Ammonium-N Persistence and Root Nitrogen Content of Annual Crops and Perennial Forage Grasses Following Pig Manure Application

By

Ahmed Agboola Lasisi

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Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

ABSTRACT

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Studies have shown that significant amounts of nitrate-N is leached beyond root zones of annual crops while small amounts of nitrate-N is leached beyond the root zones of perennial forage grasses. This study investigated short-term ammonium-N persistence and root nitrogen content of annual crop and perennial forage grasses following application of pig manure to a sandy loam soil at Carman, Manitoba. Results showed that ammonium-N in liquid pig manure (LPM) amended treatment peaked four days after manure application (DAM) in perennial cropping system (PCS; 50 - 74 kg ha^{-1}) and annual cropping system (ACS; 18 - 29 kg ha^{-1}) in 2014 and 2015. Ammonium-N persisted up to 7 DAM in LPM amended PCS, but did not persist beyond 4 DAM in LPM amended ACS. Ammonium-N measured in solid pig manure (SPM) amended ACS and PCS was low throughout the sampling days in both years. There was a greater percentage increase in accumulation of nitrate-N at 15 - 30 cm soil depth of LPM and SPM amended ACS than PCS. In both years, dry weight below-ground plant biomass ranged from 5,258 to 9,627 kg ha^{-1} at 0 - 60 cm depth in PCS while that of ACS ranged from 1,088 to 1,456 kg ha^{-1}. Also, root N content in PCS ranged from 43 to 118 kg N ha^{-1} in both years while that of ACS ranged from 9 to 20 kg N ha^{-1}.

In conclusion, ammonium-N persisted longer in PCS than ACS in the short-term and total plant N was greater in PCS than ACS. Greater total plant N in PCS than ACS was

mainly due to its greater root N content rather than above-ground N uptake. The order of magnitude of the difference in root N content (34 to 98 kg N ha^{-1}) between ACS and PCS was sufficient to account for the 20 to 60 kg N/ha of nitrate-N leached in ACS in previous study at the same site.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Pig Manure

Pig manure has the potential for improving crop production by supplying plant essential nutrients and improving soil physical properties (Dorff and Beaulieu 2014). Manitoba Conservation (2006) reported that Manitoba produced about 8.6 million pigs in 2005. This translates into 17.2 million tons of manure per year based on Larson (1991), who estimated that 2 tons of manure was produced per pig annually (Nikièma et al. 2013). Of the 11,650,600 acres of cropland in Manitoba, the pig industry requires between 474,000 and 742,000 acres of this crop land for the application of the pig manure produced as nitrogen (N) amendment. Thus, available pig manure is sufficient to fertilize approximately 6% of the farmlands in Manitoba to meet N requirement of crops (Manitoba Conservation 2006). Pig manure application to farmland, when managed properly is an economical and environmentally sustainable method of utilizing manure for the supply of essential plant nutrients and for recycling plant nutrients (Choudhary et al. 1996).

1.1.1 Pig Manure Handling

Pig manure is mainly managed in solid or liquid forms. Solid pig manure (SPM) contains more than 25% solid content or less than 80% water by weight (Schoenau et al.

2006). It is a combination of feces, urine, bedding materials and food residues and is generated when the pigs deposit their excreta directly on the beddings placed on shelter floors which allow liquid content to drain off before being stockpiled (Schoenau and Assefa 2004). Bedding is usually made of cereal straw that is chopped and spread on to the floor. In some cases, no bedding is used and the pig excreta is periodically scraped off the floor and then stockpiled. Solid pig manure has a lesser offensive odour in storage and during application, a greater nutrient retention, and lower quantity to handle when compared to liquid pig manure (Schoenau and Assefa 2004).

Liquid pig manure (LPM) contains no bedding and has a large amount of water. Liquid pig manure has water content of greater than 90% by weight or less than 5% by weight solid content (Schoenau et al. 2006). It is a combination of urine, water and feces. In this case, pigs pass their excreta on the concrete floor of the barn which is then flushed by large amounts of water into a reservoir where it is stored in concrete tanks below ground; concrete or steel tanks above ground; or lined earthen storages. Although LPM has a greater offensive odour than SPM in storage, it allows for ease of mechanization, is more uniform and provides greater precision in application and a lower labour requirement (Schoenau and Assefa 2004).

1.2 Nitrogen in Soil and Pig Manure

Nitrogen (N), both in the soil and pig manures, exists in two general forms: organic N and inorganic N. Freshly excreted manure has a high portion of organic N which is later converted to an inorganic form for plant uptake (Bakhsh et al. 2005). The inorganic form of

N in manure is mainly ammonium as nitrate formation is minor (Manitoba Pork Council 2007).

1.2.1 Fates of N in soil

Nitrogen in soil and manure undergoes various transformations which are influenced by biotic and abiotic factors. The processes of N transformation include mineralization, nitrification, denitrification and immobilization, plant uptake and/or loss in soil or atmosphere.

1.2.1.1 Mineralization and Immobilization

Mineralization of organic nitrogen in the soil is an ammonification process in which organic nitrogen is converted into ammonium (inorganic N) by soil organisms (Addiscott 2005). Mineralization occurs when organisms in soil feed on organic materials that contain more N than they require for their own growth. This process makes inorganic N available for plant uptake and results in formation of OH^- which slightly increases the pH of the soil (Table 1.1; Amlinger et al. 2003; Addiscott 2005).

Table 1.1 Microbial transformation of organic N in soil.

Process	Reaction	Examples of soil organisms
Mineralization		
	$\text{(Humus)R-NH}_2 + H_2O \longrightarrow NH_3 + \text{R-OH}$	Heterotrophic bacterial
	$NH_3 + H_2O \longrightarrow NH_4^+ + OH^-$	
Nitrification		
	$2\ NH_4^+ + 3O_2 \longrightarrow 2NO_2 + 2H_2O + 4H^+$	*Nitrosomonas spp*
	$2NO_2 + O_2 \longrightarrow 2\ NO_3^-$	*Nitrobacter spp*

(Amlinger et al. 2003; Addiscott 2005).

Immobilization is the reverse process of mineralization where inorganic form of N is converted into the organic form of N. Soil microorganisms convert inorganic N to organic N in the process of building cellular materials such as protein, cell walls and nucleic acid (Myrold and Bottomley, 2008). The process of immobilization reduces the amount of inorganic N that is available for plant uptake as it makes it temporarily unavailable.

Both mineralization and immobilization are affected by C/N ratio and the biochemical quality of the carbon source (Trinsoutrot et al. 2000). They occur simultaneously in soil by a process called mineralization/immobilization turnover.

1.2.1.2 Nitrification

Nitrification is a microbial process by which ammonium or ammonia is sequentially oxidized to nitrite and then to nitrate (Addiscott 2005; Sahrawat 2008). Although a few heterotrophic microorganisms such as *Bacillus badius* have been identified to carry out nitrification in soil usually at much slower rate, *Nitrosomonas spp* and *Nitrobacter spp* are examples of obligate chemoautotrophic bacteria that are responsible for nitrification in soil (Sahrawat 2008). Nitrification is an integral part of the N cycle that converts the relatively immobile N (NH_4^+) to the highly mobile nitrate (NO_3^-) form (Subbarao et al. 2006). The process of nitrification acidifies the soil due to the release of protons (H^+) (Table 1.1).

Nitrification process is affected by a number of factors including soil texture and structure, soil aeration status, cation exchange capacity, pH, moisture, temperature, organic matter, substrate (NH_4^+) and microorganism (Subbarao et al. 2006). Several of these factors are interrelated with respect to nitrate formation and stabilization.

1.2.1.3 Nitrogen Uptake by Plants

Nitrogen uptake by plants is an important indicator of plant growth (Xin et al. 2014). Nitrogen uptake in a plant's life-history follows a sigmoid function with a small N requirement during the germination period, followed by large N demand during vegetative growth and reproductive growth and finally a small N need when the reproductive organs reach maturity (Meisinger and Delgado 2002; Delgado and Follett 2010).

Nitrogen is mainly taken up by plants in inorganic forms, ammonium and nitrate and the form of uptake is dependent on several factors which include soil aeration status, plant species, cultivars, growth stage and NH_4^+/NO_3^- tolerance level of plants (Fageria and Baligar 2005; Maathuis 2009; Li et al. 2013).

1.2.1.4 Loss of N in Soil

Pathways for N loss in the soil are leaching, volatilization, surface runoff and denitrification (Fageria and Baligar 2005). Nitrate is usually lost through runoff, leaching or denitrification process while ammonium is adsorbed to the surface of soil particles and is not readily leached beyond the root zone of plants, but may be volatilized as ammonia gas when the soil pH becomes high (Bakhsh et al. 2005).

1.3 Nitrate Leaching

Leaching refers to the downward movement of nutrients such as N in water through the soil profile and beyond the root zones of plants (Zhaohui et al. 2012). Leached N may subsequently enter and pollute groundwater or remain beyond the reach of plants within the soil profile. Nitrate is the dominant form of inorganic N that is leached (Dinnes et al. 2002) and agriculture is a major contributor of nitrate pollution in water (Randall and Mulla 2001). Nitrate susceptibility to leaching is mainly due to its solubility and mobility conferred on it by its negative charge which does not allow it to be held by the negatively charged soil particles (Meisinger and Delgado 2002; Cicek et al. 2015). Nitrate leaching occurs when the accumulation of nitrate in the soil profile is followed by or coincides with a period of large water input (Di and Cameron 2002; Mulla and Strock 2008). Nitrate leaching is influenced by a combination of factors which include rate of nitrification, type of soil and porosity, hydrology, soil texture, soil water content, residual nitrate before manuring or fertilizing, the intensity of activity of various soil microorganisms, tillage practices, cropping system and manure type or N source (Meisinger and Delgado 2002; Dinnes et al. 2002; Beaudoin et al. 2005; Jia et al. 2014).

Nitrate leaching can result in poor water quality, eutrophication methemoglobinemia (Townsend et al. 2003; Elrashidi et al. 2005; Rosenstock et al. 2014). As such, in regions where groundwater serves as a source of drinking water, protecting such water from nitrate pollution by agricultural activities is vital to public health.

1.3.1 Management of Nitrate Leaching

A sustainable agricultural system is defined as "one which offers continued productivity without degrading either the base resource or ecosystems that are influenced by the agricultural system" (Cransberg and McFarlane 1994). Therefore efficient, sustainable, and sound management practices of nitrate that account for soil properties, hydrology and crop requirements are needed to increase crop yields per unit area and protect adjacent environments.

1.3.1.1 Water Management Techniques

Research has shown that the volume of water that flows through the soil is a primary factor in N loss (Tan et al. 1993; Drury et al. 1996). It is one of the two most important factors that determine nitrate leaching. When water is in excess of the soil water holding capacity, dissolved nutrients like nitrate move with water through the soil pores. Thus, efficient water use management is a fundamental practice for managing nitrate leaching.

1.3.1.2 Nutrient Management Techniques

Nutrient management is the second of the two main factors that influence nitrate leaching. Nutrient management involves the appropriate implementation of 4Rs nutrient management concept. The 4Rs are right time, right rate, right placement and right source (The Fertilizer Institute 2016).

1.3.1.2.1 Right Time

Nitrogen application should be synchronized with phases of plant growth and demand for N. Randall and Mulla (2001) applied fertilizer N in fall and spring to continuous corn during a 6-year period and found that fall application had an average corn yield that was 8% lower than spring applied N and annual nitrate leaching increased by 36% compared to spring fertilizer N application. Also, van Es et al. (2006) found a greater nitrate leaching potential from fall manure applications than spring manure applications on a 3 year continuous maize planting. Therefore, N should be applied closer to the time of planting in order to reduce the potential for nitrate leaching.

1.3.1.2.2 Right Rate

The amount of applied N is a basic factor that affects nitrate leaching. The appropriate rate of N varies from one crop to another and should take the residual soil nitrate into consideration (MAFRI 2007). Applying manure N at a rate that exceeds plant requirement will increase the risk of nitrate leaching. Nikièma et al. (2013) reported a significant increase in nitrate leaching when manure N was applied on wheat at a rate beyond the intermediate N rate for wheat.

1.3.1.2.3 Right Sources

Nitrogen source must be considered in managing N as it determines the availability of nitrate in soil. The use of manure as the sole source of nitrogen for crop production has been reported to result in a greater potential of nitrate leaching than inorganic N source (Allen et al. 2006). Bakhsh et al. (2005) compared nitrate leaching between pig manure and urea ammonium nitrate on a continuous corn system and reported that there was 53% more nitrate leached from pig manure when compared to the urea ammonium nitrate in tile water.

1.3.1.2.4 Right Placement

Manure or fertilizer N should be applied to the soil in a way that will enable efficient uptake by plants in order to improve yield and reduce its potential for loss to the environment. While fertilizer N is mainly applied by banding and broadcast methods, manure is usually applied by injection (liquid manure only) and broadcast method with or without incorporation (MAFRI 2007; Manitoba Pork Council 2007). Broadcasting involves the spreading of N on the soil surface which may then be incorporated into the soil. Broadcasting with incorporation increases immobilization and nitrification while if not incorporated, ammonium is susceptible to volatilization (Malhi et al. 1996; MAFRI 2007).

In annual crops, applied manure is usually incorporated into soil whereas in established perennial grasses, manure broadcast without incorporation is the most common method of fertilization (MAFRI 2007). Lack of manure incorporation in established perennial grasses may create a high potential of ammonium toxicity in soil due to low dilution effect of manure with soil. This may then be toxic to nitrifying organisms in soil and thereby inhibiting nitrification process (Koper et al. 2010).

1.3.1.3 Chemical Management Techniques

Nitrification inhibitors and controlled release fertilizer can be used to manage nitrate leaching in soil. These chemicals help to retain N in the ammonium form for a longer period, allowing it to be retained on cation exchange sites. These nitrification inhibitors such as dicyandiamide (DCD) and nitrapyrin are most likely to be economical in soils where nitrate-N losses are significant (Subbaro et al. 2006; Norton 2008). Also, *Brachiara humidiol*, a tropical pasture grass, has been reported to release exudates from its roots that inhibit the nitrification process by inhibiting the growth of nitrifying bacteria (Gopalakrishnan et al. 2009).

1.3.1.4 Crop Management Techniques

Plants with greater rooting depths can be used to manage nitrate leaching as they are able to capture nitrate before it is lost beyond the root zone. Deeper rooting systems result in lower nitrate loss in the soil while shallower rooting systems increase the tendency for nitrate leaching (Meisinger and Delgado 2002). Cover crops reduce nitrate leaching in the soil by immobilizing the nitrate into an organic form thereby reducing nitrate concentration in the soil resulting in a leaching reduction of about 70% for non-legume cover crops and 23% for legume cover crops (Meisinger and Delgado 2002). Perennial grasses have been used to manage nitrate leaching as they take up nutrients and water more efficiently, resulting in a greater potential for sustainable agriculture (Cox et al. 2006). High N use under vigorous growing grasses can also minimize nitrate leaching in soil (Vetsch et al. 1999). In this case, the extensive root systems of the perennial grasses can take up the nitrate and water throughout a large soil volume before it escapes from the root zone.

1.4 Perennial Grasses

Perennial crops are potential solutions to the challenges associated with annual crops in terms of nutrient management. They are more efficient in storing carbon, maintaining soil and water quality, preventing leaching, and providing valuable ecological benchmarks for agricultural environmental sustainability than their annual crop counterparts (Cox et al. 2006; Glover et al. 2010; Pimentel et al. 2012).

While annual crops require seedbed preparation, favourable weather during narrow planting windows and precisely timed inputs and management, once established perennial plants re-emerge at the beginning of the growing season without such additional efforts (Cox et al. 2006). As such, they efficiently absorb water and nutrients in the early part of the growing season before annual crops are seeded or while annual crop seedlings lie relatively inactive in the soil. Brown et al. (2000) reported that under all climate conditions, perennial grasses generally consume more nutrients and water than annual crops which thereby reduces runoff. They also reported that temperature increases of 3 - 8^o C increased the yield of a C_4 perennial grass (switchgrass), reduced its cold stress and extended its growing season while it decreased yields of annual crops such as corn, sorghum and soybean due to the resulting heat stress. Perennial plants such as sorghum (Sorghum spp.) emerge 4 weeks earlier than annual sorghum plants from large, starchy and cold-tolerant rhizomes (DeHaan et al. 2005). This earlier growth enabled the perennial plants to take up the nutrients and water before they are lost beyond the root zone.

