NO_3^- -N, and this was assumed to be a result of nitrification of the large content of NH_4^+ -N present in the digestate (Goberna et al., 2011). Ammonium represents about 20% of the TS total N, and nitrification of this ammonium may have contributed to the high NO_3^- -N content observed after the second year of the current study. The increased soil NO_3^- -N associated with injected TS compared to the broadcasted and incorporated TS may have partially been related to conserving NH_4^+ -N by reducing N loss via volatilization processes. It was previously reported that band injection of organic materials is an effective method in reducing NH_3 volatilization (Huijsmans et al.; 2003). The experimental site area experienced excessive and unusually high rainfall amounts in the summer of 2010 (Fig 1), and resulted in leaching of NO_3^- -N to greater depths, as seen in the fall 2010 samples. Despite the high crop removal of N in both growing seasons, the larger amount of NO_3^- -N residing in soil after crop harvest in the second year indicates that repeated application of TS at the high rates could pose an environmental risk of NO_3^- -N accumulation in soil profiles, increasing leaching potential to groundwater.

Addition of TS resulted in increased available P in soil. This is in line with other previous findings. In a study similar to the one conducted here, application of TS derived from sorghum feedstock resulted in a significant increase in residual available P (Jenkins et al., 1988). The same was also observed under controlled environment conditions, where TS enhanced soil content of residual available P (Qian et al., 2011). The TS used here had a high content of total P similar to that found in liquid animal manures. This increases its fertilizer value as a source of P, as shown by its significant impact on crop P uptake. However, just like for many animal manures, repeated application of TS based on N requirement may lead to excessive supply of P that exceeds crop demand, due to the narrow N:P ratio in TS. The continued increase in soil content of available P after crop harvest, especially at the high rates of application, is of environmental concern for the potential build-up of P in the soil profile.

The inherent high fertility and organic matter content of the Black Chernozem soil used in this study may have resulted in the absence of a clear effect on some selected parameters, such as organic C and available K. The Black Chernozemic soil has a high content of native soil organic matter, and the amount of added organic matter as TS was likely not adequate to produce a measurable significant difference in soil organic C content. It was reported elsewhere that soil C content was not a sensitive indicator of changes in the C pools in high organic matter soils receiving organic materials (Eghball et al., 2004).

The lack of a significant impact of TS addition on soil pH and salinity (EC) in the current study is consistent with findings in a study in which addition of distillery effluent for three consecutive years to a clay soil with a pH of nearly 8.0 did not affect the soil pH, attributing this to the high buffering capacity of the studied soil (Hati et al., 2007). However, in an early greenhouse study, application of distillery waste anaerobic digester effluent to a relatively acidic soil increased soil pH (Sweeney and Graetz, 1991). In the current study, a pH of 8.0 indicates that this soil may contain free CaCO_3 , and thereby has a high buffering capacity against pH alteration (Bache, 1984). This is consistent with the results obtained from a growth chamber study where a similar TS was used (Qian et al., 2011). Sweeney and Graetz (1991) also observed no effect on trace metals content in soil receiving distillery effluent. The low concentration of trace metals added with TS, and the high soil pH that remained unaltered may explain the soil content of extractable trace metals being unaffected by TS application.

The reported effects of distillery effluent on soil biological properties (e.g. enzyme activities and microbial biomass) are inconsistent in the literature. This is likely related to the variations in chemical composition among the by-products used, in addition to differences in soil conditions among the studied soils, and the time period that had elapsed between amendment and measurement. For instance, fresh beet vinasse was found to have a negative impact on dehydrogenase activity and microbial biomass (Tejada et al., 2007), whereas another study found that addition of three distillery effluents varying in their chemical characteristics resulted in increased dehydrogenase activity in soil (Singh et al., 2003). It was also reported that microbial biomass increased in soil treated with distillery effluent addition (Hati et al., 2007). In the current field study, the TS derived from wheat grain had no negative effect on biological properties of the Black Chernozemic soil measured in the fall of the year after crop harvest. This indicates that TS did not suppress soil microbial activity. An interesting finding was that urea fertilizer amendment tended to reduce soil content of MBC and MBN in the first year of the study, but not the second year. Unfortunately measurements of MBC and MBN were not made throughout the growing season. A longer-term field study may help clarify these effects and their origin.

8.7 Conclusion

The results of the current study indicate that repeated application of TS can lead to build-up of residual NO_3^- -N and extractable P in soil. Such accumulations of NO_3^- -N and P in

soil could result in potential risks of NO_3^- -N leaching and P runoff. Under annual application practice, release of nutrients from TS application likely can persist into the following year, due to its high decomposability in soil. Injection of TS appears to result in better retention of added N than broadcast application. Reduced frequencies of TS application with continuous crop production might be a better solution to allow maximum utilization of available nutrients left in soil from previous applications. A low rate application may be recommended especially under repeated yearly application regimes. There were no significant impacts of two years of TS application on pH, salinity, organic C or extractable metals content at this site. Addition of TS did not show any negative impact on measured microbial parameters (dehydrogenase enzyme and microbial biomass). A further field study monitoring the effects of several years of TS amendment may be useful to better understand the effect of TS addition on soil microbial community structure and activity.

9. BIOCHAR AND GLYCEROL APPLICATION TO A PRAIRIE SOIL IN THE FIELD: CROP RESPONSE AND CHANGES IN SOIL BIOLOGICAL AND CHEMICAL PROPERTIES

9.1 Preface

Biochar (BC) is a by-product of pyrolysis of organic materials during biogas production whereas glycerol (GL) is by-product of transesterification of vegetable oils during biodiesel production. These two by-products have a high content of C, and based on this, they were grouped in one study and reported in this final chapter. The studies conducted on the impact of GL on soil nutrient release and biological properties under controlled environment conditions are covered in Chapter 3 and Chapter 4 revealed that GL application to soil may be a possible option for its utilization. Unlike GL, the number of research studies concerning soil amendment with BC continues to grow globally. A field research study was conducted to investigate direct and residual effects of BC and GL application on crop yield, nutrient uptake and changes in selected soil chemical and biological properties in a Brown Chernozemic soil. This soil type was selected because it may better benefit from C-rich substrate application due to its lower content of organic matter.

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9.2 Abstract

Unlike glycerol (GL), biochar (BC) has recently received more attention globally as a potential soil amendment, especially in highly weathered, acidic soils. However, this potential has not been well-evaluated for young neutral to alkaline soils. This study investigated the immediate and residual effects of a single application of BC and GL to a cultivated Brown Chernozem from the semi-arid region of southwestern Saskatchewan. Field plots were treated with BC and GL applied alone at $2000 \text{ kg C ha}^{-1}$, or with N (BC+N and GL+N, respectively). A treatment of 100 kg N ha^{-1} as urea (UR) was included for a comparison. Selected crop and soil parameters were measured. In the first season following a spring application of the amendments, addition of BC and GL alone had no significant impact on crop or soil parameters. However, when combined with 50 kg N ha^{-1} , BC provided similar or larger yields than those following the 100 kg N ha^{-1} treatment, suggesting that BC may have improved urea N use efficiency. The GL+N reduced crop yield and N uptake, probably due to microbial immobilization of N. However, this treatment had a significant residual effect in the second year on crop yield and N uptake, presumably because of the re-mineralization of the N that was immobilized after application in the first season. Both GL and GL+N treatments enhanced dehydrogenase activity compared to other treatments whereas BC+N significantly decreased microbial biomass C, possibly related to the depletion of soil nutrients owing to high crop growth observed in the same treatment. No further residual impacts were detected in following seasons. The soil response to application of amendments, especially BC, was less than expected according to the previous studies conducted elsewhere. A greater application rate of amendments may be required to demonstrate a response.

9.3 Introduction

Biochar (BC) is the carbon-rich by-product of the thermal breakdown of C-based feedstocks in absence of oxygen and at relatively low temperature ($< 700^{\circ}\text{C}$) using the pyrolysis process, which is employed to convert biomass into liquid, gas and BC (Lehmann and Joseph, 2009; Kwapinski et al., 2010). Application of BC to soil is not a new concept, but it has recently attracted a global interest due to its potential agronomic and environmental benefits in addition to

offering an improved organic waste management strategy. It has widely been evaluated as a possible means to improve soil fertility, increase crop productivity and reduce greenhouse gas emissions (Lehmann et al., 2011) in a variety of soils. The relatively recalcitrant nature of C in BC to microbial decomposition leads to its effective use for sequestering carbon in soil, thereby mitigating climate change (Laird, 2008; Sohi et al., 2010). In addition, BC addition was shown to decrease N₂O and CH₄ emissions from soil (Rondon et al., 2005; Yanai et al., 2007; Roberts et al., 2010) and frequently reported that crop growth can be improved by BC application (Lehmann et al., 2003; Yamato et al., 2006; Rondon et al., 2007; Steiner et al., 2007; Chan et al., 2008; Blackwell et al., 2009; Major et al., 2010).

The increases in crop productivity following BC application may occur directly through supply of essential nutrient, or indirectly through improving soil properties and functions (Lehmann and Joseph, 2009; Woolf et al., 2010). However, BC itself is not expected to supply nutrient due to its low content of available plant nutrients and the recalcitrant nature of the material. Consequently, it has been recommended that BC be supplemented with fertilizer when applied to soil (Lee et al., 2010). It has been found that the mechanisms responsible for the improvements in crop production include enhanced water and nutrient retention, improved soil structure and drainage (Jeffery et al., 2011) as well as increased cation exchange capacity, changes in pH and in electrical conductivity (EC) (Liang et al., 2006; Gundale and DeLuca, 2007; Warnock et al., 2007; Amonette and Joseph, 2009). Indirect added value from BC to nutrient efficacy is related to its ability to reduce N loss through volatilization and leaching processes via absorbance of NH₃ and retention of ammonium (Chan and Xu, 2009; Singh et al., 2010; Lehmann et al., 2003a; Major et al., 2009).

Some previous studies have reported effects of BC application on soil microbial activity and microbial community structure that are considered to be indicators of soil health and quality (Rondon et al., 2007; Warnock et al., 2007; Steiner et al., 2008). Most of the work examining the agricultural and environmental impacts of BC application, especially the ones showing positive benefits of BC application, have been limited to tropical regions (Atkinson et al., 2010; Spokas et al., 2012). Therefore, several key soil properties and functions pertinent to soil productivity in highly weathered or degraded and acidic soils are likely to show positive benefits of BC utilization (Atkinson et al., 2010). However, crop and soil responses may be different when BC is applied to soils in arid, semi-arid or temperate regions. Studies in this context need to be

geographically expanded to include regions with different soils and climatic conditions in order to create a robust body of scientific research to understand how BC effects and benefits vary geographically and temporally prior to serious contemplation of global scale implementation of BC. Currently, BC application to agricultural soils is rare in Canada, with no studies conducted on calcareous prairie soils typical of the northern Great Plains.

Glycerol (GL), also known as glycerin, is another C-rich by-product generated during the manufacture of biodiesel via transesterification of vegetable oils. It comprises a significant portion of biodiesel production in which every tonne of biodiesel produced generates 100 kg of GL. The global production of biodiesel is projected to reach over 140 billion L by 2016 with an average annual growth of 42%, which will lead to approximately 15 billion L of crude GL being generated (Fan et al., 2010). This will lead to a surplus of GL and will also have an impact on the GL market. Surplus GL by-product is dealt with as a waste material. More applications of this by-product need to be developed to help sustain biodiesel production. One example of a potential use of glycerol is its direct application to soil as amendment. This potential has received little attention.

Glycerol lacks essential plant nutrient content, such as N and P. However, one potential benefit of its agricultural use is that it could be used as a C source amendment to improve soil quality through enhancing soil organic matter content and biological activity, especially in degraded soils that contain low organic matter due to the lack of organic inputs. As shown in Chapter 3, under growth chamber conditions, addition of GL to soil led to microbial immobilization of soil N. Addition of GL also showed a positive impact on enzyme activity and soil microbial C content in a controlled environment study. These findings encourage further research studies under field conditions to help assess their potential as soil amendment.

The objective of the current study was to examine the effect of BC (derived from oat hull) and GL (derived from canola biodiesel production) applied once to a semi-arid prairie soil on crop yield, nutrient uptake, dehydrogenase activity, soil microbial biomass C and N and selected soil chemical properties over a 3-yr period.

9.4 Materials and Methods

9.4.1 Experimental site

The experiment was carried out on agricultural land in a canola-wheat rotation located near the town of Central Butte (50°47'31" N lat, 106°30'28" W long) in south-central Saskatchewan. The soil at this site is classified as Orthic Brown Chernozem (Soil Association: Ardill Loam), with a loamy texture and nearly level topography. The field at this site was cropped to hard red spring wheat in the year prior to the current study. Immediately after experimental plots were laid out in spring of 2009, selected soil properties to characterize site were determined on soil samples collected across the study area from the control plots at three soil depth (0-15, 15-30 and 30-60 cm) increments (Table 9.1). The soil is deficient in available N and P, sufficient in K, and with low organic matter content and high pH. The soil is non-saline. Climate data during the growing season for the three-yr study period were obtained from a weather station located near the experimental site (Environment Canada, 2012). Monthly cumulative rainfall and mean air temperature over the three growing seasons and the 30-yr average are given in Table 9.2.

