TRACING CROP RESIDUE DERIVED NITROGEN INTO SUBSEQUENT CROPS AND NITROUS OXIDE EMISSIONS

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ABSTRACT

Research has demonstrated that including winter wheat with under-sown red clover into corn-soybean rotations has the potential to improve crop yields and N use efficiency. Yet, the mechanisms that explain these benefits are poorly understood. One possible explanation is that the crop rotation 'diversification' improves the soil N supply or that the soil N cycle 'tightens'—thereby lowering potential N losses. To address this hypothesis, an isotope tracing experiment was setup i) to follow the fate of enriched ^{15}N residues into subsequent soil and crop N pools; and ii) to measure N_2O and CO_2 emissions, and N residue decomposition dynamics. For my field experiment, natural abundance and enriched ^{15}N urea were applied to 1 m^2 micro-plots within a 37-yr long-term trial, where I had access to the 'simple' corn-corn-soybean-soybean (C**C**SS, S**S**CC) rotations; and 'diverse' corn--corn-soybean-wheat/red clover (C**C**SWrc, S**Wrc**CC) rotations. These systems were maintained under conventional tillage or no-till. At harvest, a residue exchange operation was performed to transfer enriched ^{15}N above-ground residues to ^{15}N natural abundance micro-plots, and vise-versa, thus isolating enriched ^{15}N above- and below-ground residue contributions. Subsequent crops were harvested and used to determine above- and below-ground previous year's residue N contributions. For my soil incubation experiment in the lab, field soil cores were collected from the crop rotation and tillage treatments to establish 50 g soil microcosms that were amended with ^{15}N-enriched corn stover or roots. Soil and gas samples were periodically collected to measure crop residue decomposition dynamics (via CO_2 emissions and ^{15}N mineralization) and $^{15}N_2O$ emissions. The field trial demonstrated that crop rotation had no impact on the overall crop residue N allocated to the subsequent crop systems. In contrast, no-till and below-ground residues increased corn residual N contributions to the subsequent crop, relative to conventional tillage and above-ground residues, respectively. Regardless, below-ground residual N pool contributed more N to subsequent crops than above-ground crop residue. The incubation results demonstrated higher residue-derived N mineralization, and greater overall N_2O and CO_2 emissions from 'diverse' *vs.* 'simple' rotations. Overall, my findings indicate that crop 'diversification' enhanced soil N stocks likely due to the additional N inputs (N fertilization or N fixation). Although 'diversifying' corn-soybean rotations with winter wheat and red clover

may produce higher crop yields, it is necessary to adjust for nutrient credits or soil N surplus when applying N inputs year after year. Otherwise, N losses may be a side-effect and should be investigated at field scale.

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1. GENERAL INTRODUCTION

1.1. Introduction

Conservation agriculture practices have been implemented for thousands of years, dating as early as when the Inca empire cultivated crops in the Americas (Kosiba 2018). As farmers transitioned from self-sustaining agriculture to a global integrated commodity system, agricultural practices were intensified to achieve market demands; i.e., increased yields and lower costs. However, agricultural intensification was often achieved at an environmental cost (e.g., soil degradation, loss of biodiversity, enhanced greenhouse gas emissions) and, as a result, agricultural practices have again shifted due to public pressure to balance agronomic and economic performance with environmental outcomes that ensure crop production remains sustainable. One method believed to improve yields while reducing the agri-environmental footprint is crop 'diversification' and reduced tillage. Even though the benefits associated with these practices are well known, their impact on ecosystem service mechanisms still intrigue soil and plant scientists.

1.2. Hyphotheses and objectives

The object of this research was to analyze the influence of long-term wheat with under-seeded red clover on N cycling in corn-soybean based rotations, under conventional or no-till tillage. I hypothesized that the introduction of such crops provides ecosystems services that would result in more N supplied to subsequent crops while reducing N losses such as N_2O.