1.4.1 Perennial Grass Root Systems

Perennial crops have large root systems which allow efficient absorption of nutrients and water in the soil profile (Cox et al. 2006; Pimentel et al. 2012). Roots of perennial grasses make a greater contribution to the maintenance of soil N and soil carbon than annual crops (Glover et al. 2010). The presence of perennial grasses at the start of the growing season with a large living root system, enable them to have the potential for greater photosynthetic production than annual plants (DeHaan et al. 2005). The deep penetrating roots of perennial grasses also create an extensive biologically active rhizosphere, improve soil structure and reduce water repellency associated with annual crops (Cransberg and McFarlane 1994).

1.5 Justification of the Study

Several studies have reported the benefit of perennial forage grasses over annual crops in preventing nitrate leaching from N inputs (Cox et al. 2006; Pimentel et al. 2012). Annual crops have been reported to leach larger amount of nitrate-N when compared to perennial forage grasses (Randall and Mulla 2001; Meisinger and Delgado 2002). Many of these studies have based their reports on the soil tests of annual and perennial cropping systems after harvest. Unlike other studies, Karimi et al. (2013) used field core lysimeters to directly measure the quantity of water and nitrate-N that was lost below the root zones of annual crops and perennial forage grasses. The study clearly demonstrated that while significant nitrate-N was lost below the root zone of annual crops (20 to 60 kg ha^{-1} annum^{-1}) whether manure was added to the plot or not, very little nitrate-N was lost from the perennial forage grasses (less than 1 kg ha^{-1} $annum^{-1}$). The reasons for this disparity in nitrate-N leaching between the two cropping systems are uncertain as they cannot be attributed to their above-ground biomass and N uptake. This inconsistency between above-ground N uptake and the nitrate-N leached in the two cropping systems may be due to an underestimation of total N in the plants as no report was given on the quantity of N in below-ground plant biomass or the capacity of the below-ground plant biomass to store N.

Similarly, excess nitrate in the presence of excess moisture is known to be the main cause of nitrate leaching in the soil (Di and Cameron 2002; Mulla and Strock 2008). The persistence of ammonium in soil will reduce nitrate formation and thereafter reduce the chance of nitrate leaching. Several nitrification inhibitors have been developed to allow added ammonium to persist longer in soil as a means of preventing nitrate accumulation (Dinnes et al. 2002; Tiessen et al. 2006; Norton 2008). However, we are not aware of any study that compared the persistence of ammonium-N in annual and perennial cropping systems following pig manure application as a possible mechanism for the differences in nitrate leaching between the two cropping systems.

1.6 Objectives

The objectives of this study were:

1. To measure and compare persistence of ammonium-N between annual and perennial cropping systems following application of solid and liquid pig manures.

2. To measure and compare root nitrogen content of annual and perennial cropping systems on solid and liquid pig manures system.

These objectives were tested based on the hypotheses that:

1. Applied nitrogen in manure persists longer in the ammonium-N form in perennial cropping system thereby delaying the process of nitrification and nitrate-N accumulation.

2. Differences in nitrate-N losses through leaching measured between annual and perennial cropping systems are due to differences in their below-ground plant biomass and nitrogen content rather than above-ground biomass and nitrogen uptake.

1.7 Thesis Layout

This thesis is made up of four chapters. Chapter one (Review of literature) gives a general overview on pig manure, fates of nitrogen in soil, causes, effect and management of nitrate leaching and a brief note on how perennial forage grasses help in nutrient management. Chapter two compares persistence of ammonium-N between annual and perennial cropping systems following pig manures application. Chapter three compares total plant N between the annual and perennial cropping systems with a focus on root biomass and root N content as well as root nitrogen use efficiency between the two cropping systems to see if differences in root N content will account for the differences in nitrate-N lost through leaching between the two cropping systems measured in previous study of Karimi et al. (2013). Chapter four provides a general synthesis of this study.

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CHAPTER 2

2.0 PERSISTENCE OF AMMONIUM-N IN PIG MANURE AMENDED ANNUAL CROPS AND PERENNIAL FORAGE GRASSES

2.1 Abstract

Perennial forage grasses have been reported to prevent nitrate leaching from soil receiving manures. A 2-year study was conducted to compare short-term persistence of ammonium-N in an annual cropping system (ACS) and a perennial cropping system (PCS) following pig manures application on a sandy loam soil as a possible mechanism for the reduced loss of nitrate-N through leaching from perennial forage grasses. The experimental design was a split-plot with cropping systems (ACS and PCS) being the main plots and manure treatments [N-based liquid pig manure (LPM), N-based solid pig manure (SPM), and a check without manure addition] as subplots. In 2014 and 2015, manures were broadcast in ACS and PCS and only incorporated in ACS. Following manure application, soil samples were taken from each plot seven times in 2014 and six times in 2015 at 0 - 15 and 15 - 30 cm depths. The samples were analyzed for ammonium-N and nitrate-N concentrations. Ammonium-N in 2014 was used to generate a nitrification kinetics parameter assuming a first order kinetics. The kinetic model was validated using the ammonium-N measured in 2015. Ammonium-N of LPM amended treatment at 0 - 15 cm depth reached its peak at four days after manure application (DAM) in ACS and PCS and was as high as 50 kg ha^{-1} in 2014 and 74 kg ha^{-1} in 2015 for PCS. For the ACS, the peak ammonium-N was 29 kg ha^{-1} in 2014 and 18 kg ha^{-1} in 2015. Ammonium-N persisted up to

7 DAM in the LPM amended PCS but did not persist beyond 4 DAM in the ACS in both years. The ammonium-N measured in SPM amended treatments was low in both cropping systems after manure application in both years. There was greater nitrate-N accumulation in ACS than PCS of LPM and SPM amended treatments at both depths and years. The first order rate constants for apparent nitrification (K_n) in ACS and PCS were similar for each manure treatment. When the kinetic parameters generated in 2014 were used to simulate ammonium-N dynamics in 2015, there was a significant correlation between the measured and simulated ammonium-N only in LPM amended treatment with R^2 of 0.84 for ACS and R^2 of 0.76 for PCS.

Persistence of ammonium-N in LPM amended PCS may be due to high ammonium-N concentration created by the lack of manure incorporation. The persistence of ammonium-N in the LPM amended PCS than ACS coupled with lower percentage increase in nitrate-N may account for lower nitrate leaching in perennial forage grasses as the persisted ammonium-N may possibly be adsorbed to the soil surface, immobilized or taken up by plant rather than being immediately converted to nitrate-N.

2.2 Introduction

Nitrogen is an essential plant nutrient that is needed in relatively large amounts by plants to complete their life cycle and physiological activities (Delgado and Follett 2010; Xin et al. 2014). It is the most limiting nutrient for crop growth (Malhi et al. 2001).

Pig manure is a source of N for plant uptake. In addition to the supply of N, pig manure increases organic matter composition, water holding capacity, wet aggregate stability and cation exchange capacity of the soil (Darwish et al. 1995; Du et al. 2014). The pig industry represented the fourth largest industry in Canada in 2011 (Yan 2014). The provinces of Quebec, Ontario and Manitoba accounted for 79% of all the pigs in Canada in 2011 (Dorff and Beaulieu 2014). Pig manure may be handled in a liquid or solid form depending on the water content. Liquid pig manure is the preferred form of pig manure storage in Canada (Dorff and Beaulieu 2014) because it is easier to handle due to high level of dilution; although the high volume increases the cost associated with transportation and application (Vanotti et al. 2002; Schoenau and Assefa 2004).

Relative quantity of the different forms of N in the pig manure depends on the method of manure collection, storage and handling, presence or absence of bedding materials and type of ration fed (Choudhary et al. 1996; Lamb et al. 2014). Solid pig manure has much of its N in the organic form and liquid pig manure has a higher percentage of its N in the ammonium form (Manitoba Pork Council 2007). The Prairie Provinces' Committee on Livestock Development and Manure Management (2004) estimated that liquid pig manure has over 50% of its total N in the ammonium form. If environmental conditions are not limiting, the ammonium in pig manure may be quantitatively converted to NO_3^- by soil autotrophic bacteria in a process known as nitrification (Addiscott 2005; Sahrawat 2008; Smits et al. 2010).

The nitrification process determines the relative quantities of ammonium and nitrate in the soil and the form of inorganic N taken up by plants and/or lost to the environment (Auyeung et al. 2015; Subbarao et al. 2015). It is influenced by environmental factors such as aeration, moisture, pH, substrate and temperature (Barnard et al. 2005; Sahrawat 2008). The nitrification rate can range from 10-70 kg/ha/day under favorable temperature and moisture conditions when ammonium is not limiting (Bolado-Rodríguez et al. 2010). Also, nitrification of applied N is affected by the method of application. Van Es et al. (2006) reported that incorporation of manure rapidly converts ammonium in manure to nitrate. This can be attributed to greater contact between soil microorganisms and ammonium and a high aeration level that enhances nitrification process. High concentrations of ammonium in soil may create a toxic zone to soil organisms and delays nitrification (Koper et al. 2010). Reports have shown that some ammonia oxidizers are sensitive to high ammonia concentration in soil (Norton 2008). In the case of perennial grasses, where manure is usually surface applied without incorporation, nitrification may be delayed due to high concentration of ammonium on the soil surface. Similarly, some plants have evolved root exudates that are capable of inhibiting nitrification in soil. Root exudates from *Brachiaria humidicola* and the *Brassicaceae* family have been found to inhibit the growth of *Nitrosomonas* bacteria (Gopalakrishnan et al. 2009; Subbaro et al. 2015). This allows the plants to be adapted to taking ammonium as a source of available N for a longer period of time thereby preventing nitrate accumulation in the soil.

Unlike annual crops, perennial forage grasses have been known to reduce nitrate leaching by reducing nitrate accumulation in the subsoil (Entz et al. 2001; Campbell et al. 2006; van Es et al. 2006). A previous 3- year study conducted by Karimi et al. (2013) at the site of our current study showed that PCS significantly reduce nitrate leaching from pig manures system when compared to ACS. This may be through the inhibition of nitrification which allows ammonium-N to persist longer in soil thereby reducing nitrate-N accumulation. The exact mechanisms by which perennial forage grasses reduce nitrate leaching have not been clearly enunciated. The study was conducted to investigate and compare the persistence of ammonium-N in ACS and PCS following solid and liquid pig manure applications as a possible mechanism for the reduced loss of nitrate-N from perennial forage grasses.

2.2.1 Hypothesis

This study tests the hypothesis that applied nitrogen in manure persists longer in the ammonium-N form in perennial cropping system thereby delaying the process of nitrification and nitrate-N accumulation.

2.3 Materials and Methods

2.3.1 Field Description and Experimental Design

The study was conducted at the University of Manitoba Ian Morrison Research Station in Carman, Manitoba. The site was mapped to Hibsin soil series (Mills and Haluschak 1993). The original study was established in 2009. The general properties of the soil site are shown in Table 2.1.

The experimental design was a split-plot design with four replications (Figure 2.1). Each plot was 10 m x 10 m. Main plots were cropping systems (CS) and subplots were manure treatments (MT). The CS were annual cropping system (ACS) and perennial cropping system (PCS) and MT were: Nitrogen-based liquid pig manure application (LPM); Nitrogen-based stock-piled solid pig manure application (SPM); Check or control to which neither solid nor liquid pig manure was applied (CON). The annual plots were seeded to a canola-barley rotation from 2009 to 2015 and the perennial plots were seeded to a mixture of orchard and timothy from 2009 to 2012. In 2013, the forage perennial grass was plough down and seeded to annual crop (canola) and in the fall of 2013, the perennial forage grasses (timothy and orchard grass) were reseeded and maintained till 2015.

Table 2.1 General properties of Site soil[*]

Properties	**Carman soil (0-10 cm)**
Soil series	Orthic Black Chernozem
pH	5.8
Sand content (%)	87
Silt content (%)	5
Clay content (%)	8
Drainage	Moderately to slow permeability
Organic matter (%)	6.5
Water holding capacity	High (33 % on volume basis)
Olsen P (mg kg^{-1})	20
Bulk density (g cm^{-3})	1.2 (0 - 15 cm depth)

[*] Adapted from Sayem (2014)

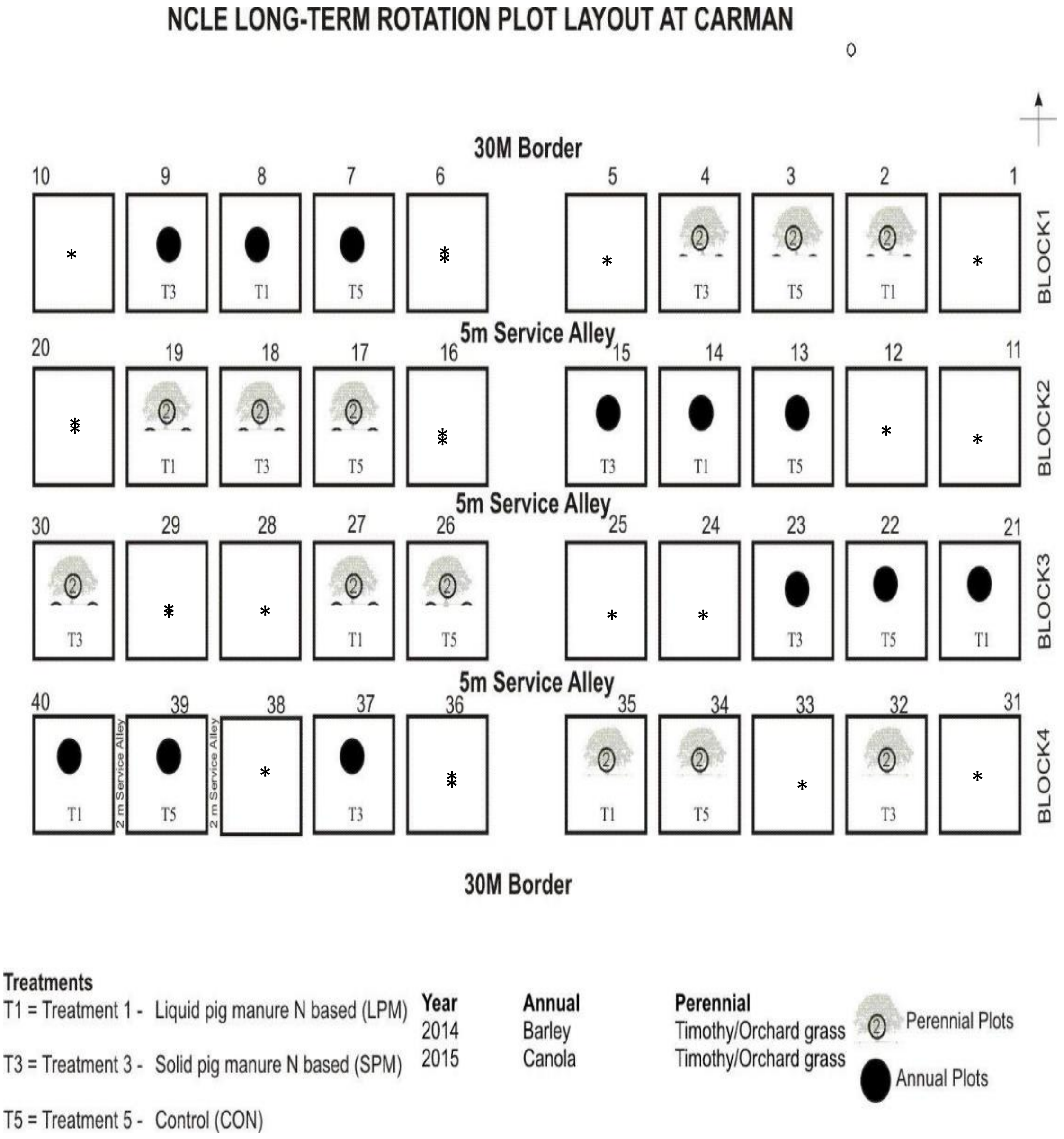

Figure 2.1. A schematic layout of the experimental plot design

* Not part of the experimental plot. NCLE, National Centre for Livestock and Environment

2.3.2 Manure Application and Seeding

Prior to manure application in each year, ammonium-N concentrations and total N (wet oxidation method of Akinremi et al. 2003) of the manures was determined (Table 2.2). This data was used to estimate the manure application rate using the MARC (2008) software to meet the N requirement of each crop taking into consideration the soil residual nitrate at 0-60 cm depth, soil type and nitrogen processes such as ammonia volatilization (Table 2.3) (Mooleki et al. 2002; MAFRI 2007). Total available organic N and ammonium-N of the LPM and SPM applied to each plot (Table 2.3) were back calculated from the manure rate using manure nutrient analyses results (Table 2.2) to estimate the total available N applied assuming 25% ammonium-N volatilization loss and 25% organic N available in the manures.