9.4.2 Experimental design

The field experiment was initiated in spring 2009. Experimental plots were laid out with a dimension of 2 m × 2 m for each plot, to which the treatments were immediately applied. The limited amount of available BC and GL amendments dictated the plot size. The amendments of BC and GL were applied based on applying equal amount of C. Thus, the experimental treatments included one rate of BC and GL applied at 2000 kg C ha⁻¹, either alone or combined with urea N. The GL was combined with 100 kg N ha⁻¹ (GL+N) whereas BC was combined with 50 kg N ha⁻¹ (BC+N), as the N content of BC applied at 2000 kg C ha⁻¹ rate added 50 kg N ha⁻¹ itself, giving a total of 100 kg N ha⁻¹. The treatments also included one rate of urea (UR) fertilizer applied at 100 kg N ha⁻¹, which is the typical rate of N applied in the area and an unamended/unfertilized plot (control). The assigned treatments were applied in a completely randomized design and replicated four times. The amendments were added only once in spring 2009, but the effects of treatments were monitored for three growing seasons (2009-2011).

Table 9.1. Selected soil properties at the beginning of the field study in spring 2009 at three soil depth increments (0-15, 15-30 and 30-60 cm)

Property	Soil depth (cm) [§]		
	0-15	15-30	30-60
NO ₃ ⁻ -N (mg kg ⁻¹)	3.9 ± 0.9	3.2 ± 0.8	3.1 ± 0.4
NH ₄ ⁺ -N (mg kg ⁻¹)	2.6 ± 0.1	2.6 ± 0.2	3.0 ± 0.1
Avail. P (mg kg ⁻¹)	10.6 ± 1.4	8.4 ± 1.3	4.9 ± 0.8
Avail. K (mg kg ⁻¹)	348 ± 36	279 ± 32	239 ± 22
OC (%)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.2
pH	7.9 ± 0.1	7.9 ± 0.0	8.0 ± 0.1
EC (dS m ⁻¹)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0

§ values presented are means (*n* = 4) followed by standard error

Table 9.2. Monthly total precipitation and mean air temperatures at the experimental site for the entire growing season for the three growing seasons (2009, 2010, 2011).

Month	Precipitation				Mean temperature			
	2009	2010	2011	30-yr Avg.	2009	2010	2011	30-yr Avg.
	-----mm-----				°C			
April	10.2	34.1	4.5	22.9	2.6	6.2	3.2	4.6
May	19.6	124.4	31.2	51.8	9.3	9.0	10.3	11.6
June	47.7	75.3	96.1	67.9	14.9	16.4	15.0	16.2
July	80.9	55.7	52.5	63.6	16.3	17.8	18.8	18.5
Aug.	48.5	43.6	14.7	44.1	15.8	16.8	18.1	17.9
Sept.	14.6	55.4	5.2	31.9	16.6	10.9	14.4	11.7

9.4.3 Amendment procurement, preparation and application

The BC material was obtained from Titan Clean Energy, Prince Albert, Saskatchewan, produced from pyrolysis of oat hulls, a by-product obtained from the oat milling process. The application of oat hull BC to soil can be a viable option for recycling nutrient contained in this by-product. A homogenous subsample of BC was collected and sent to a commercial laboratory (ALS Laboratory Group, Saskatoon, SK) to determine its nutrient content. Selected characteristics of the BC are provided in Table 9.3. Prior to field application, the bulk BC was homogenized by breaking and crushing larger chunks manually to pass through a 2 mm sieve. It was weighed, bagged and broadcast applied by hand. For the treatment in which BC was combined with mineral fertilizer, first the UR was broadcast by hand, and then the BC applied. The GL material, a thick syrupy liquid from canola-based biodiesel production, was obtained from Milligan Biotech Ltd, Foam Lake, Saskatchewan. It was stored at 4 °C prior to use. The GL used was a crude methanol-stripped product. It is a carbon rich material containing 57% total C, as determined using a Leco CNS 2000 Elemental Analyzer (Leco Instruments Limited, Mississauga, ON) with N and P below detection limits. Prior to application, the required amounts of GL were weighed out and placed in plastic containers. Then, 7 L of distilled water were added and the mixture shaken for 12 h. This step was taken to ensure homogenization and ensure even distribution when applied to each plot. The GL-water mixture was poured into a sprinkler can for application to each plot. For the treatment where GL was combined with UR, UR was broadcast by hand first, and then GL was applied immediately. Granular UR was broadcast by hand. Immediately after the application and prior to seeding, amendment treatments were incorporated with a tandem disk to a depth of 5 cm. The same day, canola (*Brassica napus* var. Invigor 5030) was seeded on May 21st, 2009 at a rate of 5.6 kg ha⁻¹ using John Deere 610 air seeder at 30 cm row spacing and 2 cm depth. On May 16th, 2010, unfertilized Hard Red Spring Wheat (var. Waskeda) was seeded at a rate of 75 kg ha⁻¹. On May 1st, 2011, a blanket application of fertilizer was made across the site at a rate of 45 kg N ha⁻¹ and 12 kg P₂O₅ ha⁻¹ as pre-plant banded fertilizer. Then, plots were seeded with canola (var. Invigor 5030) on May 10th, 2011 at rate of 5 kg ha⁻¹. At the time of seeding, an additional 20 kg N ha⁻¹ and 10 kg P₂O₅ ha⁻¹ were applied with the seed in the seed-row.

Table 9.3. Basic characteristics of oat hull biochar used in the field study.

Property	Value
C (mg g ⁻¹)	714
N (mg g ⁻¹)	20
P (mg g ⁻¹)	25
K (mg g ⁻¹)	15
S (mg g ⁻¹)	1.0
Na (mg g ⁻¹)	8.0
Ca (mg g ⁻¹)	46
Mg (mg g ⁻¹)	2.0
Cu (mg kg ⁻¹)	11.5
Fe (mg g ⁻¹)	4.0
Mn (mg kg ⁻¹)	109
Zn (mg kg ⁻¹)	80
Surface area (m ² g ⁻¹)	13.4

9.4.4 Plant and soil sample collection and analysis

Crops were harvested in late August of each year at physiological maturity. Plant samples from one square meter (1-m² samples) were cut manually 5 cm above the soil surface. The samples were dried by forced air at 45 °C, and mechanically threshed to determine seed and straw yield. Straw samples were ground to < 2 mm in a WileyTM mill and wheat grain samples were finely ground with a CycloneTM mill. Total N and P were measured by digesting the canola seed and ground wheat grain and straw samples in sulfuric acid-peroxide (H₂SO₄-H₂O₂) using a temperature-controlled digestion block (Thomas et al., 1967), followed by automated colorimetry for determination of P and the NH₄⁺-N using Technicon Autoanalyzer II (Technicon Industrial Systems, 1973). Total N and P uptake were then calculated from plant N and P contents and total dry matter yield. The total N and P uptake was not determined in crop samples collected in the final year of 2011.

Soil sample collection occurred three times during the course of the study: immediately after crop harvest in fall 2009 (September), before planting in spring 2010 (April) and again in fall 2010 (September) after crop harvest. No soil samples were collected during the cropping year 2011. During sampling, a hydraulic punch truck was used to collect bulk samples that

consisted of three soil cores taken per plot, at two soil depth increments (0-15 and 15-30). The three soil cores collected randomly from each plot for each soil depth were mixed thoroughly to generate a representative sample. A subsample was taken from each composite soil sample and immediately frozen until their use for microbial biomass analysis. The rest of the soil samples were air-dried and ground to pass a 2-mm sieve prior to laboratory analysis. The air-dried soil samples collected in fall 2009 were analyzed for inorganic N (NH_4^+ -N and NO_3^- -N) in all depths and for available P and K, organic C, electrical conductivity (EC) and pH in the 0-15 cm depth. The soil samples collected in spring 2010 were only analyzed for their content of inorganic N whereas the selected chemical parameters for analysis in soil collected in fall 2010 included inorganic N determined in the two depths and total N, total P, available P and K and organic C determined in the 0-15 cm depth. Dehydrogenase activity and microbial biomass C (MBC) and N (MBN) were determined in soil samples collected from the 0-15 cm depth for all three sampling periods (fall 2009, spring 2010 and fall 2010).

Exchangeable NH_4^+ -N and NO_3^- -N were extracted by shaking 5 g of soil with 50 mL of 2 M KCl for 1 h on rotary shaker, followed by filtration. The NH_4^+ -N and NO_3^- -N content in the KCl extracts were measured colorimetrically using a Technicon Autoanalyzer II (Keeney and Nelson, 1982). Available P and P were determined by a modified Kelowna method (Qian et al., 1994). Electrical conductivity and pH were measured in 1:1 soil:water suspension. The soil organic C content was determined using a LECO CR-12 combustion carbon analyzer (LECO Corporation, St. Joseph, MI) set at 840 °C (Wang and Anderson, 1998). Soil total N and P contents were determined by sulfuric acid peroxide digest.

Dehydrogenase activity analysis involved the reduction of 2,3,5-triphenylterazolium chloride (TTC) to triphenyle formazan (TPF) as described by Casida et al. (1964) and slightly modified by Serra-Wittling et al. (1995). Briefly, 3 mL water and 3 mL TTC were added to 3 g of air-dried soil (< 2 mm) and incubated for 24 h in darkness at 37 °C. After incubation, the suspension received 10 mL of methanol, and the content was mixed and then filtered through a glass fiber filter. Extra methanol was gradually added until the reddish color vanished from the filter, followed by dilution of the filtrate with methanol to a 100-mL volume. The intensity of reddish color produced through the reduction of TTC to TPF was measured using a spectrophotometer at 485 nm.

Soil content of MBC and MBN was determined by fumigation extraction procedure as

outlined by Voroney et al. (2008). In particular, two 25 g portions of sieved field-moist soils (<2 mm) were weighed out. The first soil portion (25 g) was fumigated with ethanol-free CHCl_3 for 24 h at laboratory temperature in a vacuum desiccator and then extracted with 50 mL of 0.5 M K_2SO_4 . The other soil portion was extracted immediately with the same extractant. Total organic C and N in both fumigated and non-fumigated (control) soil extracts were analyzed using a CN analyzer (TOC-V_{CPH}-TN Shimadzu). The values of nonfumigated samples were subtracted from those obtained from fumigated samples, and MBC and MBN were calculated using a K_{EC} factor of 0.45 for MBC (WU et al., 1990; Joergensen, 1996) and K_{EC} factor of 0.54 for the MBN (Joergensen and Mueller, 1996).

9.4.3 Statistical analyses

Prior to data analysis, raw data were subjected to normality and homogeneity of variance tests using Shapiro-Wilk and Bartlett tests, respectively. Then, a one-way analysis of variance (ANOVA) was employed to analyze treatment effects on plant and soil variables. Treatments effects were declared statistically significant at a probability level of $P \leq 0.1$ at which means were also separated by Fisher's protected LSD. As in the field study in Chapter 8, the probability level of 0.1 was used in this field study owing to variability arising from the need to hand apply the amendments, and the high variability inherently present in biological soil properties in the field. Due to differences in the crop type, the analysis was performed on each year or sampling period data separately.

9.5 Results

9.5.1 Crop yield and nutrient uptake

In the first year following the amendments application, crop yield and N and P uptake were significantly affected by treatment application (Fig. 9.1, Fig. 9.2). The impact of amendments application in spring 2009 persisted to the following growing season (spring 2010) in which the amendments addition showed a significant effect on wheat yield and N uptake (Fig. 9.1, Fig. 9.2). However, no significant impact on the measured crop variables was observed in the third growing seasons. In the first year, the greatest yield was observed for BC+N treatment and was almost double that produced by the control or BC treatments. The yield in the BC

treatment was not significantly different from that provided by the control treatment. The yield obtained from the BC+N treatment was not significantly different from that observed in the treatment of UR applied at 100 kg N ha⁻¹. For GL applied alone the mean yield was slightly but not significantly smaller than the control. However, application of GL+N significantly increased the yield compared to the control or GL-N treatment, but significantly reduced the yield when compared to a 100 kg N ha⁻¹. In the second year, the residual effect of treatments application was most evident with GL+N treatment, providing the greatest crop yield when compared to the other treatments (Fig. 9.1).

In the first year, the N uptake was the highest in UR treatment, followed by BC+N and GL+ N treatments. The N uptake was significantly higher in UR treatment than BC+N treatment (Fig. 9.2). The addition of BC or GL without N did not show a significant effect on N uptake and both were not significantly different from the control. The GL+N treatment resulted in plant N uptake that was significantly lower than that observed with UR applied alone. Similarly, the P uptake was the greatest in UR and BC+N treatments, followed by GL+N treatment (Fig. 9.2). The C-based amendments (BC, GL) applied in absence of N did not have a significant impact on P uptake, compared to the control treatment. In the second year, the residual effect of amendments applied in the first year on N uptake was observed. The N uptake was the greatest in GL+N treatment and was significantly different from that observed in the other treatments (Fig. 9.2). However, there was no residual effect on the P uptake measured in the second year of the experiment.

9.5.2 Dehydrogenase activity and microbial biomass

Dehydrogenase enzyme activity in soil samples collected in the fall 2009 after first year harvest was significantly influenced by amendment applications (Table 9.4). However, there was no residual effect on dehydrogenase activity in soil samples collected in spring 2010 or fall 2010 (Table 9.4). In fall 2009, the dehydrogenase activity was the greatest in soil treated with GL+N, followed by GL, and both treatments were significantly higher than all other treatments including the control. Biochar application with or without N did not have any significant impact on dehydrogenase activity when compared to the control.