1.3. Thesis organization

This thesis is organized in manuscript format. Chapter two provides an overview about conservation agriculture practices and nitrogen cycling. In Chapter three I present a field study where residue N (added as enriched ^{15}N) was traced in 'simple' and 'diverse' crop rotations, under conventional and no-till tillage systems. In Chapter four I present a laboratory incubation study in which I used soil and plant materials to analyze $^{15}N_2O$ and CO_2 emissions and residue N decomposition dynamics. In Chapter five, I synthesize major findings from Chapters three and four, concluding the thesis as a whole and suggesting future work pathways. The literature cited in this thesis is located in Chapter six and, finally, supplemental data analysis tables are presented in Chapter seven, including P-tables.

2. LITERATURE REVIEW

2.1. Agriculture, nitrogen, and environmental concerns

By 2050, the global population is expected to increase by and surpass the 9.7 billion mark (United Nations, World Population Prospects 2019). One consequence of this increase in population is the need for more food and fiber to meet ever-growing demands. And, in turn, the demand for nitrogen (N) fertilizer is changing. Anthropogenic reactive N creation increased from 15 Tg yr^{-1} in 1860 to 165 Tg yr^{-1} in 2000, resulting in an altered global N cycle with cascading impacts on our environment (Galloway et al. 2002). The main reason for increased reactive N production is the reliance of agriculture on fertilizer N. Yet, it is estimated that only about 12% of N fertilizer inputs are actually taken up by plants and ultimately ends in human mouths (Galloway et al. 2003). The remaining N is lost and cascades to other ecosystems. This means that there is a tremendous opportunity and need to improve N use efficiency in agronomic systems—thus minimizing reactive N cascade through unwanted N loss pathways (Smil 1999).

Population growth will also drive an increase in the emission of greenhouse gases (GHG) (Satterthwaite 2009), such as nitrous oxide (N_2O), that impact global warming and climate change (Smith et al. 2013). Agronomic systems play an important role in these emissions (Houghton et al. 1999). In Canada, for example, agriculture is responsible for 60 Mg CO_2-equivalent, representing 8.4% of the total national emissions in 2017 (National Inventory Report 1990-2017). Of this total, agriculture in Ontario is responsible for about 10 Mg CO_2-equivalent, which accounts for 5.9% of total emissions from Ontario (Environment and Climate Change Canada, 2016). Nitrous oxide emissions mostly arise from the agriculture sector which accounted for 77% of the total N_2O produced nationally in 2017 (National Inventory Report 1990-2017). These numbers demonstrate that agriculture is a major source of GHG production (which may even increase with a growing population), so any strategy to mitigate GHG emissions will help reduce the national anthropogenic GHG footprint.

Sustainable farming strategies focused on 'low impact or conservation' agriculture may not only improve N use efficiency, but also reduce nitrogenous GHG emissions from this sector (Smith et al. 2008). Examples of 'low impact' farming include: reduced- and no-till practices (RT/NT) (Six et al. 2002) and crop rotations that include legume options (Gaudin et al. 2015). Whereas these farming strategies may be applicable across many agricultural regions, each region has its own adoption peculiarities. Customization for each reality is necessary since conservation agriculture practices are adopted following regional variables (Knowler and Bradshaw 2007).

2.2. The "Northern Corn Belt"

2.2.1. Cropping systems in southwestern Ontario

Southwestern Ontario is an important Canadian grain production region that has been dubbed the 'Northern Corn Belt' because it produces more than half (59.8%) of the total corn (*Zea mays* L.) acreage in Canada (Agri-Food Canada, 2016). Soybean (*Glycine max* L.) is another major crop in Ontario (Shi et al. 2012), with 49.6% of the national soybean acres in the Province (Statistics Canada, 2017). On the other hand, wheat (*Triticum estivum* L.) is a relatively minor crop (with most production in the Prairie provinces), which in eastern Canada it is often produced in rotation with corn and soybeans (Hoss et al. 2018, Statistics Canada Census of Agriculture, 2011).