Total available N= NH_4^+-N **X** (100% - volatilization loss) + 25% organic N [1]

Table 2.2 Chemical composition of the liquid and solid pig manures applied to the plots in 2014 and 2015.

Year	Manure	Moisture (%)	Total N (%)	NH_4^+-N (ppm)	Available N (ppm)
2014	LPM	98.6	0.16	1,351	1,086
	SPM	76.6	0.71	1,350	2,440
2015	LPM	99.0	0.25	2,154	1,694
	SPM	84.0	0.58	1,177	2,014

Table 2.3 Manure type, dry target yield, residual soil nitrate-N, manure application rate, available organic N and ammonium-N fractions, total available N applied and total available N for plant uptake in 2014 and 2015.

Year	Crops	Manure	Dry target yield (kg ha^{-1})	Residual soil NO_3^--N (kg ha^{-1})	Target N (kg ha^{-1})	Manure rate (ha^{-1})	Avail. Org. N (kg ha^{-1})	NH_4^+-N applied (kg ha^{-1})	Avail. N applied (kg ha^{-1})	Total avail. N (kg ha^{-1})
2014	ACS	LPM	4,570	50.4	151	46,470.0 L	3.4	47.1	50.5	100.9
	(Barley)	SPM	4,570	52.0	151	20.5 ton	28.9	20.5	49.4	101.4
		CON	NA	NA	NA	0	NA	NA	NA	NA
	PCS	LPM	7,410	87.4	140	48,260.0 L	3.5	48.9	52.4	139.8
		SPM	7,410	101.9	140	16.0 ton	22.8	16.2	39.0	140.9
		CON	NA	NA	NA	0	NA	NA	NA	NA
2015	ACS	LPM	2,128	83.2	140	33,610.0 L	2.7	54.3	57.0	140.2
	(Canola)	SPM	2,128	109.2	140	15.8 ton	18.2	13.9	32.1	141.3
		CON	NA	NA	NA	0	NA	NA	NA	NA
	PCS	LPM	7,410	45.9	140	55,562.0 L	4.4	89.8	94.2	140.1
		SPM	7,410	84.3	140	27.0 ton	31.2	23.8	55.0	139.3
		CON	NA	NA	NA	0	NA	NA	NA	NA

NA, not applicable.

The SPM was applied to the surface of the plots manually using a pitch fork and a rake. The LPM was metered out using 20 L jugs and manually added to the plot. After the application of both manures, the manures were rototilled in the ACS to incorporate the manures to a depth of about 10 cm before seeding in each year.

The ACS was seeded to barley (Tradition) in 2014 and canola (Liberty Link, Invigor L140P) in 2015, while the PCS was seeded to a mixture of timothy (Promesse) and orchard grass (AC Nordic) at a ratio of 68:32 in the fall of the previous growing season (2013) (Table 2.4).

Table 2.4 Manure application date, incorporation date, seeding date and seeding rate in 2014 and 2015.

Year	Crops	Manure application	Manure incorporation	Seeding date	Seeding rate (kg ha^{-1})
2014	Barley	June 4th & 5th	June 6th	June 6th	108.0
	Timothy & Orchard	June 4th & 5th	NA	Sept. 23rd 2013	8.4
2015	Canola	June 1st	June 1st	June 1st	8.0
	Timothy & Orchard	June 1st & 2nd	NA	NA	NA

NA, not applicable.

2.3.3 Soil Sampling

In 2014, soil samples were collected 17 days before manure application and 4, 7, 11, 14, 18, 21, and 27 days after manure application (DAM) at soil depths of 0 - 15 cm and 15 - 30 cm using a back saver probe with 1.8 cm internal diameter. Each sample was a composite of five soil samples taken randomly within each plot. In 2015, the soil was sampled more frequently and not beyond 14 DAM because of the 2014 data that showed only a small amount of ammonium-N beyond 14 days of manure application. Soil was therefore sampled four days before manure application and 2, 4, 7, 9, 11, and 14 DAM at the depths of 0 - 15 cm and 15 - 30 cm using a back saver probe (1.8 cm internal diameter) with each sample being a composite of seven samples taken randomly within each plot.

2.3.4 Soil Analysis

Six grams of moist soil was used from each plot and was weighed into a centrifuge tube and 25 mL of 2.0 M KCl was added. The mixture was shaken for 30 minutes and filtered with a Whatman No. 40 filter paper into 30 mL Nalgene bottles (Thermo Fisher Scientific Inc., MA, USA). From the resulting filtrate, soil nitrate-N was determined by an Automated Cadmium Reduction method while soil ammonium-N was measured by an Automated Phenate method (Maynard et al. 2006) using an AQ2 Discrete Analyzer (SEAL Analytical Inc., WI, USA).

The gravimetric water content for each plot was determined by oven drying a known amount of freshly collected soil sample for 24 hours at 105°C. After 24 hours, the samples was allowed to cool and reweighed to determine moisture loss.

2.3.5 Calculations

2.3.5.1 Persistence of ammonium-N

The percentage persistence of ammonium-N ($NH_{4(p)}$) was calculated for each crop to determine which of the two cropping systems allowed added ammonium-N to persist longer.

$$NH_{4(P)} = \frac{NH_{4\_t}}{NH_{4to}} \times 100 \qquad [2]$$

Where: NH_{4_t} is the quantity of ammonium-N (kg ha^{-1}) measured in the soil at time t (where t is the days after manure application) and NH_{4_to} is the quantity ammonium-N (kg ha^{-1}) applied.

2.3.5.2 Recovery of ammonium-N

The percentage recovery of ammonium-N ($NH_{4(R)}$) was calculated as follows:

$$NH_{4(R)} = \frac{NH_{4(ap)}}{N_{in}} \times 100 \qquad [3]$$

Where: $NH_{4(ap)}$ is the apparent ammonium-N (kg ha^{-1}) (ammonium-N in the manure amended soil minus that in the control soil) and N_{in} is the apparent available N (kg ha^{-1}) which is the sum of apparent ammonium-N and nitrate-N in the plot.

2.3.5.3 Kinetic Model of Nitrification

The quantity of ammonium-N (kg ha^{-1}) that was measured with time in 2014 was used to generate nitrification kinetics parameter for each crop assuming a first order kinetics. The kinetic model was validated using the ammonium-N data in 2015. The first order kinetic model is given as:

$$\ln NH_{4\_t} = \ln NH_{4\_to} - kt \qquad [4]$$

Where: NH_{4_t} is the ammonium-N (kg ha^{-1}) measured in the soil at time t, NH_{4_to} is the ammonium-N at time zero (the applied ammonium-N), K_n is first order rate constant (apparent nitrification constant) and t is the time in days after manure application.

2.3.6 Statistical Analysis

Statistical analysis of the data was performed with PROC GLIMMIX for repeated measures using SAS 9.4 analysis software package to test for significance differences in the amount of ammonium-N and nitrate-N measured in each depth interval (0 - 15 and 15 - 30 cm) from the two CS and the three MT at various sampling time intervals (day) in each year. Significance difference in ammonium-N and nitrate-N between the two CS was tested for each MT on each sampling day. Prior to PROC GLIMMIX analysis, soil ammonium-N and nitrate-N data were checked for assumption of normal distribution using PROC UNIVARIATE. Except for nitrate-N in 2014, the data did not conform to normal distribution and they were specified for lognormal distribution in the model. Cropping systems, day, and MT were fixed effects while the block and its interactions with the fixed effects were random effects. The covariance structure used in the model was compound symmetry in which the repeated factor (day) had unequal intervals. Mean comparisons were performed using Tukey-Kramer means comparison at $P < 0.05$. A regression analysis was used to test for significant difference in K_n generated between the two CS for each MT. Also, a regression analysis was performed to test for relationship between the measured and simulated ammonium-N for each MT in 2015 at 0.05 probability level of significance.

2.4 Results and Discussion

2.4.1 Ammonium-N in Soil

In 2014, there were significant MT x day x CS interactions on soil ammonium-N at 0 - 15 cm depth (Table 2.5). The quantity of ammonium-N measured in each CS was a function of type of MT applied and day of sampling. Ammonium-N measured was significantly greater in PCS than ACS only on 7 DAM in LPM amended treatment (Figure 2.2). In contrast, there was no significant MT x day x CS interactions on soil ammonium-N at 0 - 15 cm depth interval in 2015 but there were CS x MT and day x MT interactions (Table 2.5). Similar to 2014, ammonium-N of PCS was significantly greater than that in ACS on 7 DAM only in LPM amended treatment in 2015 (Figure 2.3). In both years, there was a significant effect of day on the amount of ammonium-N in the ACS and PCS at 0 - 15 cm depth. Expectedly, the amount of ammonium-N in all the MT and CS at the first sampling date (before manure application) was low (less than 3 kg ha^{-1} in 2014 and less than 5 kg ha^{-1} in 2015) (Figures 2.2 & 2.3).

Table 2.5 Effects of cropping systems and manure treatments on ammonium-N and nitrate-N with time in 2014 and 2015 at 0 - 15 cm and 15 - 30 cm depth intervals.

Model Effect	Df	0 - 15 cm		15 - 30 cm	
		NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
2014		..………….………..P values………………………			
CS	1	0.2483	0.1272	0.4578	0.1633
MT	2	0.2232	0.0003	0.0182	<.0001
Day	7	<.0001	<.0001	0.0044	<.0001
CS x MT	2	0.2079	0.0185	0.2039	0.0249
CS x day	7	0.1562	0.2831	0.2266	<.0001
Day x MT	14	0.0008	<.0001	0.0023	0.0003
CS x day x MT	14	0.0399	0.0069	0.3584	0.0161
2015					
CS	1	0.0572	<.0001	0.0226	0.0517
MT	2	0.0012	<.0001	0.1840	<.0001
Day	6	0.0004	<.0001	0.0021	0.3137
CS x MT	2	0.0280	0.0014	0.1675	0.4689
CS x day	6	0.4382	<.0001	0.3641	<.0001
Day x MT	12	<.0001	<.0001	0.3784	0.0665
CS x day x MT	12	0.1278	0.4140	0.9438	0.0937

P values are significant at $P < 0.05$ Tukey-Kramer means comparison.

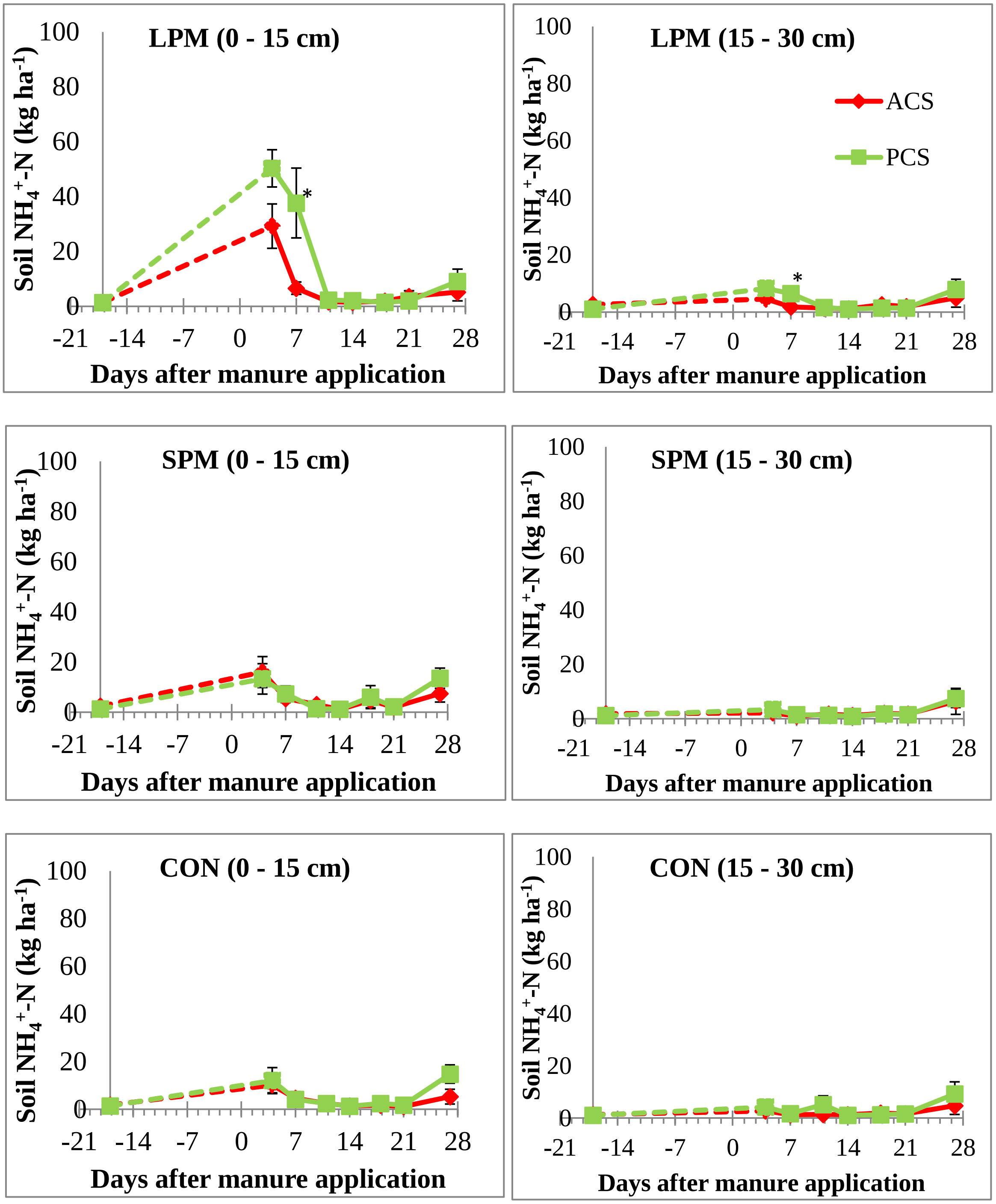

Figure 2.2 Effect of cropping systems and manure treatments on ammonium-N at 0 - 15 and 15 - 30 cm depths in 2014. Error bars are ±1 standard error of the means.
* Ammonium-N was significantly different between ACS and PCS at $P < 0.05$ Tukey-Kramer means comparison on 7 DAM.