Soil microbial biomass C content was significantly affected by amendment only in fall 2009, but not in the other two sampling periods (Table 9.4). The BC+N treatment had the lowest content of soil MBC and was significantly lower than all other treatments. The rest of the treatments were not significantly different. Similarly, soil MBN content was also significantly influenced by amendment addition only in fall 2009 (Table 9.4). Soil amended with BC+N showed the lowest content of MBN, followed by BC treatment. Both BC+N and BC treatments were significantly different from the control, which provided the greatest content of soil MBN compared to all other treatments, with the exception of GL treatment that also did not differ from the control.

9.5.3 Selected soil chemical properties

The residual inorganic N content varied with depth, with NH_4^+ -N being the dominant form of inorganic N, especially in fall 2009, the first period of sampling following the amendment application (Table 9.5). The other measured soil chemical parameters, such as extractable P and K, organic C, pH, total N and P, in fall 2009 or fall 2010 were not significantly influenced by amendments application, with the exception of EC which showed a slight but significant decrease in fall 2009 in all amended soils, compared to the control (Table 9.6).

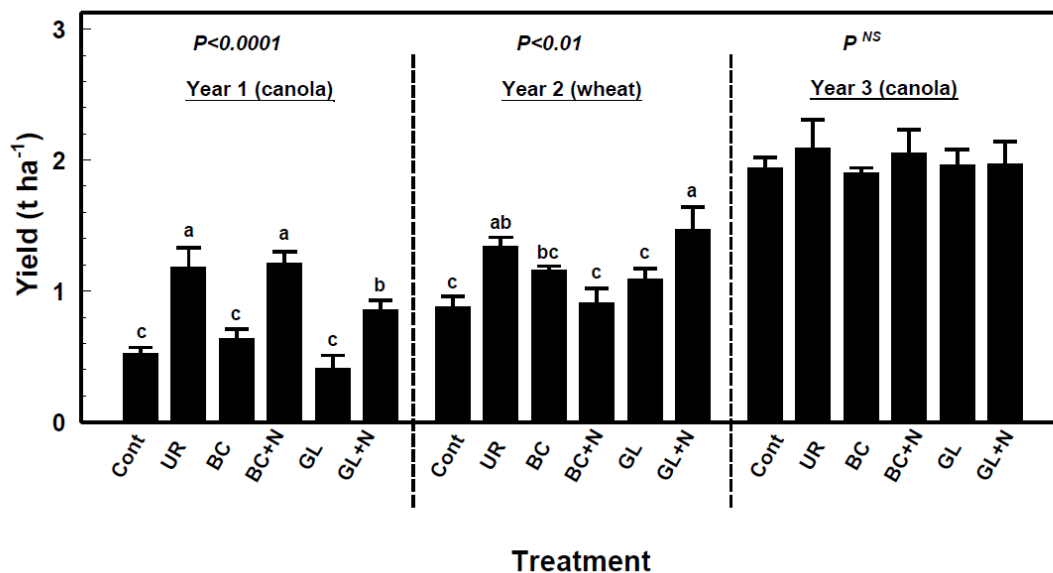


Fig. 9.1. Yield responses to treatment application during a three-yr field study in Central Butte, SK. Treatments were applied once in spring 2009 and were control (Cont), urea (UR), biochar (BC), biochar plus N (BC+N), glycerol (GL) and glycerol plus N (GL+N). For a year, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.1$). Errors bars represent standard error of mean ($n = 4$). *NS* denotes not significant at $P \leq 0.1$.

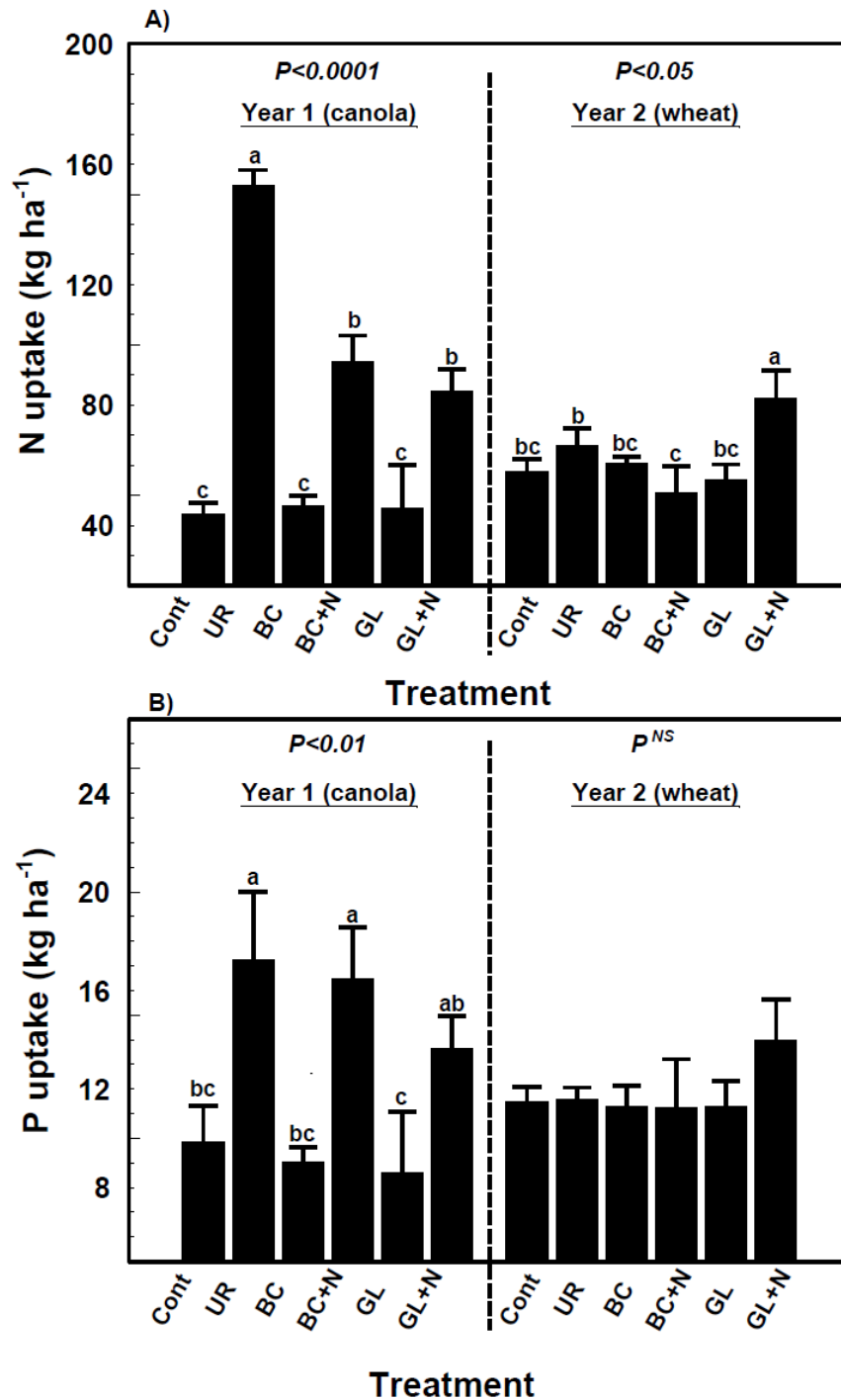


Fig. 9.2. Total plant N uptake (A) and total plant P uptake (B) responses to treatment application during a three-yr field study in Central Butte, SK. Treatments were applied once in spring 2009 and were control (Cont), urea (UR), biochar (BC), biochar plus N (BC+N), glycerol (GL) and glycerol plus N (GL+N). For a year, bars sharing the same letter among treatments are not significantly different according to LSD test ($P \leq 0.1$). Errors bars represent standard error of mean ($n = 4$). NS denotes not significant at $P \leq 0.1$.

Table 9.4. Dehydrogenase activity, microbial biomass C and microbial biomass N responses to experimental treatments during three sampling periods (mean \pm standard error).

Treatment [¶]	Dehydrogenase Activity			Microbial Biomass C			Microbial Biomass N		
	Fall 2009	Spring 2010	Fall 2010	Fall 2009	Spring 2010	Fall 2010	Fall 2009	Spring 2010	Fall 2010
	----- $\mu\text{g TPF g}^{-1}$ -----			----- $\mu\text{g g}^{-1}$ -----					
Cont	187 \pm 8b [§]	212 \pm 10	299 \pm 37	244 \pm 34a	250 \pm 63	205 \pm 20	71 \pm 13a	51 \pm 19	30.5 \pm 3.8
UR	203 \pm 19b	235 \pm 21	289 \pm 22	191 \pm 21a	259 \pm 54	211 \pm 7	38 \pm 6bc	57 \pm 23	30.7 \pm 0.9
BC	171 \pm 15b	211 \pm 10	279 \pm 22	220 \pm 57a	187 \pm 79	207 \pm 4	35 \pm 9bc	53 \pm 16	29.7 \pm 1.0
BC+N	199 \pm 16b	199 \pm 8	348 \pm 52	109 \pm 13b	220 \pm 9	235 \pm 33	24 \pm 8c	40 \pm 3	34.2 \pm 5.4
GL	247 \pm 20a	206 \pm 9	324 \pm 32	217 \pm 14a	202 \pm 30	223 \pm 18	52 \pm 7ab	41 \pm 14	32.9 \pm 3.2
GL+N	252 \pm 18a	238 \pm 4	257 \pm 13	191 \pm 18a	287 \pm 71	199 \pm 8	48 \pm 4b	69 \pm 25	28.5 \pm 2.6
<i>ANOVA</i>									
Treatment	0.020	NS	NS	0.040	NS	NS	0.020	NS	NS

[¶] Cont denotes control, UR denotes urea, BC denotes biochar, BC+N denotes biochar plus N, GL denotes glycerol, GL+N denotes glycerol plus N.

[§] Means within a column sharing the same letter are not significantly different at $P = 0.10$.

Table 9.5. Soil content of inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) at 2 soil depth increments determined in soils collected after crop harvest in 2009, before planting in 2010 and after crop harvest in 2010 (mean \pm standard error).

Treatment [¶]	0-15 cm					
	Fall 2009		Spring 2010		Fall 2010	
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$
	-----mg kg ⁻¹ -----					
Cont	6.4 \pm 1.5	3.4 \pm 0.6	3.0 \pm 0.3	5.0 \pm 0.8	6.1 \pm 1.1	4.8 \pm 0.5
UR	6.3 \pm 0.8	2.9 \pm 0.8	3.6 \pm 0.6	5.9 \pm 0.5	7.3 \pm 1.5	3.7 \pm 0.3
BC	7.6 \pm 1.4	2.9 \pm 0.7	3.3 \pm 0.3	4.0 \pm 1.2	6.0 \pm 0.9	4.1 \pm 0.2
BC+N	6.6 \pm 1.2	2.0 \pm 0.3	3.3 \pm 0.4	5.0 \pm 1.2	5.5 \pm 1.1	4.2 \pm 0.9
GL	6.3 \pm 1.2	3.3 \pm 0.7	3.3 \pm 0.4	5.9 \pm 0.6	6.0 \pm 1.1	3.9 \pm 0.2
GL+N	6.4 \pm 1.0	3.3 \pm 0.9	3.3 \pm 0.1	7.3 \pm 0.5	6.3 \pm 1.5	3.6 \pm 0.5
<i>ANOVA</i>						
Treatment	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>
Treatment	15-30 cm					
	Fall 2009		Spring 2010		Fall 2010	
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$
	-----mg kg ⁻¹ -----					
Cont	7.0 \pm 1.1	2.6 \pm 0.6	3.3 \pm 0.4	4.4 \pm 1.1	7.1 \pm 1.2	2.2 \pm 0.2
UR	7.2 \pm 1.2	1.7 \pm 0.3	3.4 \pm 0.6	4.4 \pm 1.5	8.4 \pm 1.6	2.0 \pm 0.2
BC	9.7 \pm 1.1	2.5 \pm 0.8	3.4 \pm 0.3	2.5 \pm 0.7	6.2 \pm 0.8	2.4 \pm 0.3
BC+N	5.3 \pm 1.5	2.3 \pm 0.5	3.9 \pm 0.4	4.4 \pm 1.5	6.0 \pm 0.9	3.0 \pm 0.8
GL	8.4 \pm 0.2	2.9 \pm 0.6	6.5 \pm 3.6	5.4 \pm 0.2	7.1 \pm 1.1	2.7 \pm 0.4
GL+N	7.8 \pm 1.0	2.1 \pm 0.7	3.3 \pm 0.3	4.4 \pm 0.7	6.6 \pm 1.4	2.1 \pm 0.1
<i>ANOVA</i>						
Treatment	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>

¶ Cont denotes control, UR denotes urea, BC denotes biochar, BC+N denotes biochar plus N, GL denotes glycerol, GL+N denotes glycerol plus N.

Table 9.6. Selected soil chemical properties at 0-15 cm depth determined after crop harvest in 2009 and 2010 (mean \pm standard error).