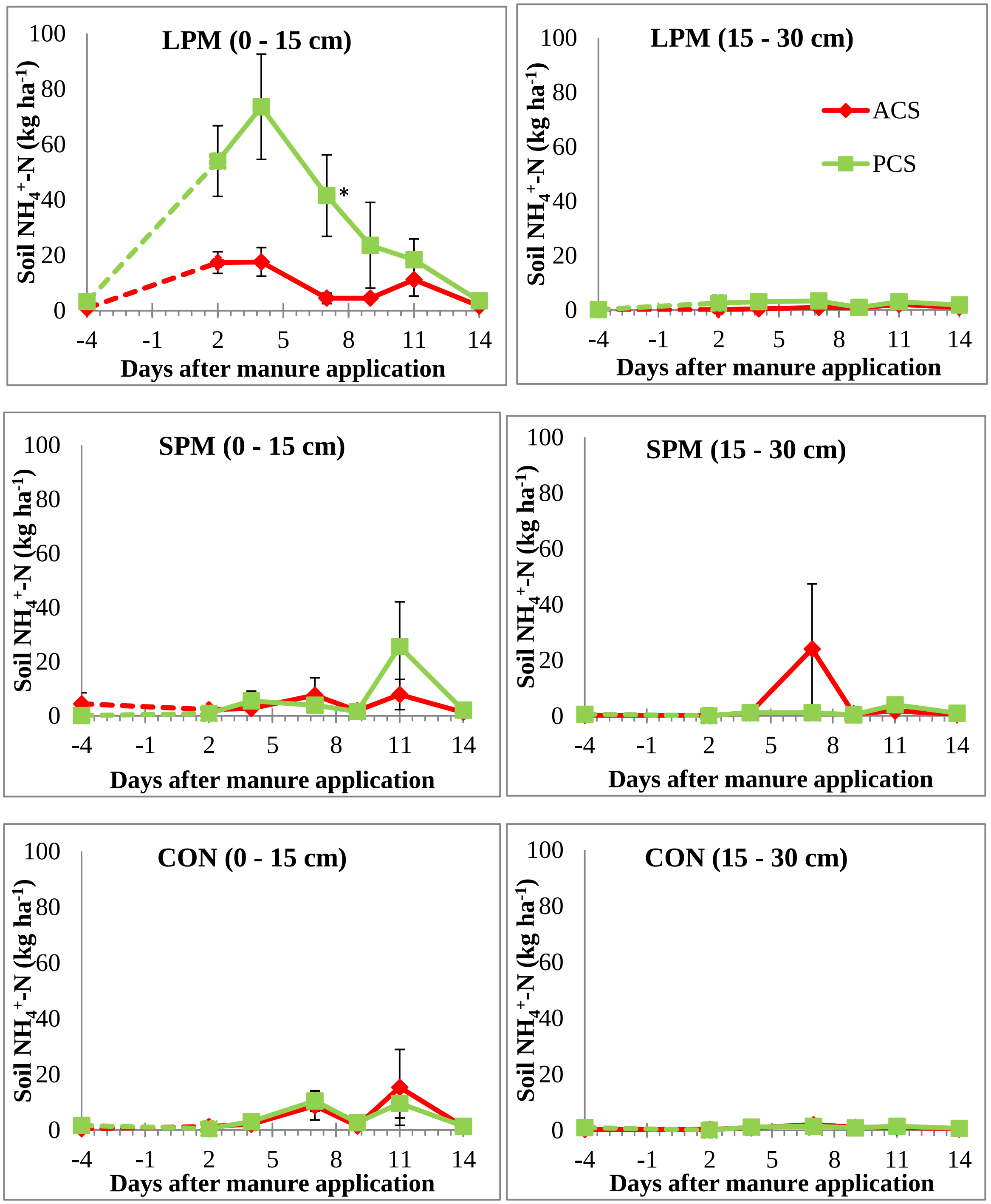

Figure 2.3 Effect of cropping systems and manure treatments on ammonium-N at 0 - 15 and 15 - 30 cm depths in 2015. Error bars are ±1 standard error of the means.
* Ammonium-N was significantly different between ACS and PCS at $P < 0.05$ Tukey-Kramer means comparison on 7 DAM.

Following manure application in 2014, large amounts of ammonium-N were measured in all plots (including the control where no manure was added) of each CS by the second sampling date (4 DAM) at 0 - 15 cm depth. The largest amount of ammonium-N was in the LPM amended treatment (50 kg ha^{-1} in the PCS and 29 kg ha^{-1} in the ACS; Figure 2.2). In 2015, large amounts of ammonium-N were measured only in the LPM amended treatment (74 kg ha^{-1} in PCS and 18 kg ha^{-1} in ACS; Figure 2.3) by the second sampling date (2 DAM) at 0 - 15 cm. Ammonium-N in LPM amended ACS and PCS reached its peak at 0 - 15 cm at 4 DAM in both years but persisted till 7 DAM only in the PCS in both years (Figures 2.2 & 2.3). This increase in the amount of ammonium-N on the second sampling date was due to the ammonium-N of the manure. The highest amount of ammonium-N in the LPM amended treatment was due to higher amounts of ammonium-N that were added with LPM than SPM whereas the least amount of ammonium-N in CON treatment on second sampling date was because the increase in ammonium-N was only due to soil mineralization as no manure was added to this treatment. The LPM amended treatments received 47 & 54 kg ha^{-1} of ammonium-N in 2014 and 2015, respectively for ACS and 49 & 90 kg ha^{-1} of ammonium-N in 2014 and 2015, respectively for PCS whereas the SPM amended treatments received 21 & 14 kg ha^{-1} of ammonium-N in 2014 and 2015, respectively for ACS and 16 & 24 kg ha^{-1} of ammonium-N in 2014 and 2015, respectively for the PCS (Table 2.3).

The significant difference in the amount of ammonium-N between LPM amended PCS and ACS was due to persistence of ammonium-N at 7 DAM in both years. In 2014, the persistence of ammonium-N was 77% and 14% in the PCS and ACS, respectively at 7 DAM. Despite the mature sward of perennial forage grasses in 2015 with a greater potential for N uptake, the persistence of ammonium-N in the LPM amended PCS was still greater than in ACS. Persistence of ammonium-N in 2015 was 46% and 8% in the PCS and ACS, respectively at 7 DAM. In the SPM amended and CON treatments, there were no significant differences in ammonium-N between the ACS and PCS on any sampling day at 0 - 15 cm in both years. Ammonium-N in SPM amended treatment was greater in the ACS than the PCS at 4 DAM in 2014 but smaller than PCS in 2015 (Figures 2.2 & 2.3). The persistence of ammonium-N in the SPM amended PCS (45%) was higher than in ACS (23%) at 7 DAM in 2014. Although the ammonium-N was slightly higher in the SPM amended PCS than ACS in 2015, ammonium-N was more persistent in ACS than PCS. From 11 to 21 DAM in 2014, both CS had low but relatively stable amounts of ammonium-N in all MT which then increased at 27 DAM, possibly due to mineralization.

There were no significant CS x day x MT interactions in the amounts of ammonium-N at 15 - 30 cm in both years (Table 2.5). The amount of ammonium-N at 15 - 30 cm depth interval was relatively low in all MT, CS and years (Figures 2.2 & 2.3). In 2014, ammonium-N was significantly different between LPM amended ACS and PCS on 7 DAM. The low ammonium-N at the 15 - 30 cm was due to the limited mobility of ammonium in soil and its adsorption to the negatively charged soil surfaces at the 0 - 15 cm (Nieder et al. 2011; Scherer et al. 2014).

The initial increase in ammonium-N after manure application followed by a decline was similar to the results reported by Calderón et al. (2005) and Ige et al. (2015) in that ammonium-N reached its peak following manure application and declined gradually within a week after manure application. The greater persistence of ammonium-N in the LPM amended PCS signified a delay in the nitrification process perhaps due to a lack of manure incorporation into the soil. This is similar to the action of nitrification inhibitor such as nitrapyrin that delays the microbial oxidation of ammonium to nitrite for some days after N application (Zerulla et al. 2001). It is possible that the lack of incorporation of manure in the PCS created zones of high ammonium concentration that inhibited activities of nitrifying organisms. Koper et al. (2010) reported that significant nitrification inhibition may occur in soil after N application due to the toxic zone created by high ammonium concentration for the oxidizing organisms. The broadcast and incorporation of manure in LPM amended ACS probably resulted in higher nitrification rate due to the dilution effect and higher contact of manure with the soil leading to higher microbial activities (Malhi et al. 1996; van Es et al. 2006; MAFRI 2007). The low ammonium-N measured from 11 to 21 DAM in 2014 in this results are similar to those of other studies that showed smaller and relative stable ammonium-N two weeks after manure application (Calderón et al. 2005; Li and Li 2014).

2.4.2 Nitrate-N in Soil

There were significant CS x day x MT interactions on nitrate-N measured at 0 - 15 cm and 15 - 30 cm depth intervals in 2014 (Table 2.5). In 2015, there were no significant CS x day x MT interactions on nitrate-N at either 0 - 15 cm or 15 - 30 cm depth interval (Table 2.5). However, there were significant effects of day on nitrate-N at 0 - 15 cm in 2014 and 2015.

Following manure application in 2014, the nitrate-N at 0 - 15 cm depth increased at 4 DAM in all the MT of ACS and PCS but not all of the increase can be attributed to nitrification from the added ammonium-N since there was also an increase in CON treatment of ACS where no manure was added (Figure 2.4). The increase in nitrate-N of CON treatment may be attributed to mineralization of soil N. In 2014, accumulated nitrate-N in the LPM amended soil was significantly greater in ACS than PCS at 7 DAM despite higher initial nitrate-N in PCS before manure application (Figure 2.4). The nitrate-N of the LPM amended ACS increased by 31% between 4 and 7 DAM and decreased by 8% in the PCS. In 2015, nitrate-N at 0 - 15 cm depth was greater in ACS than PCS in all MT until 14 DAM (Figure 2.5). The nitrate-N increased by 13% between 2 and 4 DAM in the LPM amended ACS but only increased by 1% in the LPM amended PCS in the same period (Figure 2.5). The smaller amounts of nitrate-N in the LPM amended PCS was consistent with the persistence of ammonium-N until 7 DAM. Conversely, the greater amounts of nitrate-N in the ACS was consistent with its lesser persistence of ammonium-N as indicated by the lower ammonium-N at 7 DAM (Figures 2.2 & 2.3) than in the PCS. In the SPM amended treatment, higher nitrate-N in PCS than ACS at 0 - 15 cm on 4 and 7 DAM in 2014 was mainly due to its significantly greater initial nitrate-N (before manure application) and not due to applied N as both CS had similar percentage nitrate-N increase on 4 DAM (Figure 2.4). Nitrate-N in SPM amended treatment at 0 - 15 cm declined from 7 DAM to the end of measurement in 2014 while in 2015 nitrate-N increased throughout the sampling period.

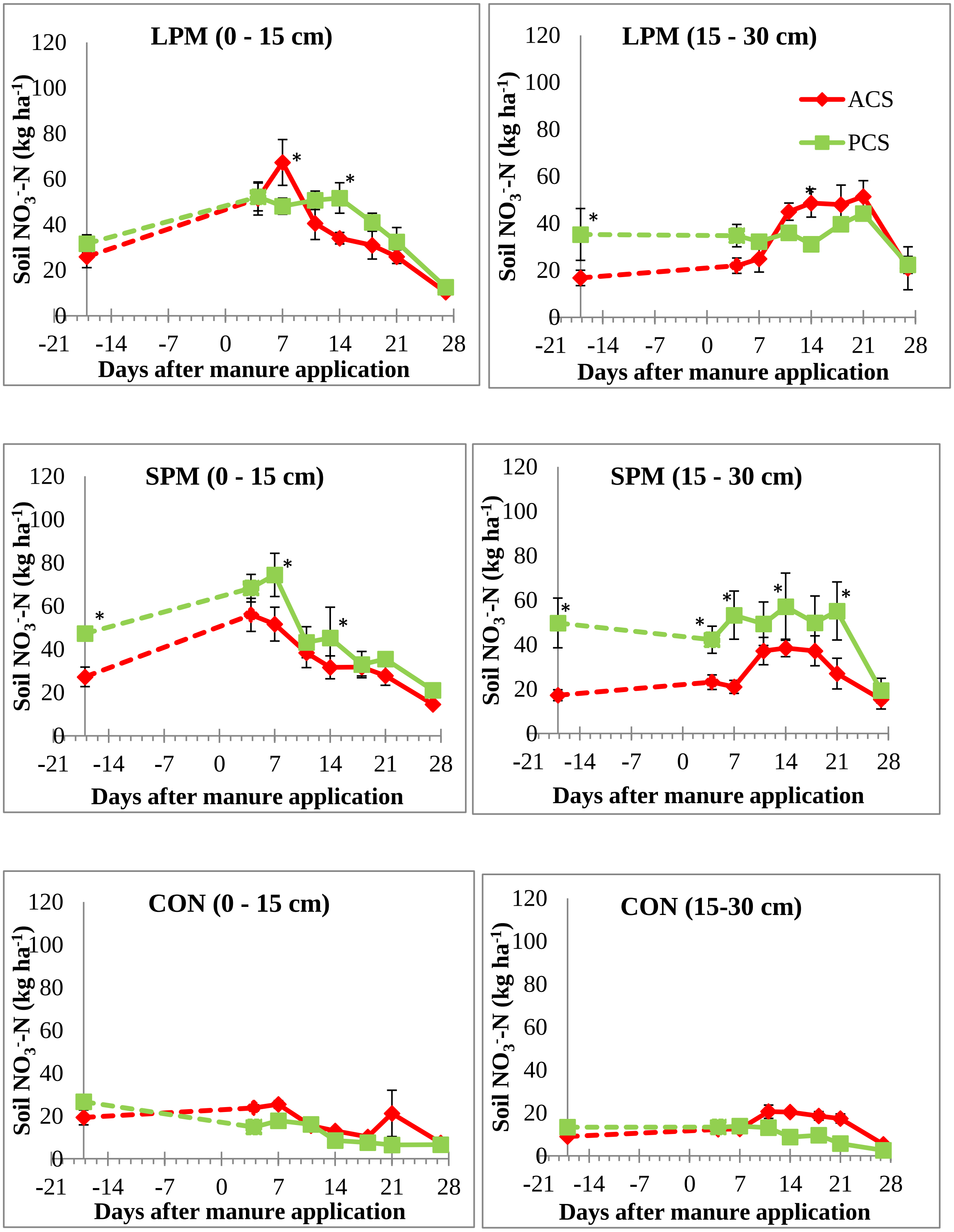

Figure 2.4 Effect of cropping systems and manure treatments on nitrate-N at 0 - 15 and 15 - 30 cm depths in 2014. Error bars are ±1 standard error of the means.

* Nitrate-N was significantly different between ACS and PCS at $P < 0.05$ Tukey-Kramer means comparison on these days.

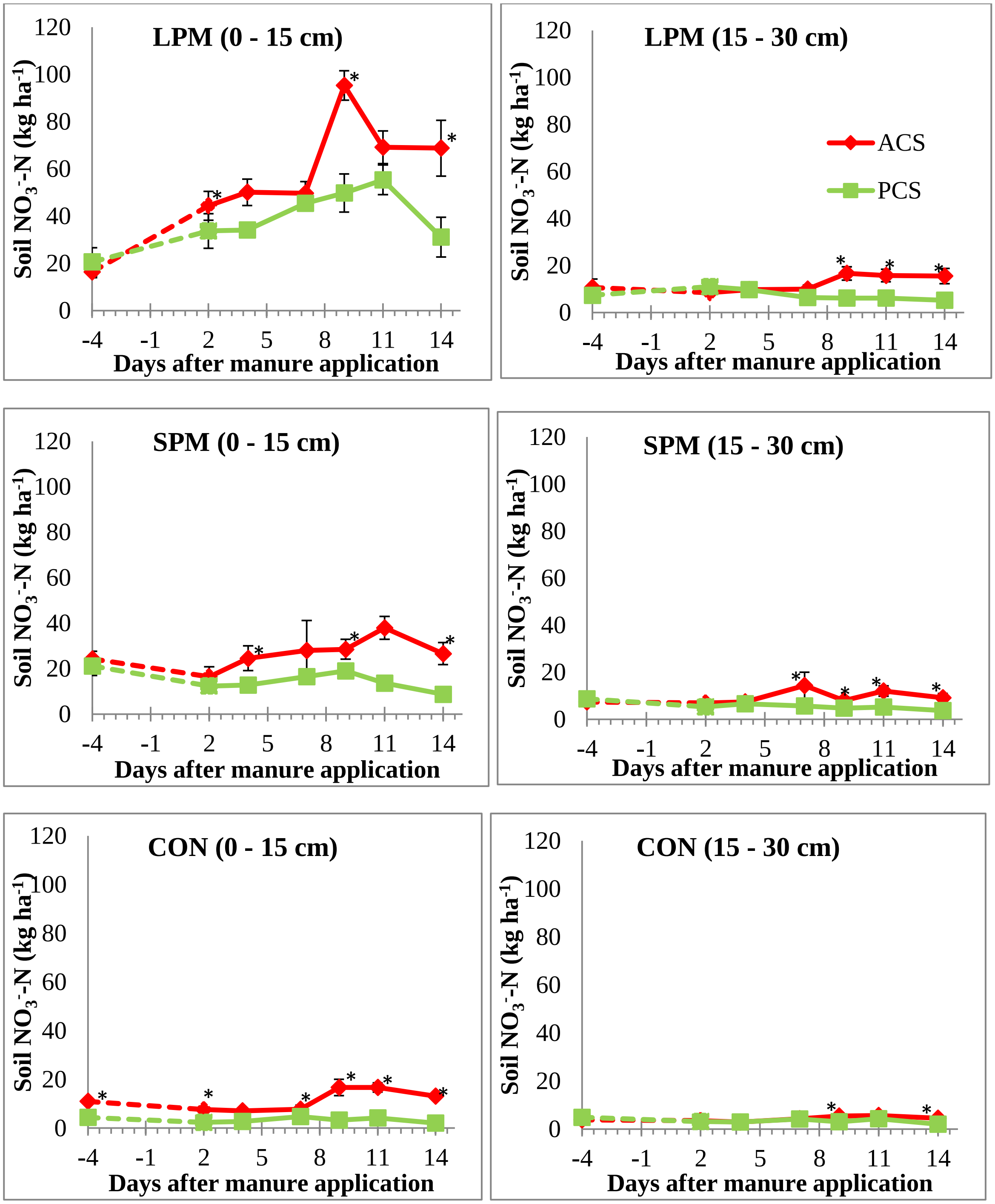

Figure 2.5 Effect of cropping systems and manure treatments on nitrate-N at 0 - 15 and 15 -30 cm depths in 2015. Error bars are ±1 standard error of the means.
* Nitrate-N was significantly different between ACS and PCS at $P < 0.05$ Tukey-Kramer means comparison on these days.

In 2014, nitrate-N at 15 - 30 cm depth reflected the movement of nitrate-N in both CS from the 0 - 15 cm depth. Nitrate-N of PCS was significantly greater than ACS at five of the eight sampling times in SPM amended treatment. The greater nitrate-N in PCS was not due to nitrification of added ammonium-N as its initial nitrate-N (before manure application) was also greater (Figure 2.4). However, the percentage increase of nitrate-N (relative to the initial measurement) was greater in the ACS than in the PCS until 21 DAM (Figure 2.4). The greater percentage increase of nitrate-N at 15 - 30 cm depth of ACS corresponded to a larger decrease in nitrate-N at 0 - 15 cm depth (Figure 2.4). In 2015, the amounts of nitrate-N between ACS and PCS at 15 - 30 cm depth were similar until 9 DAM in LPM and CON treatments when ACS had greater nitrate-N than PCS (Figure 2.5).

The distribution of nitrate-N in both CS with respect to time suggests that lower nitrate-N in the PCS in both years was due to a delay in the nitrification process and the uptake of nitrate-N as soon as it was formed. Studies have showed that nitrate accumulation can be minimized by allowing N to stay longer in the ammonium form (Zerulla et al. 2001) thereby reducing the potential for nitrate leaching. Guiraud and Marol (1992) reported that the amount of nitrate-N in the soil during the period of ammonium-N persistence due to application of nitrification inhibitor was lower than in the soil where there was no nitrification inhibitor. In another study, van Es et al. (2006) attributed the higher nitrate-N in an ACS compared to a PCS to manure incorporation which resulted in nitrate accumulation in the ACS. The growth stage of ACS compared to PCS might have also led to the greater percentage increase of nitrate-N in the 15 - 30 cm depth of ACS than PCS. This was because the annual crops were at germination stage and their N requirement was low unlike the perennial forage grasses that were at vegetative stage and required high amount of N (Delgado and Follett 2010). This greater percentage increase of nitrate-N in ACS than PCS was an indication of a greater potential for nitrate leaching in the ACS than PCS as they have been reported to leach a greater amount of nitrate (Randall and Mulla 2001; Dinnes et al. 2002; Mulla and Strock 2008).

2.4.3 Mass balance/apparent available N

Apparent ammonium-N was closer to the added ammonium-N in the LPM amended treatment compared to that in the SPM amended treatment at 4 DAM in 2014 and 2015. In both years, the LPM amended PCS had greater recovery of ammonium-N ($NH_4^+{}_{(R)}$) than the ACS up to 7 DAM (Table 2.6), particularly in 2014 when the two CS received about the same amount of available N (about 50 kg ha^{-1}; Table 2.3). Despite the greater potential for volatilization in the PCS due to lack of incorporation, $NH_4^+{}_{(R)}$ in LPM amended PCS on 4 and 7 DAM was 68 and 74%, respectively, in 2014 and 82 and 58%, respectively, in 2015. In LPM amended ACS, the $NH_4^+{}_{(R)}$ on 4 and 7 DAM was 40 and 5%, respectively, in 2014 and 30 and -19%, respectively, in 2015. In SPM amended treatment, $NH_4^+{}_{(R)}$ was greater in ACS than PCS on 4 DAM but lower than PCS on 7 DAM in 2014. In 2015, $NH_4^+{}_{(R)}$ was greater in ACS than PCS on 4 and 7 DAM (Table 2.6).

Table 2.6 Apparent soil ammonium-N and available N at 4 and 7 DAM in 2014 and 2015 at 0 - 30 cm using ammonium-N and nitrate-N at 0 - 15 and 15 - 30 cm.

Year	MT	CS	4 DAM Apparent NH_4^+-N (kg ha^{-1})	4 DAM Apparent avail. N (kg ha^{-1})	7 DAM Apparent NH_4^+-N (kg ha^{-1})	7 DAM Apparent avail. N (kg ha^{-1})
2014	LPM	ACS	21	52	2	47
		PCS	42	62	38	52
	SPM	ACS	5	40	0.3	28
		PCS	0	14	3	34
2015	LPM	ACS	15	50	-5	28
		PCS	72	88	33	57
	SPM	ACS	1	1	20	-8
		PCS	3	31	-6	-14

Higher $NH_4^+{}_{(R)}$ in the LPM amended PCS showed that much of the apparent available N was retained in ammonium-N form up to 7 DAM with little nitrate-N in both years. The pattern of $NH_4^+{}_{(R)}$ in the PCS in both years was similar to the effect of a nitrification inhibitor which retains applied nitrogen in the ammonium form for a longer period of time and reduces the potential of nitrate leaching (Subbarao et al. 2006). Similar to Tiessen et al. (2006) who showed that the use of a nitrification inhibitor (dicyandiamide) increased ammonium recovery from fertilizer N, this study also showed that perennial forage grasses can increase the recovery of ammonium when LPM is broadcast on the soil surface. Lower $NH_4^+{}_{(R)}$ in SPM treatment was possibly due to immobilization of inorganic N due to the added straw in the SPM. This is due to the presence of straw or bedding materials in SPM affecting amount of available N in the soil (Miller et al. 2010).

2.4.4 Nitrogen Transformation

The first order rate constant for apparent nitrification constant (K_n) was 0.24 day^{-1} in LPM amended ACS and PCS; and 0.21 day^{-1} for SPM amended ACS and PCS (Table 2.7). The K_n between the CS for each MT was not statistically different. Our nitrification rate constants were smaller than those obtained by Koper et al. (2010) and Habteselassie et al. (2006) who used inorganic fertilizers and compost, but they were greater than those obtained by Bolado-rodríguez et al. (2010) who used pig slurry. An earlier study by Koper et al. (2010) reported that the nitrification constant is affected by source of N applied and ammonium oxidizing organisms in the soil.

The formulated kinetic model fit the ammonium-N data of the LPM amended treatment in 2015 as indicated by the R^2 (Table 2.7). There was a significant correlation between simulated and measured ammonium-N in the LPM amended PCS and ACS in 2015 (Table 2.7). The ammonium-N was better simulated in ACS than in PCS (Figure 2.6). In contrast, the model did not fit the ammonium-N data in the SPM amended treatment of both CS. The regression showed that there was no significant correlation between the simulated and measured ammonium-N and the R^2 of the model was low (Table 2.7). This is not surprising as the SPM was placed on the soil surface in PCS and the ammonium-N in the soil incorporated ACS may be quickly immobilized.

Table 2.7 Kinetics of ammonium-N transformation in manure treatments and cropping systems.

		2014		2015	
		Model fitting		Model Validation	
Manure	**Cropping system**	**R^2**	**K_n (day^{-1})**	**R^2**	**Pr > |t|**
LPM	ACS	0.89	0.24	0.84	0.0036
	PCS	0.79	0.24	0.76	0.0103
SPM	ACS	0.84	0.21	0.05	0.6198
	PCS	0.77	0.21	0.05	0.6393

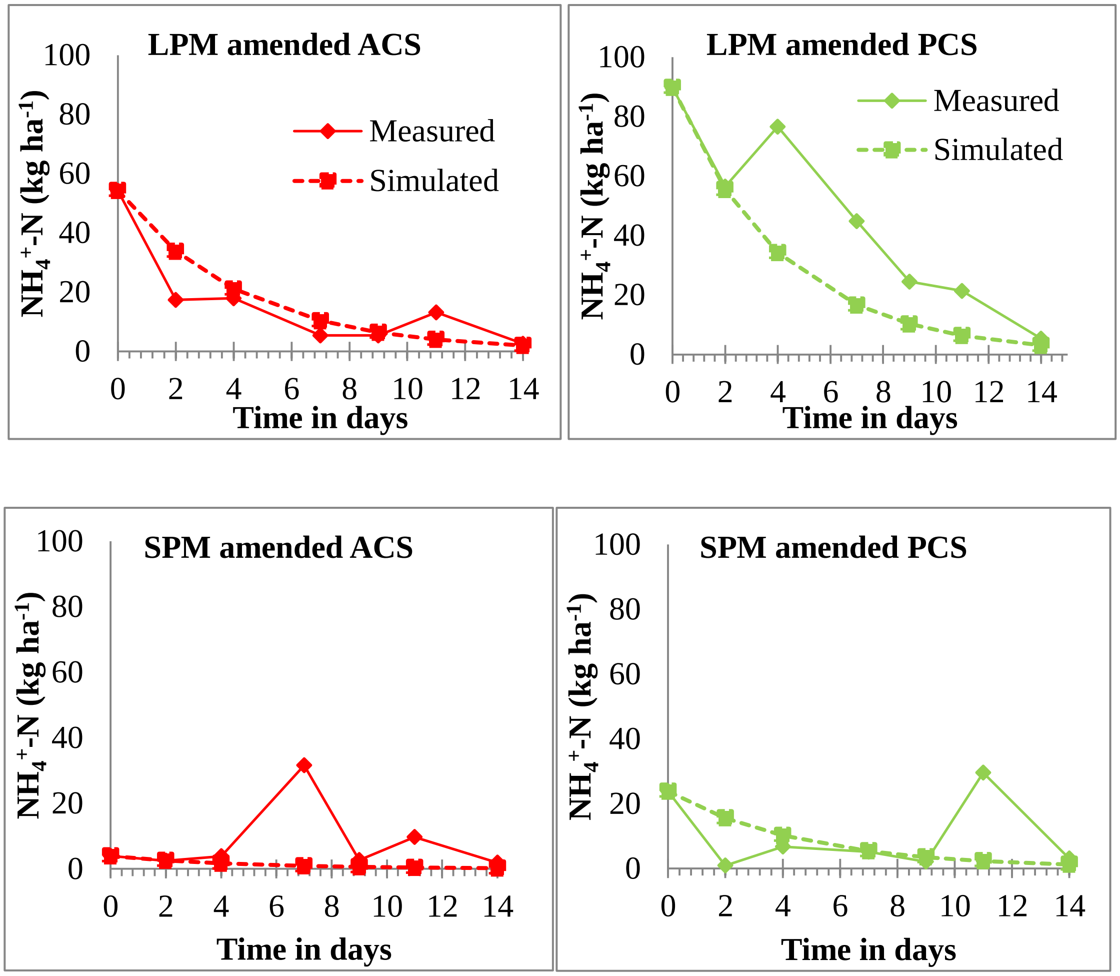

Figure 2.6 Validation of nitrification kinetics model in 2015 using the ammonium-N data (0 - 15 cm and 15 - 30 cm) obtained in 2014.

2.5 Conclusion

Studies have shown that inclusion of perennial forage grasses in a crop rotation has the potential to reduce nitrate leaching. Ammonium-N in LPM amended ACS and PCS peaked 4 DAM in both years and persisted until 7 DAM in PCS, but not beyond 4 DAM in ACS. Similarly, there was greater recovery of ammonium-N in LPM amended PCS than in LPM amended ACS until 7 DAM. Low concentrations of ammonium-N was measured in SPM amended ACS and PCS on 4 and 7 DAM in both years.

There was a greater accumulation of nitrate-N in the ACS than PCS at both depth intervals and in both years. Relative amounts of nitrate-N at both depths and in both CS is an indication of nitrification and movement of nitrate-N between layers.

The first order rate constants for apparent nitrification (K_n) in ACS and PCS were similar for each manure treatment and when the kinetic parameters generated in 2014 were used to simulate ammonium-N dynamics in 2015, there was a significant correlation between the measured and simulated ammonium-N only in LPM amended treatments.

Persistence of ammonium-N in LPM amended PCS may be as a result of high ammonium-N concentration created by the lack of manure incorporation into the soil. The persistence of ammonium-N in the LPM amended PCS than ACS coupled with lower accumulation of nitrate-N may partly account for lower nitrate-N leaching in perennial forage grasses measured by Karimi et al. (2013) as the persisted ammonium-N may possibly be adsorbed to the soil surface, immobilized into organic matter or taken up by plant rather being immediately converted to nitrate-N.

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CHAPTER 3

3.0 ABOVE- AND BELOW-GROUND BIOMASS AND NITROGEN STORAGE OF PERENNIAL FORAGE GRASSES AND ANNUAL CROPS AS INFLUENCED BY TYPE OF PIG MANURE

3.1 Abstract

A previous study on a long-term rotation at Carman, Manitoba, comparing liquid and solid pig manures showed that while significant nitrate-N was leached below the root zone of an annual cropping system (ACS), little nitrate-N was leached from a perennial cropping system (PCS). However, only above-ground biomass and nitrogen uptake were measured and this did not fully explain the differences in nitrate-N lost through leaching by the cropping systems. The aim of this study was to measure both above- and below-ground N uptake by ACS and PCS to ascertain the mechanisms involved in reduced nitrate-N losses through leaching in PCS. The experiment was established in 2009 using a split plot design with the main plot being cropping systems (CS) and subplots being manure treatments (MT). Cropping systems used were ACS and PCS. Manure treatments included N-based liquid pig manure, N-based solid pig manure, and a control with no manure addition. Manures were applied to meet the N requirement of the crops for each year. In 2014, above- and below-ground plant biomass were collected at harvest whereas in 2015, above- and below-ground plant biomass were collected at mid-season. Below-ground plant (root) biomass was collected from 0 - 60 cm at 15 cm depth intervals. The biomass was dried, weighed and sub-sampled for total N analysis. In 2014, PCS had

greater above-ground N uptake than ACS irrespective of MT whereas in 2015, above-ground N uptake of both CS was similar. In both years, root biomass ranged from 5,258 to 9,627 kg ha^{-1} in PCS while that of ACS the ranged from 1,088 to 1,456 kg ha^{-1}. Also, root N content in PCS ranged from 43 to 118 kg N ha^{-1} in both years while that of ACS ranged from 9 to 20 kg N ha^{-1}. Total plant N (sum of above-ground N and root N content) was greater in PCS than ACS in all MT in both years due to greater root N content.

Greater total plant N in PCS than ACS was mainly due to significant greater root N content in PCS rather than above-ground N uptake. The difference in root N content (34 to 98 kg N ha^{-1}) between the CS in this study was sufficient to account for the magnitude of nitrate-N (20 to 60 kg N ha^{-1}) leached in ACS in previous study. In conclusion, greater root biomass coupled with greater root N content in PCS than ACS may account for low nitrate-N leached in perennial forage grasses.

3.2 Introduction

Nitrogen (N) is an essential plant nutrient that is needed in relatively large amounts for their physiological functions and completion of their life cycle (Delgado and Follett 2010; Li et al. 2013). Nitrogen is an important index of plant growth as it improves plant biomass (Xin et al. 2014). Nitrogen is mainly taken up by plants in inorganic forms (NH_4^+ and NO_3^-) both of which move through root cells into the xylem and are transported to different parts of the plants (Li et al. 2013). It is an essential component of many organic compounds in the plant such as nucleic acids, proteins, chlorophyll and alkaloids (Maathuis 2009; McAllister et al. 2012) and N deficiency in plants is the most important nutritional disorder limiting crop yields worldwide (Fageria and Baligar 2005).