Treatment [¶]	Fall 2009					Fall 2010				
	Extract P	Extract K	Organic C	pH	EC	Total N	Total P	Extract P	Extract K	Organic C
	-----mg kg ⁻¹ -----		mg g ⁻¹		dS m ⁻¹			-----mg kg ⁻¹ -----		mg g ⁻¹
Control	7.2 \pm 2.7	291 \pm 24	10.5 \pm 1.0	7.6 \pm 0.2	0.29 \pm 0.13a [§]	1070 \pm 19	441 \pm 8	13.7 \pm 1.7	323 \pm 26	11.6 \pm 0.5
Urea	7.5 \pm 1.5	306 \pm 10	10.2 \pm 0.3	7.3 \pm 0.2	0.15 \pm 0.02b	1066 \pm 38	440 \pm 98	14.4 \pm 2.5	329 \pm 19	12.3 \pm 0.3
GL	5.7 \pm 1.0	343 \pm 24	10.6 \pm 0.6	7.5 \pm 0.1	0.13 \pm 0.01b	1087 \pm 27	448 \pm 8	12.1 \pm 1.5	364 \pm 28	11.9 \pm 0.4
GL+N	6.5 \pm 1.5	330 \pm 20	10.6 \pm 0.9	7.3 \pm 0.3	0.17 \pm 0.03b	996 \pm 10	418 \pm 7	12.7 \pm 1.5	336 \pm 20	11.5 \pm 0.5
BC	9.0 \pm 2.0	325 \pm 34	10.9 \pm 0.7	7.4 \pm 0.1	0.13 \pm 0.01b	1092 \pm 26	443 \pm 6	14.5 \pm 1.7	354 \pm 27	12.5 \pm 0.4
BC+N	5.1 \pm 0.8	357 \pm 11	10.8 \pm 0.1	7.5 \pm 0.2	0.14 \pm 0.02b	1105 \pm 52	450 \pm 13	11.6 \pm 0.7	399 \pm 23	12.7 \pm 0.8
<i>ANOVA</i>										
Treatment	NS	NS	NS	NS	0.10	NS	NS	NS	NS	NS

[¶] Cont denotes control, UR denotes urea, BC denotes biochar, BC+N denotes biochar plus N, GL denotes glycerol, GL+N denotes glycerol plus N.

[§] Means within a column sharing the same letter are not significantly different at $P = 0.10$.

9.6 Discussion

Application of BC alone at a rate of $2000 \text{ kg C ha}^{-1}$, equivalent to approximately 2.8 T ha^{-1} , did not benefit crop yield and nutrient uptake in the immediate or subsequent two growing seasons following application. This is an indication that the BC used in the current study did not itself supply nutrient for plant uptake. Similarly, Van Zwieten et al. (2010) generally found little crop response to BC addition in absence of N to acidic and alkaline soils, under controlled environment conditions. Gaskin et al. (2010) also reported limited effects of peanut hull and pine chip BC on yield and nutrient concentrations in plant, relating this to lack of N availability from BC. This may explain the absence of BC effect on crop parameters in the current study in which the nutrient, especially N and P, contained in oat hull BC was not immediately plant available following application or did not become available with time during the subsequent two growing seasons. Based on the application rate used here, the BC is assumed to add about $50 \text{ kg total N ha}^{-1}$ in addition to about $70 \text{ kg total P ha}^{-1}$. However, it appears little of any of these nutrients became available for plant, as shown in the similar N and P uptake in BC alone amended soil and the control. Nutrients, especially N, in manure-based BCs may be more available for plant compared to these in plant-derived BCs (Chan et al., 2008; Tagoe et al., 2008; Hass et al., 2011; Gaskin et al., 2010). Thus, to better benefit from BC application, additional N application may be needed as observed in the current study as well as others (Nelson et al., 2011). Joint application of BC and UR showed equivalent or greater yield and nutrient uptake than other treatments, despite having only half as much UR N added. Biochar addition is reported to sustain soil fertility when an additional nutrient source is provided, and in presence of fertilizer, it was able to improve plant growth and grain yield, compared to the fertilizer without BC (Steiner et al., 2007). Steiner et al. (2008) reported improved efficiency of mineral N fertilizer in Amazonian soil amended with BC, as shown by higher N retention in the soil and the enhanced uptake by biomass. In the current study, the treatment of 50 kg N ha^{-1} combined with BC benefited the crop yield similar or better than that in 100 kg N ha^{-1} applied alone treatment. This could be due to the ability of BC to reduce UR N losses through reduction of leaching or gaseous losses (Lehmann et al., 2003; Yanai et al., 2007).

Glycerol application tended to reduce crop yield and nutrient availability in the first growing season (spring 2009), as shown specifically by N uptake in GL+N treatment, compared

to urea applied alone treatment. This is very likely a consequence of microbial immobilization of soil N. The immobilized N in GL+N treatment in spring 2009 appeared to become remineralized and plant available during the subsequent growing season (spring 2010), resulting in higher yield and N uptake. Similarly, Qian et al. (2011) reported that N supply from UR fertilizer was adversely affected by GL application, especially at the high rates, leading to a significant reduction in plant growth and N uptake. Under growth chamber conditions, GL amendment was also shown to immobilize soil available N, as shown by small supply rates of NO_3^- -N and NH_4^+ -N measured in the soil (see Chapter 3). This indicates that GL can contribute to N reservation when co-applied with conventional fertilizer. In a recent study, GL was also found to significantly reduce N loss through minimizing nitrate leaching, owing to microbial immobilization of N (Redmile-Gordon et al., 2014).

Dehydrogenase is an intracellular enzyme participating in the biological oxidation of organic compounds in soil (Tabatabai, 1994), and has been reported to be correlated to the organic matter availability in the soil (Serra-Wittling et al., 1995; Moeskops et al., 2010). In the few studies identifying the impact of BC on soil enzymes, there are discrepancies and inconsistencies among the documented findings. Under controlled environment conditions, Ameloot et al. (2012) revealed that dehydrogenase enzyme activity increased in soil amended with BCs from pyrolyzed swine manure digestate and willow wood at 350 °C, but the enzyme activity was suppressed in the same soil amended with the BCs produced from the same feedstocks, but pyrolyzed at 700 °C. The authors related this to the higher level of volatile compounds present in BCs produced at low temperature that can stimulate enzyme activity, as also reported elsewhere (Smith et al., 2010; Bailey et al., 2011). In the current study, BC neither increased nor suppressed dehydrogenase enzyme activity, which is in line with the recent findings that used BC from wheat straw (Wu et al., 2012). Biochar C from the source used in the current study is resistant to microbial breakdown and not accessible by soil microbes, and thereby did not stimulate enzyme activity. However, GL applied alone or with N promoted dehydrogenase activity in the year of application that was significantly higher than any other treatment. This may be explained by lower recalcitrance of C in GL and greater availability for soil microbes, resulting in stimulated enzyme activity. The same was observed when GL was added at different rates but under growth chamber conditions as reported on in Chapter 3 of this dissertation.

The soil microbial biomass can enhance nutrient cycling and availability to plants following application of organic materials to soil, due to its key role in organic matter decomposition (Moore et al., 2000). It is the most labile pool of organic matter, and is frequently used as a sensitive indicator of changes in soil organic matter content (Powlson et al., 1987). Few research studies most of which were conducted under controlled conditions, have specifically evaluated the effect of BC addition on soil microbial biomass content and reported inconsistent findings. For instance, Kolb et al. (2009) found increased microbial biomass content and activity in a range of temperate soil types amended with one type of BC whereas Dempster et al. (2012) reported decreased MBC but not MBN in a coarse textured soil treated with Eucalyptus BC. In the current study and only in the fall 2009 sampling, the BC applied alone did not alter MBC, but decreased MBN content compared to the control, as also did BC plus N. However, when BC was combined with N, the content of MBC was the lowest in comparison to other treatments. The reason for the significant decrease in MBC here is not clear, but is coincident with greatest crop yield and nutrient uptake in the first growing season (2009) prior to first soil sampling period for microbial analysis. This may be related to the depletion of soil nutrients and surface soil moisture arising from high crop growth that subsequently limited microbial growth and N accumulation potential. Changes in nutrient and C availability may increase or decrease microbial biomass growth and activity, depending on soil background of nutrient and C and the microbial groups responsible for decomposition (Lehmann et al., 2011).

The amount of BC used here and the conditions of low precipitation (semi-arid environment) may limit the ability to show a clear effect on selected soil chemical properties, especially if applied only once. Application rate of BC is critical for the effects on plant and soil (Lehmann et al., 2003; Spokas et al., 2010), and as reported in most studies, the greatest positive effects of BC were observed at the rates of 100 t ha⁻¹ (Jeffery et al., 2011). However, given the difficulty in broadcasting large quantities of powdery, fine BC in the windy prairie conditions, rates less than 10 t ha⁻¹ appear more practical.

9.7 Conclusion

Addition of BC in absence of N to prairie soil at a rate of 2000 kg C ha⁻¹ (approximately equivalent to 2.8 t BC product ha⁻¹) had no significant effects on measured plant, soil and microbial parameters in this study. This indicates that this specific BC type was rather inert

material that neither supplied nutrient or caused immobilization. However, its combination with 50 kg N ha⁻¹ provided similar crop response to a treatment of 100 kg N ha⁻¹ applied urea. This suggests that BC may conserve urea N from potential losses via leaching and volatilization processes and thereby improve N use efficiency. Glycerol was more effective in its role in reducing urea N availability via microbial immobilization as shown by its impact on reducing yield and N uptake in the first growing season following amendments application. This N seemed to become released via remineralization process during the subsequent growing season as mirrored by increased crop yield and N uptake. It also enhanced dehydrogenase enzyme activity, indicating utilization of its C by soil microorganisms. Overall, the effects, especially with BC amendment, on plant and soil variables observed in the current study were generally smaller than reported in other studies. This can be due to differences in BC type, soil type and also the rate of application that is lower in this study compared to many other studies. This study indicates that GL may have a potential in reducing N losses from soil when combined with N fertilizers. This potential needs be clarified in further lab and field studies.

10. SYNTHESIS, SUMMARY AND CONCLUSIONS

10.1 Synthesis

The increasing global demand for energy leads to growing concern about the heavy reliance on fossil fuel reserves. From an environmental perspective, use of fossil fuel has a significant impact on global warming due to its role in increasing greenhouse gas emissions. As such, alternative sources of fuel are sought, emphasizing those derived from renewable resources such as biomass. Besides bioenergy production from these sources, various by-products are also generated. The type of generated by-product varies according to the various technologies that have been employed to convert organic materials to energy. Examples of these by-products include distillers' grains and thin stillage (biochemical conversion), glycerol (mechanical/chemical), ash (gasification) and biochar (pyrolysis).

Expansion in bioenergy production has resulted in accumulation of by-products. Finding a practical method of utilization that captures value from the by-products will help to sustain the bioenergy production system economically. The transition from a waste material to a resource also needs to address the many aspects of effective recycling and reutilization of materials and energy. An important consideration in the life cycle analysis of any bioenergy production system is that a portion of the plant nutrients that were utilized in the production of the bioenergy feedstock are present in the by-products and can be potentially recycled. As these by-products contain carbon and/or mineral nutrients, one possible option of utilization can be application to soil to improve soil fertility and plant production. The main objective of this dissertation was to assess the potential of using bioenergy production by-products as soil amendments to improve soil fertility and quality by determining their impact on various soil biological and chemical attributes as well as crop growth. The research work was conducted under controlled environment conditions using soils and plants grown in the growth chamber along with complementary trials conducted with the by-products under actual field conditions and, where possible, using field scale equipment.

The series of studies that were conducted and reported on in this thesis were intended to provide new, important information on the potential for amending prairie soils with bioenergy production by-products. Prior to this thesis research, little or no information existed on the effects of amending soils with these novel materials on soil chemical and biological conditions, nutrient cycling processes, plant nutrition and crop yield. An understanding of these effects however, is needed to develop recommendations for their best use.

The initial characterization of the bioenergy production by-products (BPB) evaluated in this dissertation revealed that these by-products contain varying, but considerable amounts of C and plant nutrients. This promotes their utilization as sources of plant nutrients. The availability of nutrients contained in these by-products was determined by crop yield and N and P uptake responses to application of BPB under growth chamber and/or field conditions. The crop yield response to wet distillers grains (WDG), thin stillage from fermentation (TS) and ash application was often equal to or better than that from conventional single nutrient source commercial fertilizers. However, the magnitude of BPB effects on crop yield varied among the BPB type, according to the initial composition of each by-product. Ash derived from dried distillers' grains (DDGA) was an excellent source of P as shown in its higher crop yield and P uptake responses when compared to mineral P fertilizer. However, ash derived from meat & bone meal (MBMA) was less effective as a P fertilizer (Chapter 5). The cause of this was shown to be related to the high content of stable and recalcitrant P forms in MBMA that appeared to limit P release from this type of ash (Chapter 6). The ethanol fermentation by-products WDG and TS were also shown to supply sufficient nutrients that stimulated crop yield, N and P uptake responses (Chapter 7), owing to the readily plant available nature of the nutrients contained in these by-products (Chapter 3). Specifically, TS was as effective per unit of N added as urea in enhancing crop yield, with the injection method of application being more effective than broadcast and incorporation (Chapter 7). Given that a high portion of total N in TS is in a form of $\text{NH}_4^+\text{-N}$ (Chapter 3 and Chapter 7), the injection method may have reduced N losses through volatilization. These findings indicate that best practices for land application of BPB's requires the same type of considerations as for more traditional nutrient amendments like manure, compost and commercial inorganic fertilizers: addressing the rate, composition, timing and placement in the soil. It is important to note that, just like for manures and composts, the BPB

materials generally have lower nutrient concentrations than commercial inorganic fertilizers, making them more costly to transport long distances and apply economically.