Pig manure is a source of many plant nutrients but it is mostly used to supply N to the crop (Mooleki et al. 2002). Application of pig manure to fertilize cropland can result in nitrate leaching if not well managed (Nikièma et al. 2013). Continuous use of manure as the only source of crop N requirement has been reported to have a greater potential for nitrate leaching than inorganic N sources (Allen et al. 2006) with a greater risk to water quality when application is followed by surface runoff (Smith et al. 2007). Similarly, studies have shown that the use of pig manure leads to a build-up of soil residual nitrate with little benefit to crop yield making the accumulated soil residual nitrate-N prone to leaching and groundwater contamination (Bakhsh et al. 2001).

The potential to lose N from ACS is generally high as the recovery of applied N in the above-ground biomass is an average of 50% worldwide (Delgado 2002). This creates a challenge for efficient nitrate management. Nitrate loss through leaching from ACS is reported to be about 30 to 50 times greater than losses from PCS (Randall and Mulla 2001). Therefore, the use of PCS has been proposed as an alternative management practice to prevent nitrate leaching that occur in ACS (Entz et al. 2001; Campbell et al. 2006; van Es et al. 2006). Perennial forage grasses have been reported to maintain and support microbial activities in the soil, improve water infiltration and prevent nitrate leaching when incorporated into a crop rotation (Glover et al. 2007; MAFRD 2015). Unlike annual crops that need to be seeded every season, perennial forage grasses can re-grow early in the spring and remain active into the fall absorbing water and nutrients such as nitrate before they move beyond the root zone (Campbell et al. 2006; Glover et al.

2007). Perennial forage grasses have a larger root biomass than annual crops enabling greater nitrate and water uptake per unit area over a longer period of time (Cox et al. 2006; Wang et al. 2006).

Several studies have compared nitrate leaching between ACS and PCS (Campbell et al. 2006; van Es et al. 2006; Karimi et al. 2013). Unlike many of these studies, Karimi et al. (2013) used field core lysimeters to directly measure the quantity of water and nitrate-N that was lost below the root zone of ACS and PCS. The study clearly demonstrated that significant amounts of nitrate-N was lost below the root zone of ACS (20 to 60 kg ha^{-1} annum^{-1}) whether manure was added to the plot or not while very small amounts of nitrate-N was lost from the PCS (less than 1 kg ha^{-1} annum^{-1}). The reasons for these differences in nitrate leaching between the two CS are uncertain as the above-ground biomass and N uptake did not account for the differences in nitrate leaching between the ACS and PCS. In the study by Karimi et al. (2013) only the above-ground N uptake was measured, leaving the below-ground N storage of the two CS unknown. It is possible that there are differences in the below-ground plant biomass and N storage that could account for the differences in the amount of nitrate-N that was leached between the two CS. The objective of this study was to measure and compare the above- and below-ground N between the ACS and PCS to account for the differences in nitrate-N losses through leaching between the two CS.

3.2.1 Hypothesis

The differences in nitrate-N losses through leaching between ACS and PCS are due to differences in below-ground plant biomass and nitrogen content rather than above-ground N uptake.

3.3 Materials and Methods

3.3.1 Field Description and Manure Application

The detailed descriptions of the site, plot design, manure application as well as seeding dates have been provided in Chapter 2 and they will not be repeated in this chapter. Only procedures that are pertinent to the above-ground and below-ground plant biomass and N storage study will be described.

3.3.2 Above-ground Biomass Sampling

Four random samples of the above-ground biomass were taken from each plot using a quadrat of $0.25m^2$. The quadrant was randomly placed on each plot and above-ground biomass within the quadrat area at 4 cm above-ground level was cut. For ACS, above-ground biomass was sampled at harvest in 2014 and at mid-season in 2015. Above-ground biomass of PCS was sampled at mid-season and harvest in 2014 and at mid-season in 2015. In 2014, perennial forage grasses was mowed and bailed after the mid-season sampling campaign to simulate the first cut, while final harvest represented the second cut. The above-ground biomass of ACS and PCS of each year were put in a cloth bag and hung in a drying room of temperature 30 ^{o}C until they were dried. After drying of harvest above-ground biomass in 2014, the barley was threshed and the weights of grain and straw were determined. Due to the time of canola sampling (flowering stage) in 2015, the canola was not threshed. Subsamples of dried above-ground biomass were taken from annual crops (straw + grains in 2014 and straw only in 2015) and perennial forage grasses and ground for total N analysis.

3.3.3 Below-ground Plant Biomass Sampling

Below-ground plant (root) biomass was sampled in 2014 and 2015 using the sequential coring technique (Ostonen at al. 2005). The technique involved using a hydraulic probe (Giddings, #15-TS/ Model GSRTS, Colorado) to drive a core to a depth of 60 cm to collect below-ground plant biomass between and within the rows on each plot. Below-ground plant biomass in the core was separated into four depth intervals of 0 - 15 cm, 15 - 30 cm, 30 - 45 cm and 45 - 60 cm. Below-ground plant biomass between and within the rows of each depth interval from each plot was composited as an independent sample. This was done in duplicate on each plot to give two sets of independent samples per plot. Each sample was put in nylon bags and stored in a refrigerator (2 ^{o}C) for less than a week before separating the soil from the roots. In 2014, below-ground plant biomass was collected using a core with a diameter of 5.2 cm whereas in 2015, a larger core with a diameter of 8.6 cm was used for the below-ground plant biomass sampling.

3.3.4 Root Washing

Separation of roots from soil was done in plastic buckets. The soil sample containing the roots was emptied into a 1.2 mm sieve and then sprayed with water at a moderate pressure using a spray nozzle to wash soil from roots. The sieve's mesh was small enough to hold fine roots on top of the sieve. The sample left on the sieve was then soaked in water for 5 minutes followed by gently hand-mixing the samples. After soaking, the remaining soil attached to the root was washed with the spraying nozzle until the soil was completely loosened and removed from the roots. The soil in the bucket below the sieve was repeatedly hand-mixed and sieved again to capture any root material that escaped (Jane and Jack 2010; Culman et al. 2010). Roots were placed in an open paper bag and dried at 70 °C in an oven for two weeks. The dried root weight was measured and then ground for total N analysis.

3.3.5 Total N

To measure total N, 0.4 g of dried plant material (0.2 g was used for roots below 15 cm depth due to insufficient plant materials) was weighed into a Kjedahl digestion tube and 4.4 mL of digestion mixture (containing 0.42 g Se powder, 14 g $LiSO_4.H_2O$, 350 mL 30 % H_2O_2, and 420 mL concentrated H_2SO_4) was added to the Kjedahl tube under a fume hood. The mixture was allowed to stand for one hour. After an hour, the mixture was placed in a digestion block whose temperature was raised by 4 ^oC per minute and digested for one hour at 100 ^oC, after which the temperature was raised to 350 ^oC and digested for another 3 hours. The digest was quantitatively transferred into a 50 mL volumetric flask and made to volume with de-ionized water (Akinremi et al. 2003). The resulting digest was read for total N using the Technicon auto-analyzer II (Pulse Instrumentation Ltd, Saskatoon, SK).

3.3.6 Calculation

3.3.6.1 Biomass

Above-ground biomass (S_b) in kg ha^{-1} on dry basis was calculated as the ratio of shoot (straw + grain in 2014 and straw only in 2015) to surface area. Root biomass (R_b) in kg ha^{-1} on dry basis was calculated as the ratio of below-ground plant biomass per core area.

3.3.6.2 Biomass Nitrogen

The above-ground N uptake (N_S) and the root N content (N_R) in kg N ha^{-1} of each CS was calculated as follows:

$$N_S = \frac{S_s \times \%N_s + S_g \times \%N_g}{100} \qquad [1]$$

$$N_R = \frac{R_b \times \%N_r}{100} \qquad [2]$$

Where: $\%N_s$ is the concentration of N in straw; $\%N_g$ is the concentration of N in grain (in case of barley in 2014); and S_s is the straw biomass in kg ha^{-1}; S_g is the grain biomass in kg ha^{-1} (in case of barley in 2014); $\%N_r$ is the concentration of N in the root. In 2015, there was no grain because the biomass was sampled before grain formation.

3.3.6.3 Root Nitrogen Use Efficiency

Root N use efficiency (NUE_R) was estimated to allow comparison of the ability of the roots of both CS to recover applied N. It was calculated as:

$$NUE_R = \frac{N_{R(man)} - N_{R(con)}}{N_{app}} \times 100 \qquad [3]$$

Where: $N_{R(man)}$ is the root N of the plot with manure, $N_{R(con)}$ is the root nitrogen of the control plot with no manure added and N_{app} is the available N applied as manure (Mooleki et al. 2002).

Root biomass samples for all four depths of each plot were added to estimate the root biomass and root N content.

3.3.7 Statistical Analysis

Statistical analysis of the data was performed with PROC GLIMMIX using SAS 9.4 analysis software package to test for significance differences on above-ground and root biomass as well as above-ground N uptake and root N content between the two CS and among the three MT in each year. PROC GLIMMIX was also used to test for significant differences in total plant N between the CS for each MT in each year. Prior to PROC GLIMMIX, the data were checked for the assumption of normal distribution using PROC UNIVARIATE. The above-ground biomass and total plant N in 2014 and 2015 as well as above-ground N in 2015 were normally distributed. The root N content in 2014 and 2015 as well as above-ground N in 2014 was not normally distributed and thus a lognormal distribution was specified in the model. The significant differences were tested at $P < 0.05$ using Tukey-Kramer means comparison.

3.4 Results and Discussion

3.4.1 Above-ground Biomass

In 2014, there was a significant CS x MT interaction on above-ground biomass (Table 3.1). The quantity of biomass collected in each plot depended on the kind of manure applied. In the ACS, the SPM amended treatment had the highest above-ground biomass (16,000 kg ha^{-1}) whereas in PCS, the LPM amended treatment had the highest above-ground biomass (13,000 kg ha^{-1}) (Table 3.1). Expectedly, the CON of both CS treatments had the lowest biomass. Overall, ACS had a higher, but not significantly so, above-ground biomass than the PCS in each MT (Table 3.1).

In 2015, there was no significant CS x MT interaction in the above-ground biomass (Table 3.1). There were both a significant effect of MT and CS on the above-ground biomass. The manure treated plots (LPM and SPM) were significantly greater than CON treatments. Unlike 2014, the PCS had significantly higher above-ground biomass than the ACS in LPM and SPM treatments.

Table 3.1 Effect of manure treatments, cropping systems and their interactions on above-ground biomass (dry basis) and N uptake in 2014 and 2015.

		Above-ground biomass (kg ha^{-1})		Above-ground N uptake (kg N ha^{-1})	
		2014 Harvest	**2015 Mid-season**	**2014 Harvest**	**2015 Mid-season**
Model effect	**Df**	……………………………**P values**	……………………………		
CS	1	0.0545	0.0036	0.0351	0.4108
MT	2	0.0003	<.0001	<.0001	<.0001
CS x MT	2	0.0410	0.0630	0.5480	0.0174
Group means					
CS x MT					
ACS[y]	LPM	15,000 a	7,000 b	200 ab	110 a
	SPM	16,000 a	7,000 b	250 a	140 a
	CON	9,000 bc	4,000 c	83 c	46 b
PCS	LPM	13,000 ab	12,000 a	270 a	160 a
1st & 2nd cut	SPM	12,000 abc	11,000 a	280 a	140 a
	CON	9,000 c	6,000b c	120 bc	44 b
CS					
ACS		14,000 a	6,000 b	180 b	99 a
PCS		11,000 a	10,000 a	230 a	110 a
MT					
LPM		14,000 a	10,000 a	240 a	140 a
SPM		14,000 a	9,000 a	270 a	140 a
CON		9,000 b	5,000 b	100 b	45 b

Means with the same letter within a column are not significantly different at $P < 0.05$ using Tukey-Kramer means comparison.

[y] Barley in 2014 (straw + grain); canola in 2015 (No grain in canola as it was harvested before grain formation).

The results obtained were consistence with Karimi et al. (2013) who reported that the biomass of PCS was smaller than that of ACS in its first year of establishment but greater than the ACS in its second year of establishment. The higher biomass in the PCS than the ACS in 2015 could be attributed to its earlier re-growth of perennial grasses in the spring far ahead that of the annual crops (Cox et al. 2006). The time of sampling may also have contributed to the difference in biomass between the two years as the crops were sampled at harvest in 2014 and at mid-season in 2015, where either the annual crop (canola) may not have reached its peak biomass production and/or canola is less productive than barley.

3.4.2 Above-ground N Uptake and N Removal

There were no significant CS x MT interactions in the above-ground N uptake at harvest in 2014 (Table 3.1). Cropping system and MT both had significant effects on above-ground N uptake (Table 3.1). Above-ground N uptake was greater in the PCS than in the ACS (Table 3.1). This was in spite of the ACS having a greater biomass, indicating that the concentration of N in the above-ground biomass of the PCS was greater than that in the ACS (Tables 3.2 & 3.3). In both CS, the manure treated plots (LPM and SPM) had significantly greater above-ground N uptake as well as N removal than CON plot even (Tables 3.2 & 3.3).

Table 3.2 Barley grain yield (dry basis), N concentration and removal at harvest in the ACS in 2014.

MT		Dry grain yield (kg ha^{-1})	N (%)	N removal (kg N ha^{-1})
LPM		7,382 ab	1.9 ab	143 a
SPM		8,693 a	2.2 a	193 a
CON		4,076 b	1.6 b	65 b
Model effect	**Df**	**…………………………..P values………………...**		
MT	2	0.0104	0.0206	0.0039

Means with the same letter within a column are not significantly different at P < 0.05 using Tukey-Kramer means comparison.

Table 3.3 Forage perennial grass yield (dry basis), N concentration and N removal at harvest in the PCS in 2014.

MT		Total yield (kg ha^{-1})	N conc. (%)		N removal
		1st & 2nd cut	1st cut	2nd cut	(kg N ha^{-1})
LPM		13,000 a	1.9 a	2.2 a	271 a
SPM		12,000 a	2.1 a	2.7 a	283 a
CON		9,000 b	1.8 a	1.1 b	122 b
Model effect	**Df**	**………………………. P values…………………………….**			
MT	2	<.0001	0.4857	<.0001	<.0001

Means with the same letter within a column are not significantly different at P <0.05 using Tukey-Kramer means comparison.

Higher N removal by both ACS and PCS may be attributed to the continuous application of manure to these plots (from 2009 to 2014). The continuous application of the manure built up the organic soil N of these manure treated plots (Adesanya 2015) which then mineralized to increase yield especially in SPM amended treatment. This potential of manure treated plots to mineralize N must be taken into consideration in the subsequent application of manure to these plots.

In 2015, there was a significant CS x MT interaction in the above-ground N uptake (Table 3.1). This was as a result of the LPM having greater N uptake than SPM in PCS with the reverse in ACS. Manure treated plots had significantly greater above-ground N than CON treatment in both CS (Table 3.1). Despite the earlier establishment of PCS and greater biomass at this time of the season than ACS, the two CS had similar above-ground N uptake (Table 3.1). This was due higher N concentration in the biomass of ACS than in PCS despite its lower biomass (Table 3.4).

Table 3.4 Above-ground biomass (dry basis), N concentration and uptake at mid-season in 2015.

CS	MT	Above-ground biomass (kg ha^{-1})	N conc. (%)	N uptake (kg N ha^{-1})
ACS^{z}	LPM	7,000	1.6 ab	110
	SPM	7,000	2.0 a	140
	CON	4,000	1.4 b	46
PCS	LPM	12,000	1.3 b	160
	SPM	11,000	1.2 b	140
	CON	6,000	0.7 c	44

^{z}Canola in 2015.
Means with the same letter within a column (in N conc.) are not significantly different at $P < 0.05$ using Tukey-Kramer means comparison.

3.4.3 Below-ground plant (Root) Biomass

There were no significant CS x MT interactions on root biomass in 2014 and 2015 (Table 3.5). Similarly, there was no significant difference in the root biomass among MT. There was a significant effect of CS on root biomass in both years (Table 3.5).

Irrespective of the MT, root biomass was significantly greater in the PCS than the ACS in both years (Figure 3.1). In 2014, the PCS root biomass on dry basis was 8,212, 5,678 and 5,258 kg ha^{-1} for LPM amended, SPM amended and CON treatments, respectively, while the ACS root biomass on dry basis was 1,387, 1,217 and 1,181 kg ha^{-1} for LPM amended, SPM amended and CON treatments, respectively.