Evaluating short and longer term effects of land application of any new amendment is relevant to agronomic and environmental sustainability. The repeated application over two-yr of fermentation by-products like TS also increased soil NO_3^- -N and available P contents measured each year, especially after the second year of application, indicating the persistence of nutrient release from TS organic matter turnover (Chapter 8). The rapid C and N turnover to CO_2 and N_2O after BPB application to soil also shows that these are easily decomposable materials. However, the evolved CO_2 and N_2O from soil amended with BPBs did not exceed those from soil receiving conventional amendments (urea and dehydrated alfalfa). This is important to document, as greenhouse gas emissions that may be produced following land application are an important consideration in the overall environmental impact of any bioenergy production system.

Unlike N or P rich by-products like stillage or ash, biochars from pyrolysis (BC) and glycerols from transesterification (GL) are C-rich by-products, making them unique and novel soil amendments. Thus, it was hypothesized that their application was not expected to directly influence crop nutrition, but may affect some soil biological processes that can ultimately impact crop yield and nutrient uptake. The BC had no clear effect on crop yield and nutrient uptake, whereas GL tended to reduce crop yield in the initial year of application (Chapter 9). This can be attributed to microbial immobilization of soil N, as was also shown under controlled environment conditions (Chapter 3). While immobilization may be considered detrimental when it coincides with crop demand for N, it could also be beneficial in helping reduce losses of excess nitrate present in the soil if it occurs in late fall after harvest or in early spring after snowmelt. As such, timing of glycerol application could be adjusted to regulate levels of nitrate present in the soil.

10.2 Summary of Findings

Addition of decomposable organic materials to agricultural soils can have impact through release of C and N and subsequent transformations of the released nutrients that can influence available nutrient supply for plants and greenhouse gas emissions from the soil. The main objective of the first study of this research was to examine the direct effects of application of selected bioenergy production by-products including wet distillers' grains, thin stillage and

glycerol on greenhouse gas emissions (N_2O and CO_2) and nutrient supply rates ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) using Plant Root SimulatorTM resin membrane probes over a short term incubation under controlled environment conditions (Chapter 3). Selected soil enzyme activities and microbial biomass content response to the same amendments were also investigated (Chapter 4). In comparison to reference amendments of urea and dehydrated alfalfa, urea fertilizer produced the greatest amount of N_2O , followed by WDG and TS whereas the lowest N_2O production was observed with GL and dehydrated alfalfa. The bioenergy by-products tested here were found to have significant impacts on release of available nutrient, with the urea treatments providing the highest NO_3^- -N supply rate. The TS treatments supplied the highest rate of NH_4^+ -N, followed by WDG compared to the other by-products. The WDG treatments were able to provide the greatest supply of $\text{PO}_4\text{-P}$ in comparison to the other amendments. Microbial N immobilization was associated with glycerol treatments applied alone. The results of this study indicate that these bioenergy by-products can be suitable soil amendments as a result of their ability to supply nutrients and result in N_2O emissions per unit of N added that did not exceed that of the conventional urea fertilizer. The same by-products were also found to have a positive impact on soil enzyme activity and microbial biomass content under the same controlled environment conditions (Chapter 4). Alkaline phosphatase activity was significantly enhanced by WDG, GL+N, urea and alfalfa addition, especially at low and medium rates of addition. All amendments significantly increased dehydrogenase and protease activity and all amendments with exception of TS significantly increased microbial biomass C and N. The reduced effect of TS on these parameters is attributed to less organic carbon added relative to nitrogen in this amendment. Overall, addition of bioenergy processing by-products to soil stimulated microbial growth and enzyme activity; supporting their potential use as soil amendments to recycle plant nutrients and enhance soil biological activity.

Ash is another bioenergy by-product that is generated during gasification of organic materials to produce biogas. The ash is rich in phosphorus and can be utilized as P fertilizer for crops in prairie soils. The effectiveness of MBMA and DDGA as P fertilizer was assessed via evaluating their direct effect on canola growth, P uptake and apparent P recovery after addition to a P deficient soil under controlled environment conditions (Chapter 5). In addition, P forms residing in the soil following ash application were also investigated using a sequential extraction procedure (Chapter 6). After a growth period of 5 weeks, the DDGA was the most effective ash

type and provided biomass yield, P uptake and apparent P recovery better or similar to that of a mineral (mono-calcium phosphate) fertilizer, indicating high availability of its P. The MBMA had a limited effect on measured crop variables, suggesting that a significant portion of this ash P is not as readily available for plant uptake. The lower availability of soil P derived from MBMA compared to DDGA was explained by the results of sequential extraction to speciate the soil phosphorus. The fractionation revealed that recalcitrant P fractions (HCl-Pi and residual-P) were the dominant forms of P in soil receiving MBMA (Chapter 6). This is attributed to the presence and formation of Ca-P compounds of low solubility with this MBMA treatment. The high content of calcium (25%) in the MBMA leads to formation of Ca-P compounds. Clearly the source and composition of the feedstock greatly influences the behavior and value of the amendment as a fertilizer.

Thin stillage (TS), the aqueous by-product generated from the distillation of ethanol following fermentation, is a potential source of plant nutrient that may be easily and effectively land applied using equipment that was developed for low disturbance injection of liquid manure slurries. Thus, a field trial was conducted involving direct addition of TS to a Black Chernozem soil over a two-yr period using two methods of application: broadcast and incorporation, and injection (Chapter 7). For both seasons of the study, at equivalent N rate the TS provided similar or greater crop yield and nutrient recovery compared to conventional urea fertilizer, especially when injected. This is explained by the contribution of other plant nutrients, such as P and S in TS, its relatively high plant available NH_4^+ -N content and rapid mineralization of the organic N component. The injection of TS appears to be a more effective application method compared to broadcasting and incorporating likely through reducing volatile N loss, and placing nutrient closer to the growing crop roots when injected in bands in soil. The TS did not show any adverse effect on measured crop parameters even at the high rate of application. In this field trial, the effect of TS on residual NO_3^- -N was more pronounced in the second year, leading to high content of NO_3^- -N after crop harvest, especially with injected TS at the high rate of application (Chapter 8). Soil residual available P content also significantly increased in both years, but was greater in the second year. This reflects the high plant availability of N and P in TS and a persistence of release of nutrient through TS organic matter decomposition. This is in accordance with the high nutrient supply rates observed with TS when applied to a Brown Chernozemic soil under controlled environment conditions (Chapter 3). In selected treatments, microbial biomass

was higher in TS than urea treatments, but not significantly different from the control. The other measured soil parameters remained unchanged for both years. The results of this study suggest that land application of TS through injection with conventional liquid manure injection equipment can be an effective solution for TS management that recycles nutrients contained in the feedstock grain. However, this field study also indicates that attention should be given to rates of application over time, as continuous application of TS may contribute to NO_3^- -N and P accumulation in soil beyond the year of application via carryover of unused inorganic forms and also possibly through increased microbial mineralization.

Pyrolysis of organic materials and transesterification of vegetable oils are popular technologies that are employed to produce biogas and biodiesel, respectively. Production of bioenergy using these techniques also results in carbonaceous by-products, including biochar (BC) in case of pyrolysis and glycerol (GL) in case of the transesterification process. These bioenergy by-products are C-rich substrates that can be suitable soil amendments. Their recycling may especially benefit soils with lower organic matter contents, such as those found in the Brown soil zone of the prairies. Direct and residual effects of BC and GL application on crop growth and selected soil chemical and biological properties in a Brown Chernozemic soil were evaluated over a three-yr period in a field trial (Chapter 9). In the first season following a spring application of the amendments, addition of BC and GL alone had no significant impact on crop or soil parameters. However, when combined with 50 kg N ha^{-1} , BC provided similar yields to the $100 \text{ kg urea N ha}^{-1}$ treatment, suggesting that BC may have improved urea N use efficiency. The GL+ $100 \text{ kg urea N ha}^{-1}$ treatment had lower crop yield and N uptake than the urea treatment alone; a result of microbial immobilization of N. However, this treatment had a significant residual effect in the second year on crop yield and N uptake, presumably because of the re-mineralization of the N that was immobilized after application in the first season. Both GL and GL+N treatments enhanced dehydrogenase activity compared to other treatments. No further residual impacts were detected in the following two growing seasons. The results of this study showed that the response of the Brown Chernozemic to application of amendments, especially BC, was smaller than expected based on previous studies conducted elsewhere such as in tropical regions. A greater application rate of amendments may be required to allow for a better response.

10.3 Conclusion and Suggestions for Future Research

Overall, it is concluded that bioenergy production by-products have great potential as a new means to add nutrients to the soil for improved soil biological and chemical conditions, more efficient nutrient recycling, and improved crop growth. Effectiveness of these products depends on the rate of application, the source and composition of feedstock, and the timing and method of application. Attention should be given to these aspects when making practical management decisions. Some identified future research needs related to utilization of bioenergy by-products as soil amendments are listed below:

- Char and ash research should to be extended geographically to include more trials in arid and semi-arid regions.
- Long-term effects of application of biochar in combination with mineral fertilizers need to be carried out to investigate its ability to conserve mineral N and reduce losses to environment through volatilization, denitrification and leaching over the long term; e.g. several years.
- Evaluation of combinations of biochars with other amendments like manure and stillage.
- Effect of variables such as feedstock type, temperature and oxygen status on biochar physical and chemical characteristics and efficacy when added to soil. This can be conducted by comparing several biochar types produced from different feedstocks under different conditions and examining their effect in the field.
- Engineered solutions and technology for handling and application of chars and ashes to soil in an efficient manner, as these were found to be very difficult to manage under field conditions.
- Most studies with ashes and chars are either conducted under controlled environment conditions or under field conditions but only for a short-term (usually ~ two years). This does not give an appropriate period to make inferences about the potential for heavy metal accumulation in soils and phytotoxicity.
- Land application of glycerol has potential to conserve N fertilizer through microbial immobilization as well as its ability to increase organic matter content. This should be further investigated under controlled environment and field conditions. Glycerol might also be used as composting additive as a source of carbon.

- Application of distillers' grains and thin stillage to land directly as organic fertilizer needs to have an economic and lifecycle evaluation to determine if land application is a viable alternative to feeding distillers' grains and thin stillage to animals.

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12. APPENDICES

12.1 Appendix A. Crop and Soil Response to Fertilization with Distillers' Grains Derived Manure in Saskatchewan Soil

A.1 Preface

As noted in the literature review of this dissertation, utilization of bioenergy production by-products (BPB), especially DG, TS and GL, for animal feeding is a common practice. However, this use will result in a significant portion of nutrient contained in the DG ending up in the soil when the animal manure is ultimately land-applied. The main focus of the dissertation is on the direct application of BPB to soil. However, indirect effects through land application of animal manure from cattle that are fed DG were considered worthwhile to evaluate and are reported on in the appendix. Therefore a field-based comparison of the crop and soil responses to application of manure derived from feeder cattle fed DG in the ration in comparison to regular feed grain ration was conducted. A two-yr field study was carried out in east-central Saskatchewan on a Black Chernozemic soil in which crop and soil response to fertilization with manure from cattle fed DG versus manure from cattle fed regular feed grain ration were investigated.

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A.2 Abstract

Including distillers' grain, a by-product of ethanol production, in animal diets has been shown to increase nutrient content in manure. This can increase manure value as organic fertilizer but could also contribute to environmental degradation if not accounted for in manure management plans. A two-yr field trial was conducted on a Black Chernozem soil to evaluate crop and soil responses to application of manure from cattle fed distillers' grain (DGM) in comparison with manure from cattle fed the standard western Canadian finishing diet based on barley (*Hordeum vulgare* L.) grain (BGM). The experimental treatments were: two types of manure (DGM or BGM) applied at two rates (15 or 30 Mg ha⁻¹) using two methods of application (broadcast followed by incorporation or band injection). An unamended control was also included for comparison. Manure addition in general promoted grain and straw yield increases, and increased plant N and P uptake in both years by 30-50%. However, manure type had no consistent effects on crop responses, with the exception of N and P recoveries in which N recovery was higher in BGM treatment whereas P recovery was higher in DGM treatments. The absence of manure type effect in the first year is consistent with the similar chemical composition of both manures. Overall, a higher rate of application provided higher crop yield and N and P uptake in both years. Placing solid manure below the soil surface in bands was slightly better in promoting yield and N uptake than broadcasting followed by incorporation in both years. Manure application in general increased soil residual NO₃⁻-N and available P contents at 0-15 cm depth. Manure type had no significant impact on selected soil chemical properties (inorganic N, available P, K, OC, Cu, Zn, Cd, pH and EC) after the two-yr of manure application.

A.3 Introduction

The growing interest in producing energy from sustainable sources to reduce dependency on fossil fuel has resulted in increased production of ethanol and related by-products, one of which is distillers' grains (DG). The DG is generated when original grains are subjected to fermentation process to convert grain starch into ethanol, followed by distillation and centrifugation processes. As a result of starch removal, the nutrients contained in the original

grains are concentrated in higher amount in the resulting by-product of DG. Using this by-product as animal feed has been a common practice and accounts for the second largest source of income after ethanol fuel for the ethanol-production plant (Bonnardeaux, 2007). It has been recognized as an excellent source of protein and P when included in animal diets (Erickson et al., 2005; Harris et al., 2008). In cattle feeding, it is estimated that only about 10% of N and 20% of P present in feed is retained by the beef animal, and the rest is excreted in feces and urine (Bierman et al., 1999). As a result, it is expected that manure produced from animals fed DG will differ in its characteristics compared to that derived from animals fed regular grains. In particular, the distillers' grain derived manure will contain higher nutrient content, especially N, P and S, due to the higher level of these nutrients in DG by-product (Hao et al., 2009).

Several manure characteristics and its fertilizer value are influenced by livestock diet (Eghball, 2002; Hao et al., 2009). In a study conducted at Lethbridge, Alberta, manure chemical composition was reported to be significantly affected by including wheat (*Triticum aestivum* L.) dried distillers' grains with solubles (DDGS) in finishing feedlot cattle diets (Hao et al., 2009). These authors found that the manure produced was significantly affected in its nutrient content by DDGS inclusion in the animal diets, resulting in significant increases in total N, water soluble NH_4^+ -N and total P. Similarly, another study showed that increasing amount of wet distillers' grains with solubles in feedlot cattle diets resulted in increased N, P and S content in cattle manure (Spiehs and Varel, 2009). The effect of including DG in animal diets is not only confined to increasing the quantity of nutrient excreted; it may also influence the forms of these nutrients, especially soluble P (Spiehs and Varel, 2009; Ebeling et al., 2002). This can have a positive effect in terms of enhancing nutrient availability and crop growth when this manure is used as a fertilizer. Higher nutrient content in DG derived manure also means it has higher agronomic value as organic fertilizer via lower handling and transportation costs per unit nutrient. However, it could have a negative environmental impact by increasing the potential for nutrient leaching and runoff if not properly managed. Higher N and P content has to be considered in the farm nutrient management plans in order to reduce the susceptibility of the nutrients to loss to surface water or air.

There has been little or no work, especially under field conditions, comparing the behaviour of cattle manure from distillers' grain- to barley (*Hordeum vulgare* L.) grain-based diets. In a growth chamber study to investigate nutrient uptake by barley forage and nutrient

accumulation in soil treated with manure produced from DDGS in comparison to soil treated with manure produced from cattle fed regular grain (REG), Benke et al. (2010) reported that soil total P and available P contents were higher in DDGS treatments than REG and un-amended control treatments. These authors also found that barley forage N and P uptake and yield were higher in DDGS than in REG treatments. Therefore, the objective of the study reported on this paper was to expand on the work by Benke et al. (2010) by investigating in the field the effect of applying distillers' grain derived manure collected from feedlot pens at two rates using two different methods of application in comparison with barley grain derived manure on crop yield, nutrient uptake and recovery over two years along with soil available nutrient (N, P, and K) content, organic C, pH, salinity and heavy metals (Cu, Zn and Cd) after the second year of application.

A.4 Materials and Methods

A.4.1 Experimental site

This two-yr experiment was conducted from fall 2008 to fall 2010 near the town of Dixon (52°13'3.2" N lat, 105°11'41.3" W long) in east-central Saskatchewan, Canada. After experimental plot layout in fall of 2008, soil samples were collected only from the control plots at three soil depth increments (0-15, 15-30 and 30-60 cm) for the purpose of the field soil characterization (Table 1). The predominant soil at the site is classified as a Black Chernozem (Cudworth Association) of clay-loam texture. The average particle-size distribution in the 0-60 cm depth was 28% sand, 23% silt and 49% clay determined using pipette method (Gee and Bauder, 1986). The site has nearly level topography and considered productive agricultural land in Saskatchewan (Stumborg et al., 2007). The field was cropped to barley (*Hordeum vulgare* L.) in the year prior to current study. Basic characteristics of the field soil are provided in Table A.1. Climate data during the growing season for the two-yr study period were retrieved from the nearest weather station located at Pilger, approximately 15 km from the experimental site (Environment Canada, 2012). Monthly cumulative rainfall and mean air temperature over the two growing seasons and the 30-yr average are summarized in Fig. A. 1.

A.4.2 Experimental design

The field experiment was established in fall 2008 with experimental treatments consisting of two manure types: distillers' grain fed cattle manure (DGM) and barley fed cattle manure (BGM), two rates of application (low and high) and two methods of application: broadcast followed by incorporation, and band injection. The rates of fresh manure application were 15 and 30 Mg ha⁻¹ (wet weight basis) that are referred to as low and high rate, respectively. The selected application rates were based on typical rates of manure product applied per hectare in Western Canada. It could have been a N-based or a P-based rate, but it was decided to utilize manure product weight to represent typical application rate ranges in Western Canada. An undisturbed check was included as a control for comparison. Treatments were arranged in a randomized complete block design with four replications. Each treatment plot had dimension of 3 m width × 6 m length.

Table A.1. Selected soil properties at the start of the field study in fall 2008 in samples collected from control plots at three soil depth increments (0-15, 15-30 and 30-60 cm)

Property	Soil depth (cm) [§]		
	0-15	15-30	30-60
NO ₃ ⁻ -N (mg kg ⁻¹)	7.4 ± 0.1	4.8 ± 0.1	4.5 ± 0.2
NH ₄ ⁺ -N (mg kg ⁻¹)	3.1 ± 0.7	5.0 ± 0.1	4.8 ± .4
Avail. P (mg kg ⁻¹)	4.5 ± 0.8	2.2 ± 0.1	1.7 ± 0.3
Avail. K (mg kg ⁻¹)	288 ± 26	132 ± 14	98 ± 18
OC (mg g ⁻¹)	27 ± 1.0	13 ± 1.0	7.0 ± 1.0
pH	7.8 ± 0.2	7.8 ± 0.1	7.9 ± 0.2
EC (dS m ⁻¹)	0.4 ± 0.1	2.1 ± 0.7	4.2 ± 0.5
Sand (%)	28.3 ± 0.1	24.2 ± 0.7	30.8 ± 10.0
Silt (%)	20.2 ± 1.0	25.0 ± 2.3	24.6 ± 3.6
Clay (%)	50.6 ± 1.0	51.0 ± 1.7	44.7 ± 6.3

§ values presented are means followed by standard error

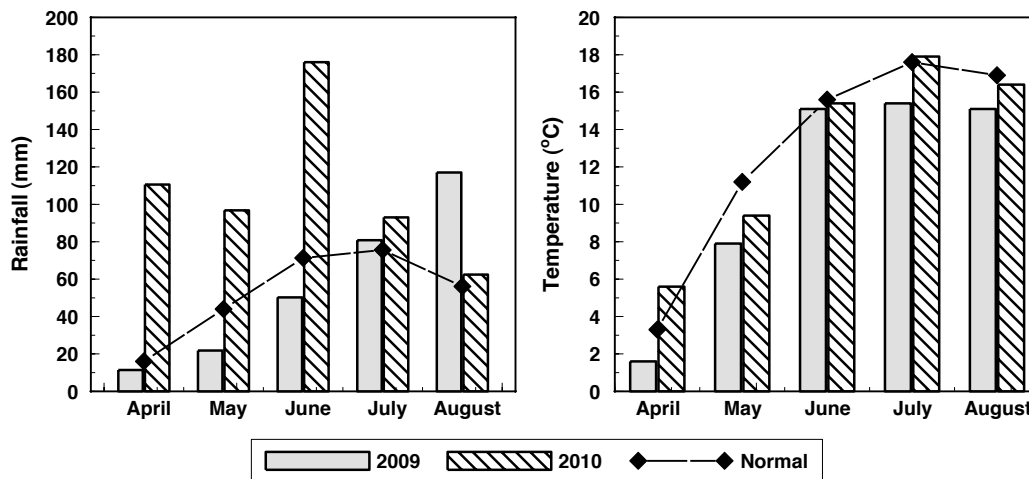


Fig. A.1. Monthly total rainfall and mean air temperatures at Humboldt for the 5-month growing season for both years of the study (2009, 2010). The 30-yr rainfall and temperature averages (normal) are also included.

A.4.3 Manure procurement and application

The two types of manure were collected from the feedlot pens at the University of Saskatchewan Beef Cattle Research Unit in early fall of each year. For DGM, cattle were fed a diet that consisted of 40% wet distiller grain (wheat-based), 45% barley grain, 5% mineral supplement and 10% silage. For the control manure (BGM), cattle were fed a diet containing 85% barley grain, 10% barley silage and 5% mineral supplement. This is a typical diet used in the western Canadian feedlot industry (Hao et al 2009). Feedlot cattle manure used in the study was surface manure pack consisting of straw, fecal material and urine deposited in University of Saskatchewan Department of Animal Science experimental feed trial pens over the ration feeding period that was then scraped off during pen cleaning at the end of the experiment. The manure was then stockpiled for approximately 4 months and mixed by hand prior to application in the field. Prior to manure application to soil, a composite sample from each manure type was collected in a 5-L plastic container, thoroughly mixed to reach appropriate homogeneity and

stored in the freezer (-20 °C) until their chemical composition analyses. Two representative subsamples from each manure type were then taken to perform the analysis. Basic characteristics of both manures are provided in Table A.2.

Manure was applied in the preceding fall (October) of each growing season. It was applied using Prairie Agricultural Machinery Institute (PAMI, Humboldt, SK) prototype solid manure applicator (Landry et al. 2011). The broadcast and incorporation of manure consisted of applying the manure on the soil surface and then immediately incorporating it with a chisel plow cultivator using one pass with 30 cm sweeps on a 20 cm row spacing, followed by harrowing.

Table A.2. Basic characteristics of distillers' grains manure (DGM) and barley grain manure (BGM) used in the field study. All contents are expressed on a fresh wet weight basis.

Property	Manure Type			
	DGM	BGM	DGM	BGM
	2009		2010	
Total N (mg g ⁻¹)	14.7	16.0	15.4	10.5
NH ₄ ⁺ -N (mg kg ⁻¹)	355	251	193	134
Total P (mg g ⁻¹)	2.7	5.1	5.5	4.5
Avail. P (mg kg ⁻¹)	136	98	49	48
Total K (mg g ⁻¹)	9.2	12.2	10.3	8.5
Total S (mg g ⁻¹)	1.5	3.0	3.4	2.7
Total Na (mg g ⁻¹)	1.8	2.9	1.7	1.5
Total Ca (mg g ⁻¹)	13.6	19.8	16.8	18.4
Total Mg (mg g ⁻¹)	5.5	9.3	7.3	7.2
Total Cu (mg kg ⁻¹)	37.5	66.5	29.0	32.0
Total Fe (mg g ⁻¹)	17.0	8.4	11.7	10.0
Total Mn (mg kg ⁻¹)	199	410	300	200
Total Zn (mg kg ⁻¹)	11.5	24.5	132	113
Moisture (%)	45	11	22	26

The band injection of solid cattle manure was performed using the PAMI prototype applicator in which manure was applied in six subsurface bands to a depth of 10 cm. Detailed information about the application techniques of solid manure are described by Landry et al. (2011). Manure was applied during the month of October of 2008 and 2009. Total nutrients in manure were

determined at a commercial laboratory (ALS Laboratory Group, Saskatoon, SK). Extractable-NH₄-N and -PO₄³⁻-P content in both manures were measured by shaking 5 g of manure with 15 mL of deionized water for an hour on a rotary shaker at low speed. The suspension was then filtered and the filtrate was analyzed for NH₄⁺-N and PO₄³⁻-P using automated colorimetry (Technicon Autoanalyzer II, Technicon Industrial Systems, 1978). Rates of nutrients applied in both years were calculated based on nutrient content and rate of manure applications (Table A.3). The field was seeded to Lillian hard red spring wheat on May 9th of 2009 at a rate of 128 kg ha⁻¹ and to BrettYoung 719 Roundup Ready canola (*Brassica napus* L.) on May 19th of 2010 at a rate of 6 kg ha⁻¹. Herbicides were applied to control annual and broadleaf weeds in-crop using standard practices described in Guide to Crop Protection 2009, Saskatchewan Ministry of Agriculture.

Table A.3. Manure nutrients applied at both rates of application (15 and 30 T ha⁻¹) for both years of the study.

Treatment			N	P	NH ₄ ⁺ -N	AP	K	S
Manure Type	Rate	Year	-----kg ha ⁻¹ -----					
	T ha ⁻¹							
DGM	15	2009	221	41	5.3	2.04	138	23
	30		442	82	10.7	4.08	276	46
	15	2010	231	83	3.0	0.74	155	51
	30		462	166	6.0	1.48	310	102
BGM	15	2009	240	77	3.8	1.47	183	45
	30		480	154	7.5	2.94	366	90
	15	2010	158	68	2.0	0.72	128	41
	30		316	136	4.0	1.44	256	82

A.4.4 Plant and soil sample collection and analysis

Both years, the crop was harvested when it reached physiological maturity in September. Duplicate 1-m² plant samples per plot were cut manually at 5 cm above the soil surface. The samples collected were dried by forced air at 45 °C, the total biomass weighed, and mechanically

threshed using a stationary thresher followed by weighing to determine yield. Straw samples were ground to < 2 mm in a WileyTM mill and grain samples were finely ground with a CycloneTM mill. Total N and P were measured by digesting the ground grain and straw samples in sulfuric acid-peroxide (H₂SO₄-H₂O₂) using a temperature-controlled digestion block (Thomas et al. 1967), followed by automated colorimetry for determining P and the NH₄⁺-N using a Technicon Autoanalyzer II (Technicon Industrial Systems, 1973). Total N and P uptake were then calculated from plant N and P contents and total dry matter yield. Apparent N recovery (ANR) and apparent P recovery (APR) were calculated according to Gagnon et al. (1997) as:

$$\text{ANR or APR} = \frac{TNUTP - TNUC}{\text{Total N or P applied}} \times 100$$

where TNUTP denotes total N or P uptake for a given treatment plot, TNUC is the total N or P uptake in control plot and total N or P applied is the amount of N or P applied for the crop year.