Table 3.5 Effect of manure treatments, cropping systems and their interactions on root biomass (dry basis) and root N content in 2014 and 2015.

		Root biomass		Root N content	
Model effect	**Df**	**2014**	**2015**	**2014**	**2015**
		………………………… **P values**		…………………..	
CS	1	0.0010	<.0001	<.0001	<.0001
MT	2	0.2163	0.0584	0.0019	0.0035
CS x MT	2	0.6622	0.9112	0.4462	0.4654

P values are significant at $P < 0.05$ using Tukey-Kramer means comparison.

In 2015, PCS root biomass was 9,184, 9,627 and 6,094 kg ha^{-1} for LPM amended, SPM amended and CON treatments, respectively, while the ACS root biomass was 1,456, 1,428 and 1,088 kg ha^{-1} for LPM amended, SPM amended and CON treatments, respectively. In both years, over 80% of the root biomass in both CS was in the 0 - 15 cm depth interval with very small amount of biomass beyond the 0 - 15 cm depth (Figure 3.2).

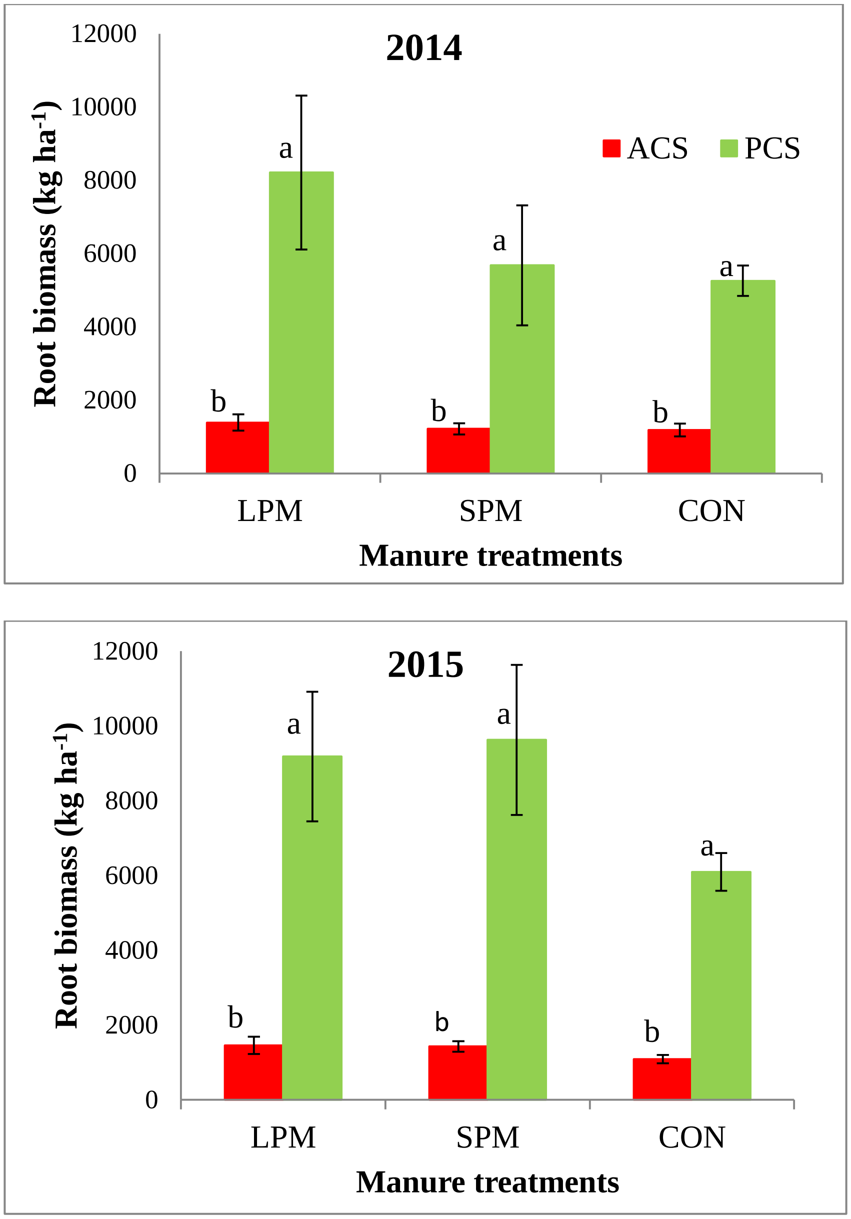

Figure 3.1 Effect of cropping systems and manure treatments on root biomass (dry basis) in 2014 and 2015. Error bars are ±1 standard error of the means. Means with the same letter are not significantly different at $P < 0.05$ using Tukey-Kramer means comparison.

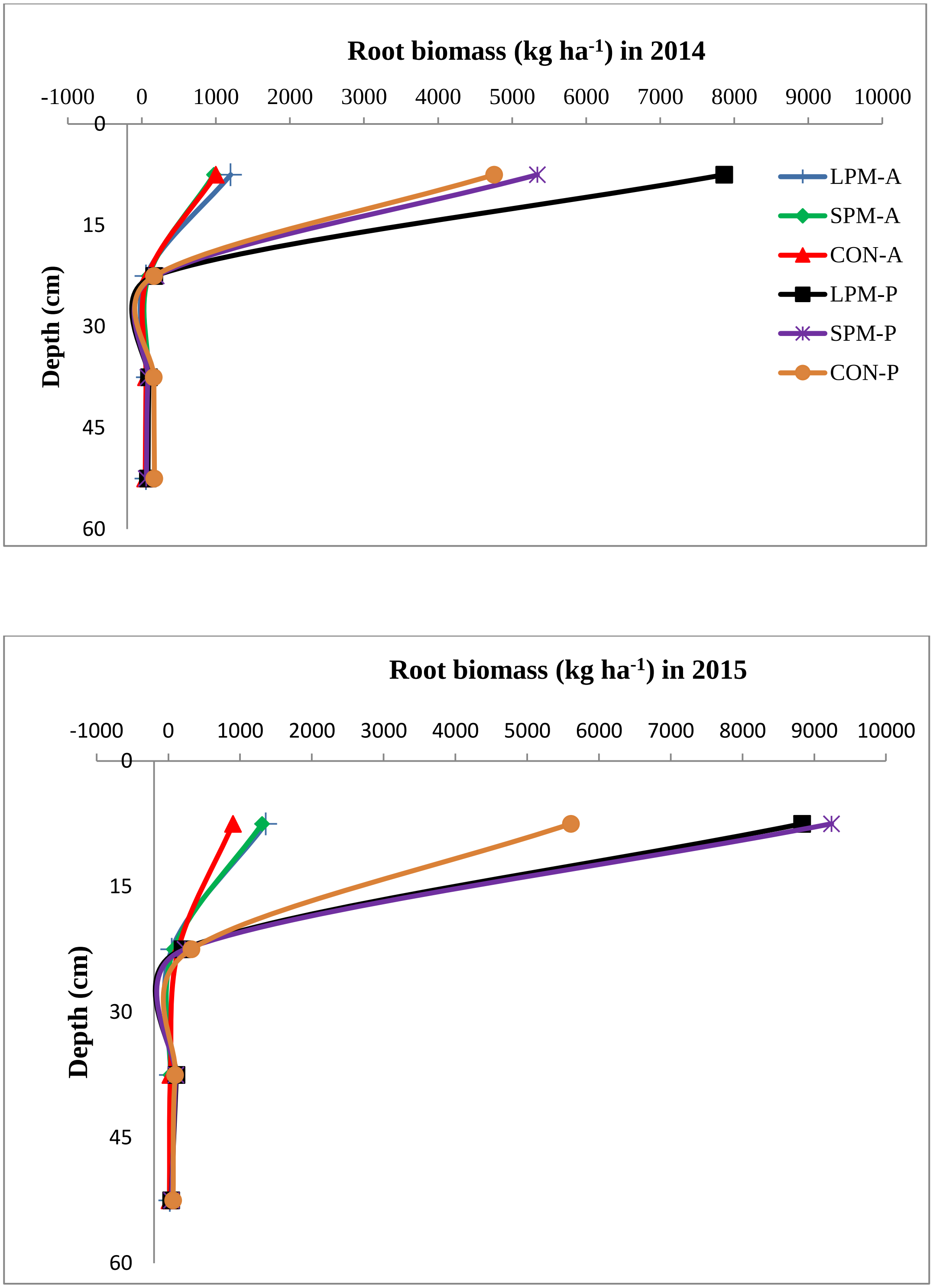

Figure 3.2 Effect of cropping systems and manure treatments on root biomass (dry basis) per depth. Where: A is ACS and P is PCS.

Greater root biomass in the PCS corroborated the results of Dupont et al. (2014) in a long-term study between PCS and ACS where the root biomass of the PCS was between three to seven times greater than the root biomass of ACS. Even when the two CS have similar above-ground biomass, Monti and Zatta (2009) reported that PCS had a root biomass that was about six times greater than the ACS. The greater root biomass of the PCS also contributed to their greater root biomass (Glover et al. 2007). Ferchaud et al. (2015) reported a positive correlation between root density and water use efficiency in soil layers of annual and perennial crops. This positive correlation between root density and water use efficiency will enhance water and N uptake by perennial forage grasses, both of which will reduce nitrate leaching. Also the presence of much of this root biomass at 0 - 15 cm (Figure 3.2), will enable the perennial forage grasses to efficiently absorb water and N at soil surface, thereby preventing leaching of nitrate and preserving the environment (Brown et al. 2000; DeHaan et al. 2005; Glover and Reganold 2010).

In addition to nutrient uptake, the large root biomass of perennial forage grasses could help to protect the soil against erosion that may result in nutrient transport to nearby surface water bodies. Glover et al. (2007) reported that timothy was 54 times more effective than annual crops in preventing soil erosion as the roots can hold soil aggregates together.

3.4.4 Root Nitrogen Content

There were no significant CS x MT interactions on root N content in 2014 and 2015, while individually both had significant effects on root N content in both years

(Table 3.5). Root N content of the ACS was not affected by manure addition in either years (Figure 3.3) but the PCS that received LPM amendment had significantly greater root N content than the CON treatment in 2014. There were no significant differences in root N content of SPM amended and CON treatments of PCS (Figure 3.3). In 2015, there was no significant difference between root N content of LPM and SPM amended PCS while both were significantly greater than CON treatment (Figure 3.3). In both CS, root N content measured in 2014 was greater than that in 2015 except in SPM amended PCS. The difference in root N content may be attributed to differences in time of sampling in both years.

Root N content of the PCS was significantly greater than root N content of ACS irrespective of MT in both years (Figure 3.3). In 2014, root N content of PCS was 118, 77 and 54 kg N ha^{-1} for LPM amended, SPM amended and CON treatments, respectively. Root N content of ACS was 20, 19 and 12 kg N ha^{-1} for LPM amended, SPM amended and CON treatments, respectively, in 2014. In 2015, root N content of the PCS was 100, 97 and 43 kg N ha^{-1} for LPM amended, SPM amended and CON treatments, respectively, while root N content of ACS was 14, 13 and 9 kg N ha^{-1} for LPM amended, SPM amended and CON treatments, respectively.

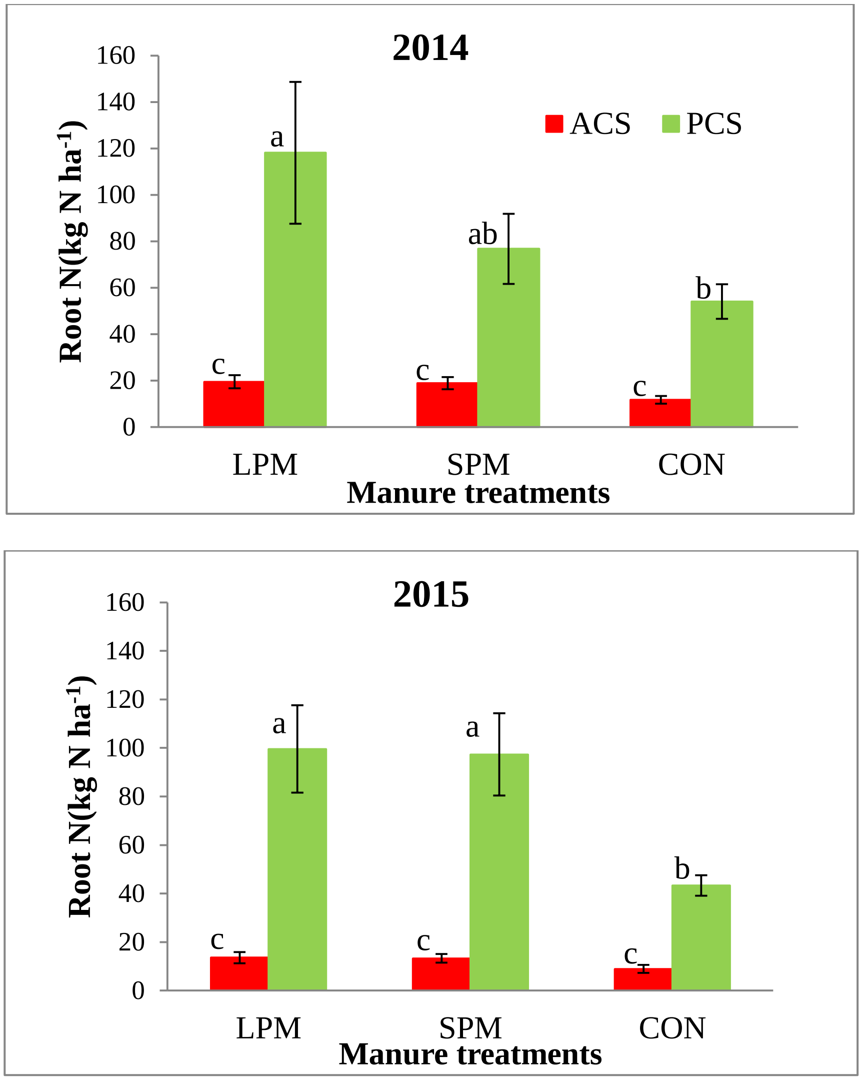

Figure 3.3 Effect of cropping systems and manure treatments on root N content in 2014 and 2015. Error bars are ±1 standard error of the means. Means with the same letter are not significantly different at $P < 0.05$ using Tukey-Kramer means comparison.

The amount of nitrogen in the root reflected amount of root biomass in the different treatments. There was a greater root biomass in PCS than ACS which dictated the greater root N content of the PCS than the ACS. A study that compared related annual and perennial species (*Lesquerella)* showed that the perennial crop (*L. mendocina*) had greater root N and root biomass than its annual counterpart (*L. fendleri*) (Ploschuk et al.

2005). Greater root N content in CON treatment of PCS than LPM amended ACS showed the greater efficiency of roots of these perennial forage grasses to mop up nutrient in the soil. Greater root biomass in CON treatment of PCS was able to compensate for the reduced availability of N in the CON treatment of PCS compared to the LPM amended ACS. Earlier studies have shown that plants have an adaptive feature to allocate greater biomass to their roots in soils with low plant nutrients (Nielson et al. 2001; Lynch and Brown 2008) which will enable greater uptake of nutrient. The uptake of N by roots is therefore not only dictated by available N in the soil but also the accessibility of nutrients to the roots. This greater root N content of PCS could be the means by which perennial grasses reduce nitrate leaching.

3.4.5 Root Nitrogen Use Efficiency

The root of a crop is known to be the primary medium of nutrient uptake by plants from soil. In both LPM and SPM amended treatments, root nitrogen use efficiency (NUE_R) of the PCS was greater than that of the ACS in 2014 and 2015. While NUE_R was 122 and 58% in LPM and SPM amended PCS, respectively, the NUE_R was 15% in LPM and SPM amended ACS in 2014. Similarly, the NUE_R in 2015 was 60% in LPM and 98% in SPM amended PCS while it was 8% in LPM and 14% in SPM amended ACS. Roots of the perennial forage grasses were between 3 to 7 times more efficient in recovering N applied to the soil than roots of the annual crops. This efficient use and storage of applied N by forage perennial grass roots could help to minimize or prevent nitrogen loss beyond the root zone and can account for the differences in nitrate-N level in the soil after the growing season. The higher NUE_R in PCS showed that the differences in nitrate-N through nitrate leaching may be attributed to the N content of the root biomass.