Soil samples were collected immediately after crop harvest in September 2010 at the end of September. A hydraulic punch truck was used to collect soil cores (three per plot), which were separated into four soil depths increments (0-15, 15-30, 30-60 and 60-90 cm) and the soil from each depth was mixed to make one composite soil sample per plot for each of those depths. The soil samples were air-dried and ground to pass a 2-mm sieve prior to laboratory analysis. The air-dried soil samples were then analyzed for organic C, inorganic N (NH₄⁺-N and NO₃⁻-N), available phosphorus and potassium, electrical conductivity (EC) and pH. The organic C content was directly measured using a LECO CR-12 combustion carbon analyzer (LECO Corporation, St. Joseph, MI) set at 840 °C (Wang and Anderson 1998). Exchangeable NH₄⁺-N and NO₃⁻-N were extracted by shaking 5 g of soil with 50 mL of 2 M KCl for 1 h on rotary shaker, followed by filtration. The NH₄⁺-N and NO₃⁻-N content in the KCl extracts were measured colorimetrically using a Technicon Autoanalyzer II (Keeney and Nelson, 1982). Available phosphorus and potassium were determined by a modified Kelowna method (Qian et al., 1994). Electrical conductivity and pH were measured in 1:1 soil:water suspension. Soil available Cu, Zn and Cd were extracted by ammonium bicarbonate (AB)-diethylenetriaminepentaacetic acid (DTPA) as described by Lipoth and Schoenau (2007). Extracted Cu, Zn and Cd were then determined using atomic absorption spectrometry (Baker and Amacher, 1982). Inorganic N

($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) was the only soil parameter measured at the four soil depths increments (0-15, 15-30, 30-60 and 60-90 cm) whereas the rest of the soil parameters were only measured in the upper soil layer (0-15 cm).

A.4.5 Statistical analysis

Before selecting the appropriate analysis procedure, Shapiro-Wilk and Bartlett tests were applied to check data normality and homogeneity of variance, respectively. Log-transformation was implemented on data that were not normally distributed and showed lack of variance homogeneity. Statistical analyses were conducted using the MIXED model procedure to determine the effects of the main treatments factors which included manure type, rate of application, method of application and all interactions on soil and crop dependant variables. However, the interactions among factors were not reported here in the tables due to the absence of significant impact. Manure type, rate of application and method of application were treated as fixed effects whereas block was treated as a random effect in the model. Treatment effects were considered significant at a probability level of $P \leq 0.1$ at which means were also separated using LSD test procedure. The analyses were conducted on each year's data separately due to differences in the crop type.

A.5 Results

A.5.1 Climatic conditions

Total rainfall (281 mm) in the 2009 growing season (April to August) was close to the long-term average (263 mm), especially during the month of April, before seeding, which showed similar rainfall to the long-term average and May, during seeding, and June which both exhibited less rainfall than the long-term average (Fig. A.1). Unlike the first growing season, the entire region experienced unusually high rainfall during the 2010 growing season. The total rainfall during the first three months of the 2010 growing season was about 66% higher than the long-term average. The total rainfall during the 2010 growing season was approximately 539 mm, nearly double the amount received in 2009 and more than double the long-term average. The excess moisture observed in 2010 growing season was expected to impact crop performance.

The monthly mean air temperature for both growing seasons did not differ greatly from the long-term average (Fig. A.1).

A.5.2 Manure characteristics

Both manure types applied in the fall of 2008 for the 2009 season showed relatively similar total N content whereas the total P content was slightly higher in DGM (Table A.2). However, the available forms of N ($\text{NH}_4^+\text{-N}$) and P were considerably higher in DGM than BGM. Manures applied in the fall of 2009 for the 2010 season were generally different in their chemical composition, especially their TN and TP content. The TN and TP content in DGM was approximately 27% and 20%, respectively, higher than in BGM. The $\text{NH}_4^+\text{-N}$ content was slightly higher in DGM than BGM whereas the available P content was almost similar in both manures.

A.5.3 Crop yield response

Crop response to manure type was not significant (Table A.4) while rate and method of application had significant effects on crop responses (Table A.4). The effect of manure application was more evident when manure was applied at the high rate regardless whether it was broadcasted or injected. Wheat grain yields at the high rate of manure application were significantly higher ($P < 0.05$) than the control. At equivalent rate, injection method tended to provide a higher yield than the broadcast and incorporated.

Manure rate of application had a significant impact on canola seed yield (Table A.5); however, neither manure type nor method of application had a significant influence on crop yield. Treatment effects on crop yield were more pronounced in the year 2010 (Table A.5) compared to year 2009 (Table A.4). Grain yield of canola was significantly higher for all treatments than that of the control. The high rate of application of both manure types tended to produce higher canola yield when compared to the low rate of application regardless of the method of application. This increase in yield was more evident with injected DGM, where the high rate treatment was significantly higher than the low rate treatment (Table A.5).

A.5.4 Nutrient uptake response

Crop N and P uptake was not significantly affected by manure type whereas the manure application rate significantly affected both parameters (Table A.4). The application method had only a significant impact on N uptake, but not P uptake (Table A.4). The greatest N uptake was observed in plots receiving the high application rate of manure (Table A.4). This value tended to be higher still when manure was injected. High rate of application treatments provided significantly higher N uptake than the control. Total P uptake was found to be significantly higher in soil treated with high rate of manure application when compared to untreated control (Table A.4).

In the year 2010, manure type had also no significant impact on either N or P uptake (Table A.5). It was only the application rate factor that showed a significant effect on N and P uptake (Table A.4). The effect of the treatments on plant N uptake was more evident in the year 2010 ($P < 0.001$) (Table A.5), compared to the year 2009. With the exception of DGM injected at the low rate treatment, N uptake from all treatments were significantly higher than the control. The highest N uptakes were obtained with the high rate of manure addition regardless of the method of application. Broadcast or injected DGM at the high rate resulted in significantly higher N uptake than that of injected DGM applied at the low rate. However, this was not the case for the BGM type. Canola P uptake from manure applied treatments were significantly higher than from the control in the year 2010. The P uptake tended to be greater when both manures were applied at the high rate especially when broadcasted (Table A.5). The treatment effects on canola P uptake in 2010 were more pronounced compared to wheat P uptake in 2009. Additionally, the P uptake among all treatments in 2010 (12.9 to 40.2 kg ha⁻¹) were much higher than values in 2009 (8.2 to 13.5 kg ha⁻¹).

A.5.5 Apparent N and P recovery

Manure type and rate of application did not significantly affect ANR; however, method of application showed a significant impact on ANR in the year 2009 (Table A.4). Overall, injected manure resulted in higher ANR compared to broadcasted manure. In contrast to the year 2009, manure type showed a significant effect on ANR in the year 2010 (Table A.5). Similar to the year 2009, method of manure application showed a significant impact on ANR in the year

2010; however, rate of application remained insignificant (Table A.5). When averaged across all treatments, DGM type application resulted in lower ANR (22%) compared to BGM type (34%) in the year 2010. In the same year, broadcasted manure resulted in higher ANR (32%) in comparison to injected manure (24%), when averaged across all treatments.

The apparent P recovery (APR) data for the years 2009 and 2010 are presented in Table A.4 and Table A.5, respectively. In 2009, APR was not affected by manure type, rate and method of manure application (Table A.4). However, manure type and rate of application were shown to have a significant effect on APR in the year 2010 (Table A.5). When averaged across all treatments, APR was higher in DGM treatments (26%) than BGM (21%). The average of low rate treatments showed greater APR (26%) compared to the high rate treatments (20%). Overall, nutrient recovery was higher in 2010 than in 2009 (Table A.5).

A.5.6 Soil response-changes in soil chemical properties

Residual inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) data collected from different depths after harvest in the second year are provided in Table A.6. The inorganic N content varied with depth and among treatments. The soil $\text{NO}_3^-\text{-N}$ content was highest near the surface (0-15 cm). At the 0-15 cm depth, method of manure application had a significant impact on $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ (Table A.6). However, inorganic N content was not affected by manure type or rate of application (Table A.6).

The broadcast and incorporation method of manure application showed a higher $\text{NO}_3^-\text{-N}$ content, averaging 24 kg $\text{NO}_3^-\text{-N ha}^{-1}$ across all treatments, compared to 15 kg $\text{NO}_3^-\text{-N ha}^{-1}$ when manure was injected in the 0-15 cm. However, the $\text{NH}_4^+\text{-N}$ content was slightly greater when manure was injected (11 kg $\text{NH}_4^+\text{-N ha}^{-1}$) than broadcast (10 kg $\text{NH}_4^+\text{-N ha}^{-1}$) (Table A.6). Unlike the surface (0-15 cm), inorganic N was dominated by $\text{NH}_4^+\text{-N}$ in the lower 3 soil sampling depths (Table A.6). The $\text{NH}_4^+\text{-N}$ content increased with soil depth until the 30-60 cm increment whereas the $\text{NO}_3^-\text{-N}$ content remained the highest in the upper soil profile (0-15 cm). The $\text{NO}_3^-\text{-N}$ content was significantly influenced by the method of manure application at the depth of 15-30 cm and the rate of application at the depth of 60-90 cm (Table A.6). At those depths, the broadcast method showed a slightly higher $\text{NO}_3^-\text{-N}$ content (11 kg ha^{-1}) than injection (9 kg ha^{-1}) at 15-30 cm depth, and the high rate of application gave a greater $\text{NO}_3^-\text{-N}$ content (12

kg ha⁻¹) compared to that of the low rate (9 kg ha⁻¹) at the 60-90 cm depth, when averaged across all treatments.

Table A.4. Grain yield of wheat, total plant N uptake, total plant P uptake, apparent N recovery (ANR) and apparent phosphorus recovery (APR) responses to experimental treatments during first growing season (2009) of the two-yr field trial.

Treatment [¶]			Grain yield	N uptake	P uptake	ANR	APR
Manure Type	Rate	Method	t ha ⁻¹	kg ha ⁻¹		%	
Control	0	Control	1.19 e [§]	47 d	8.2 d	na [∫]	na
DGM	L	BRC	1.41 cde	53 cd	9.3 cd	5.0 d	2.6
		INJ	1.51 bcde	56 bcd	9.6 bcd	12.2 abc	3.2
	H	BRC	1.83 ab	71 abc	12.3 ab	14.5 ab	4.2
		INJ	1.96 a	80 ab	13.5 a	14.8 ab	5.3
BGM	L	BRC	1.38 de	55 cd	9.8 bcd	7.9 cd	2.6
		INJ	1.74 abcd	67 abc	11.9 abc	18.1 a	5.9
	H	BRC	1.78 abc	70 abc	12.8 a	12.8 abc	3.6
		INJ	2.07 a	82 a	12.8 ab	10.3 bcd	3.6
ANOVA				<i>P</i> value			
Manure Type (MT)			0.403	0.346	0.280	0.288	0.780
Rate (R)			0.000	0.000	0.001	0.308	0.447
Method (M)			0.053	0.077	0.327	0.035	0.214

∫ Not applicable.

§ Means within a column sharing the same letter are not significantly different at *P* = 0.10.

¶ DGM denotes distillers' grains manure, BGM denotes barley grain manure, BRC denotes broadcast and incorporate, INJ denotes band injection.

Table A.5. Grain yield of canola, total plant N uptake, total plant P uptake, apparent N recovery (ANR) and apparent phosphorus recovery (APR) responses to experimental treatments during second growing season (2010) of the two-yr field trial.

Treatment [¶]			Grain yield	N uptake	P uptake	ANR	APR
Manure Type	Rate	Method	t ha ⁻¹	kg ha ⁻¹		%	
Control	0	Control	0.61 d [§]	79 e	12.9 e	na [‡]	na
DGM	L	BRC	1.51 abc	147 bcd	31.5 bcd	27.6 b	33.9 a
		INJ	1.14c	117 de	24.5 d	14.6 b	20.4 bc
	H	BRC	1.72 ab	190 a	39.5 ab	23.0 b	24.6 abc
		INJ	1.84 a	187 ab	40.2 a	22.2 b	25.2 abc
BGM	L	BRC	1.51 abc	157 abcd	33.1 abc	46.3 a	28.5 ab
		INJ	1.38 bc	137 cd	28.5 cd	33.5 b	21.7 bc
	H	BRC	1.80 a	176 abc	36.1 abc	29.1 b	16.5 c
		INJ	1.62 ab	164 abc	34.6 abc	25.4 b	15.3 c
ANOVA				P value			
Manure Type (MT)			0.808	0.826	0.711	0.026	0.091
Rate (R)			0.004	0.001	0.002	0.207	0.082
Method (M)			0.208	0.129	0.185	0.089	0.109

[‡] Not applicable.

[§] Means within a column sharing the same letter are not significantly different at *P* = 0.10.

[¶] DGM denotes distillers' grains manure, BGM denotes barley grain manure, BRC denotes broadcast and incorporate, INJ denotes band injection.

Soil available P content in the 0-15 cm depth, determined after harvest in 2010, is shown in Table A.7. The available P content was significantly affected by the rate of manure application whereas manure type and method of application did not have a significant impact on the soil available P content (Table A.7). Treatment means comparison showed that only injected manure at the high rate was significantly higher than most of the other treatments. When averaged across all treatments, the high rate of manure addition resulted in a higher soil available P content in the 0-15 cm depth (51 kg ha^{-1}) compared to the low rate of application (30 kg ha^{-1}).

Manure type did not have any significant effect on the other soil parameters (available K, Cu, Zn, Cd, OC, pH, EC) measured in the current study (Table A.7). However, the application rate and method had a significant impact on Zn and Cu, respectively (Table A.7). The high rate of manure application resulted in a slightly higher residual Zn content (3.4 kg ha^{-1}) compared to that of the low rate (2.8 kg ha^{-1}). The Cu content was slightly higher in manure injection treatment (2.0 kg ha^{-1}) than broadcast (1.7 kg ha^{-1}).

Table A.6. Soil content of inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) at 4 soil depth increments determined after crop harvest of the second growing season (2010) of the two-yr field trial.

Treatment [¶]			Soil depth (cm)							
			0-15 cm		15-30 cm		30-60 cm		60-90 cm	
			NO ₃ -N	NH ₄ -N	NO ₃ -N	NH ₄ -N	NO ₃ -N	NH ₄ -N	NO ₃ -N	NH ₄ -N
Manure Type	Rate	Method	-----kg ha ⁻¹ -----							
Control	0	Control	11.4 c [§]	11.3 ab	7.6 d	11.90	9.60	27.4	7.6 b	35.4
		BRC	23.1 ab	12.9 abc	9.6 abcd	22.20	10.10	47.5	7.9 b	41.7
		INJ	13.6 bc	9.7 bc	8.7cd	15.10	9.40	48.0	7.9 b	40.2
DGM	H	BRC	21.3 abc	8.8 c	12.1 a	14.40	11.30	25.2	14 a	20.4
		INJ	15.4 bc	11.2 abc	9.8 abcd	15.10	12.00	39.8	11.3 ab	37.4
		BRC	29.8 a	8.8 c	10.6 abc	10.80	12.00	34.7	8.9 b	43.2
BGM	L	INJ	13.5 bc	11.3 abc	9.2 bcd	14.80	11.60	16.3	9.9 ab	39.3
		BRC	21.9 abc	8.6 c	11.5 ab	11.10	11.10	35.5	10.8 ab	40.1
		INJ	16.7 bc	13.6 a	9.1 bcd	16.40	11.70	43.6	9.9 ab	36.30
ANOVA			P value							
Manure Type (MT)			0.268	0.505	0.922	0.107	0.162	0.855	0.957	0.434
Rate (R)			0.537	0.682	0.120	0.443	0.260	0.217	0.032	0.224
Method (M)			0.000	0.038	0.018	0.451	0.905	0.184	0.670	0.749

§ Means within a column sharing the same letter are not significantly different at $P = 0.10$.

¶ DGM denotes distillers' grains manure, BGM denotes barley grain manure, BRC denotes broadcast and incorporate, INJ denotes band injection.

Table A.7. Selected soil properties at 0-15 cm depth determined after crop harvest of the second growing season (2010) of the two-yr field trial.

Treatment [¶]			P	K	Cu	Zn	Cd	OC	pH	EC
Manure Type	Rate	Method	kg ha ⁻¹					mg g ⁻¹		dS m ⁻¹
Control	0	Control	17.3 b [§]	467	1.7 b	2.1 b	0.25	24	7.9	0.9
	L	BRC	29.6 b	592	1.7 ab	2.8 ab	0.28	26	8.0	1.2
		INJ	30.2b	526	2.0 ab	3.0 ab	0.29	26	8.0	1.0
DGM	H	BRC	49.9 ab	625	1.8 ab	3.3 ab	0.23	23	7.9	1.3
		INJ	66.1 a	655	1.9 ab	3.6 a	0.26	23	8.0	1.2
	L	BRC	30.5 b	577	1.7 b	2.8 ab	0.26	26	8.0	1.0
INJ		31.6 b	540	1.9 ab	2.6 ab	0.28	26	7.9	1.1	
BGM	H	BRC	41.5 ab	558	1.7 ab	3.7 a	0.28	27	8.2	0.9
		INJ	46.4 ab	666	2.1 a	3.1 ab	0.29	26	7.9	1.8
ANOVA			P value							
Manure Type (MT)			0.472	0.977	0.955	0.717	0.287	0.120	0.616	0.772
Rate (R)			0.000	0.103	0.606	0.051	0.238	0.200	0.516	0.801
Method (M)			0.419	0.835	0.018	0.963	0.160	0.664	0.243	0.338

§ Means within a column sharing the same letter are not significantly different at $P = 0.10$.

¶ DGM denotes distillers' grains manure, BGM denotes barley grain manure, BRC denotes broadcast and incorporate, INJ denotes band injection.

A.6 Discussion

A.6.1 Manure characteristics

The relatively similar composition of both manures applied the first year may have been the result of dilution by bedding materials used in the cattle feedlot pens. The other possible reason that may explain the lack of anticipated higher nutrient content in the DGM compared to that of BGM is the higher moisture content, which was about four times higher than that of BGM. However, the available forms of N (NH_4^+ -N) and P were considerably higher in DGM than BGM. This is in agreement with other studies that found that forms of manure nutrient can be influenced by inclusion of distillers' grains in animal diets (Ebeling et al., 2002; Sphiehs and Varel, 2009). Hao et al. (2009) also found a significant increase in water soluble NH_4^+ -N content in manure produced from animal fed dried distillers' grain with solubles.

Unlike the manures applied in the fall of 2008, the manures applied in the fall of 2009 for the 2010 season generally showed differences in their chemical composition; especially TN, TP and NH_4^+ -N contents. The higher content of some nutrients in DGM is in line with previous findings (Ebeling et al., 2002; Hao et al., 2009; Sphiehs and Varel, 2009).

A.6.2 Crop responses

The absence of manure type effects on wheat yield and total plant N uptake is consistent with similar initial nutrient content of both manures, especially in the first year. Despite the higher NH_4^+ -N content in DGM than BGM in the first year, DGM treatments did not lead to higher yield or plant N uptake. In comparison to other findings, several studies that compared crop and nutrient uptake responses to addition of fresh and composted beef cattle manures that differed in their chemical and physical composition have reported inconsistent results. Other researchers found that yield and N and P uptake responses to addition of fresh and composted manures to two soils with different properties were dependent on soil type (e.g., Xie and MacKenzie 1986). This may explain the lack of significant differences between the two types of manures used in the current study in which the soil used for the field study exhibits relatively high background levels of N, P and organic matter in addition to fine texture. These soil properties may explain the lack of response to the manure treatments. Mooleki et al. (2004) also found less response of

N uptake and grain yield to manure application in a soil that had relatively higher background levels of available N when compared to another soil that had low available nutrient and organic matter content.

The greater N uptake observed with the high rate of manures application in both years is consistent with the higher amount of available N ($\text{NH}_4^+\text{-N}$) associated with manure application at the high rate. Similarly, greater P uptake in high rate treatments is explained by the higher available P -in soil arising from the high rate of manure application.

In general, overall treatment effects on all crop responses were more pronounced in the second year with canola, a crop with a higher nutrient demand than wheat (Malhi et al. 2008). Unlike the first year, all treatments produced significantly higher canola yield and N and P uptake than the control, including the low rates of manure application. This can also be attributed to additional N supplied through the mineralization of residual organic N applied in the previous year (Pratt et al. 1973). Eghball and Power (1999) revealed that N availability in the first and second year after feedlot cattle manure addition was 40 and 15%, respectively. Similarly, Mooleki et al. (2004) reported that effect of single application of animal manure continued for the second year, producing greater wheat yield.

In contrast to the first year, the second year of the study showed that manure type had a significant impact on ANR and APR, in which ANR in BGM treatments was higher than in DGM treatments and APR in DGM treatments was greater than in BGM treatments. Overall, nutrient recoveries were higher in the second year, which could be a result of a contribution from the previous year and the higher nutrient requirement and uptake potential of canola versus wheat. The higher APR with the low rate of manure application treatments in the second year is consistent with the previous studies that found phosphorus use efficiency was usually greater with a low rate of manure application (Eghball and Sander 1989; Miller et al. 2009).

A.6.3 Soil responses

The available N content at the 0-15 cm depth, especially $\text{NO}_3^-\text{-N}$, was generally affected by manure application regardless of manure type. The absence of significant effects of manure type on available N remaining in soil after second year crop harvest can be related in part to the similar initial total N content of both manure types in the first year. Although initial available N content in DGM was higher than in BGM in the first year, and initial total N content in DGM

was considerably higher than in BGM in second year, soil available N did not differ between the two types of manure treatments. The very wet conditions the region experienced in the second year is an important factor that may have contributed to additional soil N losses. In a similar study but under controlled conditions, Benke et al. (2010) found that barley grain manure and dried distillers' grain with solubles manure showed similar effects on soil total N content after harvest and attributed this to the similar content of initial total N. These authors also found that the two types of manure treatments did not differ in their effects on soil available N content even though initial available N content in DGM was about three times higher than in BGM. They related this to volatile NH_3 loss after amendment addition that might be higher from DGM than BGM at the high soil pH since the amount of NH_4^+ -N and proportion of available N in NH_4^+ -N form was greater in DGM than in BGM. This may in part explain the significant impact of manure application method in this study where NH_4^+ -N was higher in injected manure treatments than broadcast and incorporation treatments in which soil conditions would promote more NH_3 volatilization (pH = 7.8 at 0-15 cm depth). However, the broadcast and incorporation treatments had higher NO_3^- -N content than injected method treatments. This may be related to the higher N uptake observed with the injected method treatments especially in the first year. The dominance of NH_4^+ -N over NO_3^- -N in lower depths is likely related to the NH_4^+ -N being fixed and bound in clay mineral interlayers. The NH_4^+ -N fixation process in the field study may explain the build-up of the NH_4^+ -N pool in the soil profile in which NH_4^+ -N ion is protected against nitrification and subsequent leaching (Nieder et al. 2011). It was also reported that NH_4^+ -N content increased with soil depth due to decreasing soil organic matter content (Zhang et al. 2003; Nieder et al. 2011). This is in agreement with the current study findings. The soil in this study has a relatively high clay content, and NH_4^+ -N fixation is greater in clay soil than in sandy soil (Chantigny et al. 2004).

Despite the fact that total P content was slightly higher in DGM than BGM in the second year, manure type did not have a significant impact on post harvest soil available P content. This is in contrast to results of Benke et al. (2010) which found that, under controlled conditions, DGM treatments had greater amount of soil total and available P content than BGM treatments, and this increase was attributed to the higher total and available P contents in DGM than in BGM. Generally, manure treatments in the present study contributed to higher available P present in soil after harvest, and this was significantly affected by the rate of application. This

observed increase in soil available P is in agreement with previous long-term field studies that reported increases in soil available P after addition of animal manures to agricultural soils (Sharpley et al. 1984; Motavalli and Miles 2002; Hao et al. 2008).

The lack of significant treatments effect on other measured soil properties, such as K, Cd, OC, pH and EC under field conditions may be partially related to the relative similarity of both manures' chemical properties. In addition, the Black Chernozemic soil is rich in organic matter and nutrients, and therefore high background levels of soil nutrient and carbon may explain the absence of significant treatment effect on most measured soil properties. The higher organic matter and clay content of this soil would buffer against pH changes (Wu and Powell 2007). Mooleki et al. (2004) also found that there was no change in soil pH following 4 years of cattle manure application to a Black Chernozemic soil. Chang et al. (1990) reported a significant decrease in pH after 11 years of annual feedlot cattle manure to a Brown Chernozem. However, Hoyt and Rice (1977) mentioned that cattle manure might have a buffering impact against decreases in soil pH, when added to a Luvisolic soil.

The rate of manure application had a significant impact on extractable Zn level in soil, in which higher Zn content was found in soil treated with a higher rate of manure ($3.4 \text{ kg Zn ha}^{-1}$, mean across treatments) compared to treatments with the low application rate ($2.8 \text{ kg Zn ha}^{-1}$). Compared to manure injection method, the lower Cu content associated with broadcast and incorporation may result from a greater Cu interaction and fixation with reactive soil constituents like carbonates and clays as manure is in greater contact with soil constituents under the broadcast than banded injection method. Qian et al. (2003) observed no significant differences in total Cu and Zn after repeated application of cattle manure on Black Chernozemic soils and related this to the relatively low amounts of Cu and Zn added over the years to cause any significant build-up in soil. However, the same authors found a slight increase in moderately labile Cu and Zn after manure addition; but this increase was only observed with large amounts of added cattle manure.

A.7 Conclusion

Manure derived from cattle fed distillers' grain in the ration had different characteristics compared to manure derived from cattle fed the regular barley grain ration, especially for manures collected in the second year of the trial. The similar composition of both manures

particularly in the first year may be related to dilution with bedding materials. The effects of distillers' grain manure on most measured crop and soil parameters did not differ greatly from that of regular barley grain fed cattle manure. The only crop variable that was higher in DGM treatments was the apparent P recovery; this may be due to higher total and soluble P content associated with DGM. The injection method of manure application appeared to be more effective in yield response and promoting N uptake and recovery than broadcast and incorporate, suggesting that band injection application may conserve N, increase NH_4^+ -N in the upper soil depth (0-15 cm) and promote N uptake. Application of manure at the high rate resulted in higher yield and nutrient uptake; however, it also led to a higher NO_3^- -N content at the lower soil depth (60-90 cm), suggesting some downward leaching of soil nitrate under the unusually wet conditions of 2010.

Future work is needed to evaluate DDGS manures derived from cattle fed different DDGS sources, e.g., corn (*Zea mays* L) versus wheat studies on their effect on manure properties and soil, and crop response to these manures is also required. Increasing DDGS inclusion in animal diets to a higher ratio, e.g., higher than 40% and evaluating this increase on manure properties may be of interest.