3.4.6 Total Plant N

Total plant N is the sum of above-ground N uptake and root N content of each CS. There was no significant CS x MT interaction in the total plant N in 2014 whereas in 2015 there was a significant CS x MT interaction in total plant N (Table 3.6). The CS x MT interaction in 2015 was because total plant N was significantly greater in PCS than ACS in LPM and SPM amended treatments and not significantly greater in CON treatment. Total plant N was greater in PCS than ACS in both years (Figure 3.4). Total plant N in PCS ranged from 180 to 390 kg N ha^{-1} in 2014 and 87 to 259 kg N ha^{-1} in 2015. In the ACS, the range was from 95 to 269 kg N ha^{-1} in 2014 and 55 to 153 kg N ha^{-1} in 2015.

Table 3.6 Effect of manure treatments, cropping systems and their interactions on total plant N in 2014 and 2015.

		Total plant N	
Model effect	**Df**	**2014**	**2015**
		……………P values……………	
CS	1	<.0001	0.0112
MT	2	0.0019	<.0001
CS x MT	2	0.4462	0.0075

P values are significant at $P < 0.05$ using Tukey-Kramer means comparison.

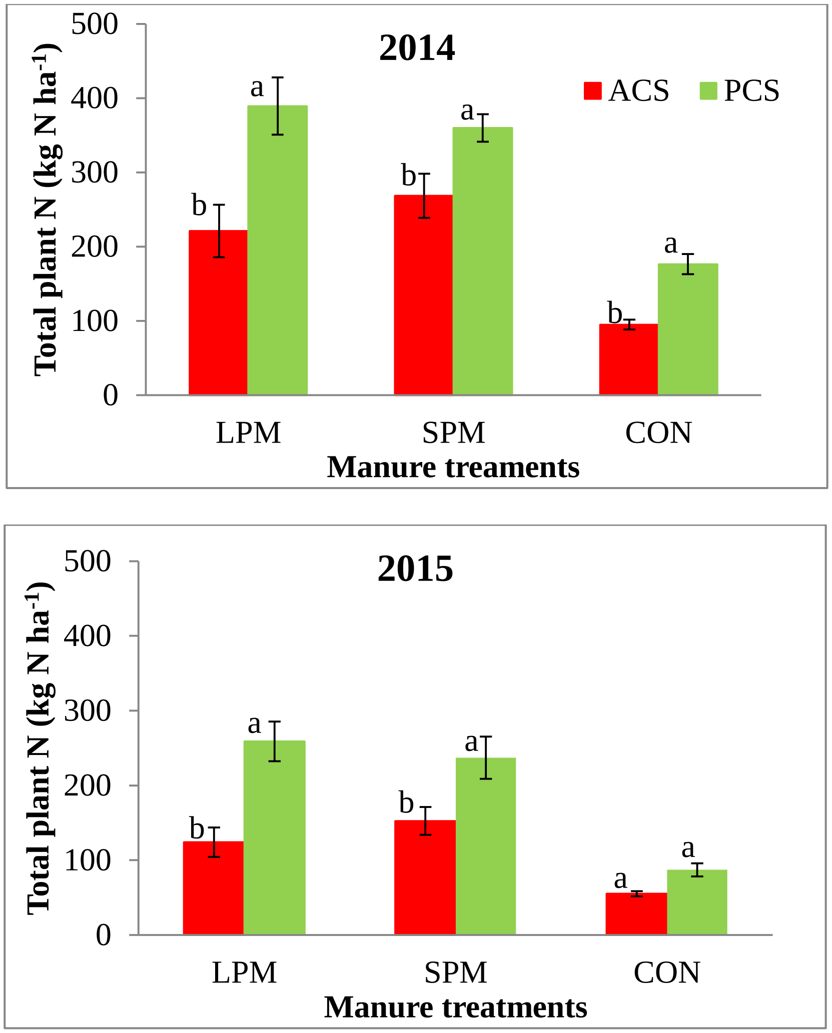

Figure 3.4 Effect of cropping systems and manure treatments on total N in 2014 and 2015. Error bars are ±1 standard error of the means. Means with the different letters within a manure treatment are significantly different at $P < 0.05$ using Tukey-Kramer means comparison.

Higher total plant N in PCS shows the efficiency of perennial forage grasses to take applied N from the field. Earlier studies by Cox et al. (2006) and Glover and Reganold (2010) have reported that perennial grasses are more efficient in nutrient utilization than annual crops and thereby reduce nitrate leaching. The higher total plant N in PCS shows that the difference in nitrate-N lost through leaching between the two CS cannot be accounted for by measuring above-ground N uptake alone. Karimi et al. (2013) has demonstrated that the differences in above-ground N uptake between the ACS and

PCS could not account for the differences in the magnitude of nitrate-N leaching (20 to 60 kg ha^{-1}) between the two cropping system. Thus, measuring the root N content with above-ground N uptake shows that the significant nitrate-N lost through leaching in ACS may be due to its significantly lower root N content than that in PCS as shown in Figure 3.3. The magnitude of the differences in root N content (34 to 98 kg N ha^{-1}) between annual crops and perennial forage grasses in this study are sufficient to account for the magnitude of the differences in nitrate leaching measured by Karimi et al. (2013) over her three years study.

3.5 Conclusion

Studies have reported that perennial forage grasses can prevent or reduce nitrate-N leaching in agricultural systems. However, none of these studies have been able to account for the lower nitrate-N lost through leaching in PCS when compared to ACS. This study compared above- and below-ground plant N uptake between ACS and PCS to see if increase N storage in below-ground biomass of PCS will account for differences in nitrate leaching with solid and liquid pig manures. In 2014, ACS had a higher, but not significantly so, above-ground biomass than the PCS whereas in 2015 the PCS had greater above-ground biomass than the ACS. In 2014, the PCS had a greater above-ground N uptake than the ACS whereas in 2015, PCS and ACS had similar above-ground N uptake. This shows that a greater above-ground biomass does not necessarily translate into a greater N uptake.

Root biomass showed a large distinction between the ACS and PCS. In 2014, root biomass in the PCS ranged from 5,258 to 8,212 kg ha^{-1} while that in the ACS ranged from 1,181 to 1,387 kg ha^{-1}. As such, root N content ranged from 54 to 118 kg N ha^{-1} in PCS and from 12 to 20 kg N ha^{-1} in ACS. Similarly, PCS root biomass in 2015 ranged from 6,094 to 9,627 kg ha^{-1} when the ACS root biomass ranged from 1,088 to 1,456 kg ha^{-1}. This root biomass in 2015 led to a root N content that ranged from 43 to 100 kg N ha^{-1} in PCS and 9 to 14 kg N ha^{-1} in ACS. Total plant N was greater in PCS than ACS in all MT in both years. Total plant N was greater in first year than second year due to the difference in time of sampling.

Greater total plant N in PCS than ACS was mainly due to significantly greater root N content in PCS. The difference in root N content (34 to 98 kg N ha^{-1}) between the CS in this study was sufficient to account for the magnitude of nitrate-N (20 to 60 kg N ha^{-1}) lost through leaching in ACS in previous study. Greater root biomass coupled with greater root N content in PCS than ACS may account for low nitrate leaching in perennial forage grasses. Whether root N storage by the perennial forage grasses is permanent or can be mobilized in the future is unknown and warrants further studies.

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4.0 GENERAL SYNTHESIS

Sixty percent of the farmland in Manitoba is classified with a higher and moderate level risk class of nitrate contamination by the Indicator Risk of Water Contamination by nitrate-N (De Jong et al. 2007). Therefore, efficient management of nitrate-N on farmland is crucial for environmental sustainability of agricultural production systems. Various studies have shown that significant amounts of nitrate-N is leached beyond the root zones of ACS while little nitrate-N is leached beyond the root zone of PCS (van Es et al. 2006; Karimi et al. 2013). However, the mechanisms by which perennial forage grasses prevent nitrate leaching have not been clearly explained.

This study investigated ammonium-N persistence and root N content of perennial forage grasses and annual crops following pig manure application. The objectives of this study were achieved by testing two hypotheses. The first part which was reported in Chapter 2 was based on the hypothesis that ammonium-N persisted longer in PCS thereby delaying the process of nitrification and nitrate-N accumulation. Ammonium-N and nitrate-N concentrations of the ACS and PCS were measured and compared during a short period of time (2 - 4 weeks) following pig manure application. The second part of this study was reported in Chapter 3 and it was based on the hypothesis that differences in nitrate-N losses through leaching between ACS and PCS were due to differences in below-ground plant biomass and nitrogen storage. Above- and below-ground plant biomass were collected at harvest in 2014 and at mid-season in 2015.

The results showed that ammonium-N reached its peak 4 DAM in LPM amended ACS and PCS in 2014 and 2015. The ammonium-N persisted until 7 DAM in the PCS treated with LPM, but did not persist beyond 4 DAM in the ACS in both years.

Ammonium-N measured in SPM amended PCS and ACS was small and similar at 4 and 7 DAM in both years. Relative amounts of nitrate-N in the two CS at 0 - 15 cm depth interval were consistent with the persistence of ammonium-N in LPM amended ACS and PCS in first 7 DAM in both years. There was a greater percentage accumulation of nitrate-N in the ACS than the PCS at 15 - 30 cm in all MT. Although incorporation of manure may increase nitrification in the ACS, the infiltration of LPM into the soil might compensate for the lack of manure incorporation in PCS, as such, the persistent of ammonium-N and concomitantly smaller nitrate-N in the PCS might not be completely due to a lack of manure incorporation. Also, manure incorporation did not yield any significant difference in ammonium-N in SPM amended ACS and PCS.

The apparent nitrification parameters generated in 2014 fit well for LPM amended PCS and ACS when used to simulate ammonium-N in 2015 but not for SPM. The generated K_n may be used to estimate ammonium-N persistence on LPM amended treatment. It may be that the SPM amended treatment requires another model to estimate its apparent nitrification.

In 2014, ACS had a higher, but not significantly so, above-ground biomass than the PCS whereas in 2015 the PCS had greater above-ground biomass than the ACS. Above-ground N of PCS was higher than that of ACS in 2014 in all MT but similar in SPM and CON treatments in 2015. In both years, root biomass ranged from 5,258 to 9,627 kg ha^{-1} in PCS while that of ACS ranged from 1,088 to 1,456 kg ha^{-1}. Also, root N content in PCS ranged from 43 to 118 kg N ha^{-1} in both years while that of ACS ranged from 9 to 20 kg N ha^{-1}. Total plant N was greater in PCS than ACS in all MT in both years and total plant N was greater in first year than second year for each CS and MT due to the difference in time of sampling.

The implication of greater persistence of ammonium-N in PCS than ACS is that the presence of perennial grasses in the field which limit manure incorporation into the soil may help delay the nitrification process thereby allowing available N to be taken up in the ammonium form, adsorbed to soil surface or immobilized. Also, the presence of larger root biomass in PCS than ACS, with over 80% of the root biomass at the 0 - 15 cm depth may allow the root of perennial forage grasses to take up larger quantity of N in the surface soil layer before it is moved below this depth. With higher root N content in PCS than ACS, the reduced nitrate-N losses through leaching in PCS may be accounted for by the nitrogen stored in plant roots. Earlier study by Karimi (2013) has shown a difference of 20 - 60 kg N ha^{-1} losses between ACS and PCS through leaching, a difference that the above-ground N uptake could not account for. This was because in some years, ACS had greater above-ground N than PCS while in other year PCS had more above-ground N. Yet, nitrate-N losses through leaching both in soil and lysimeters were always significant in ACS with very little loss in PCS. With this high root biomass in PCS which resulted in a difference of 34 to 98 kg N ha^{-1} in root N content between the two CS which is the same order of magnitude as the differences in nitrate leaching between the two CS. The presence of the roots at spring time in the perennial forage grasses must have encouraged uptake of nutrient at the soil surface before it moved to deeper depths. This may be another reason why there was little percentage increase in nitrate-N accumulation at the 0 - 15 and 15 - 30 cm depth intervals in the PCS as the large root biomass has the potential to take up nitrate as soon as it was formed. This study clearly showed that above-ground

N uptake only cannot be used to account for differences in nitrate-N leaching between ACS and PCS. Majority of the differences in total plant N between the two systems were due to the root N content. Incorporation of perennial forage grasses in annual crop rotations can be used by farmers to manage the soil nitrate-N level.

4.1 Limitation and Future Study

The study was carried out with a difference in the method of manure application between the two CS. The manure could not be incorporated on the PCS because it was seeded in the fall of 2013 while the manure was applied in summer of 2014 and 2015 when the annual crops were to be planted. A similar study that will not incorporate manure in the two CS is needed to verify if ammonium-N will still persist longer in PCS than ACS.

Also, ammonium-N persistence between the two CS needed to be compared using synthetic ammonium-N fertilizer source and nitrification inhibitors to confirm if the ammonium-N persistence in PCS is comparable to that provided by synthetic nitrification inhibitors.

Furthermore, future study may also compare the root N content between ACS and PCS over a longer period of time to know if perennial grasses can sustain their greater root N or not. It will also be of interest to examine what happens to this stored root N when a perennial grass system is converted to an annual cropping system and if nitrate leaching is accentuated when a perennial cropping system is converted to an annual cropping system.

4.2 References

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APPENDIX

I. Above- and below- ground sampling dates in 2014 and 2015.

Year	Above-ground sampling	Below-ground sampling
2014	July 23rd (1st cut of perennial grasses) August 27th	Sept. 15th
2015	July 23rd & 27th	July 23rd & 27th

II. Root biomass and root N concentration for each sampling depth in ACS and PCS in 2014 and 2015.

			2014		2015	
CS	**MT**	**Depth (cm)**	**Root biomass (kg ha^{-1})**	**N conc. (%)**	**Root biomass (kg ha^{-1})**	**N conc. (%)**
ACS	LPM	0-15	1199 b	1.4 bcd	1357 bc	0.9 kljm
		15-30	58 cde	1.3 cde	49 ghij	1.6 cb
		30-45	74 cde	1.5 bc	29 hij	1.6 cbd
		45-60	55 cde	1.4 bcd	21 ij	1.3 gefd
	SPM	0-15	973 b	1.5 bc	1307 c	0.8 lmno
		15-30	92 cde	1.8 ab	76 fghij	1.5 bcde
		30-45	92 cde	1.8 ab	28 hij	1.7 cb
		45-60	60 de	2 a	17 j	1.2 fghi
	CON	0-15	1000 b	1 ed	901 cd	0.7 nop
		15-30	79 cde	1.2 cde	140 fghij	1.2 efgh
		30-45	57 cde	1 cde	30 hij	1.4 cdef
		45-60	46 e	1.4 bcd	16 hij	2.7 a
PCS	LPM	0-15	7863 a	1.4 bcd	8832 a	1.1 ghi
		15-30	169 cd	1.1 cde	200 efg	1.1 ghij
		30-45	98 cde	1.5 bc	113 efgh	0.9 klmn
		45-60	82 cde	2.1 a	39 hij	1 ijkl
	SPM	0-15	5341 a	1.4 bcd	9242 a	1.1 hijkl
		15-30	193 c	1.5 bc	234 def	1 ghijk
		30-45	83 cde	1.4 bcd	105 efghi	1.1 ghi
		45-60	61 cde	1.2 cde	46 hij	1.9 a
	CON	0-15	4759 a	1 cde	5611 ab	0.7 mnop
		15-30	166 cd	1.2 cde	321 cde	0.7 ponm
		30-45	162 cd	1.3 cde	99 efgh	0.6 p
		45-60	170 cd	0.9 e	67 fghij	0.7 po
	Model effect	**Df**	**…....…………………. P values…………………**			
	CS	1	0.0015	0.0034	<.0001	<.0001
	MT	2	0.8116	<.0001	0.8357	<.0001
	CS x MT	2	0.0381	<.0001	0.8647	<.0001
	Depth	3	<.0001	0.0037	<.0001	<.0001
	CS x depth	3	0.0002	0.1414	0.0152	<.0001
	MT x depth	6	0.4285	<.0001	0.2673	<.0001
	CS x MT x depth	6	0.1884	<.0001	0.9964	<.0001