Due to the large quantities of corn stover that remain in the field after harvest (Wilhelm et al. 2004), many growers use conventional tillage (CT) to help breakdown the stover for improved seedbeds. Conventional tillage helps to bring the above-ground residues closer to microbes, and exposes the organic matter for more rapid decomposition (Gregorich et al. 1998). Among CT practices, moldboard ploughing has been a common form of tillage used in Ontario in past decades (Fox and Dickson 1990). One of the main issues resulting from many tillage years is gradual surface deterioration, exposing soils to erosion (Shi et al. 2012). A proposed way to mitigate this issue is making use of the 'low impact' NT practice which minimizes soil erosion rates compared to conventional plowing practices (Ruttan 1999).

2.2.2. Environmental stewardship programs in Ontario

The "soil health" concept emphasizes that soils are living ecosystems. The term soil health has been defined as the capacity of soil to function by sustaining biological productivity, environmental quality, animal and plant health (Karlen et al. 1997). To improve or maintain soil health in farming systems, the Environmental Commissioner of Ontario (ECO) recommends implementing conservation practices to protect and enhance soil life, such as keeping the soil covered with plants or residues, maximizing crop diversity, avoiding soil disturbances, increasing the presence of live roots all year, and adding soil organic amendments wherever possible (Environmental

Commissioner of Ontario Report, 2016). These measures may be defined as best management practices (BMP) and can potentially sequestrate from 50 to 1000 kg of carbon (C) per hectare in a year (Lal 2004). Regarding soil C stocks, ECO recommends developing a protocol that would reliably estimate soil-C levels in Ontario and track it over time. With these estimates the local government could link the cost of crop insurance and C levels, creating incentives that would recognize farmers who have adopted BMPs (Environmental Commissioner of Ontario Report, 2016).

To increase the adoption of BMPs, the ECO report suggests providing BMP users with financial support for up to 10 years – it is suggested that this would offset any potential yield losses within the transitional management period due to reduced excesses of N availability in newly converted NT systems (Rice et al. 1986). The ECO also recommends that Ontario sign up for the '4/1000 (4 per mille) French Initiative' that would ease the conversion to a soil health approach in local agriculture (Environmental Commissioner of Ontario Report, 2016).

With 40% of cropland adopting 'low input' or 'soil health' conservation practices, BMPs in Ontario would provide approximately 10% of yearly GHG reductions in that province (Environmental Commissioner of Ontario Report, 2016). Certain BMPs, such as NT for example, have the potential to not only sequestrate C but also reduce fertilizer N inputs by minimizing soil N losses (West and Marland 2002). The implementation of this conservation tillage practice had also demonstrated to reduce N_2O emissions at spring thaw (Wagner-Riddle et al. 2007).

Another practice that could represent a BMP in Ontario is the introduction of wheat into corn-soybean based rotations (Gaudin et al. 2015), which could potentially impact in reduced crop N inputs. Underseeded red clover with winter wheat has proven to be another important tool in combination with corn-soybean rotations with potential to sustain system resilience and maximizing input use efficiency (Gaudin et al. 2013).

2.3. Agronomic management

2.3.1. Monoculture and crop rotation

Monoculture is a common practice characterized by continuously producing a single crop in a field. Producing the same crop year after year in continuous monoculture systems may result in yield losses, diminished soil-C levels, increased erosion vulnerability, and increased risk for crop diseases and pests (Ketcheson 1980; McDaniel et al. 2016). Due to these issues, continuous cropping has long since been replaced by growing multiple crops in rotation, where the crop species from season to season—typically in a 2- to 4-year rotation cycle. Evidence from temperate studies suggests that breaking continuous monoculture systems can produce ecosystem services benefits such as i) improving crop yields by *ca.* 14% in cereal experiments (Kirkegaard et al. 2008); ii) positively influencing disease pressure by breaking the life cycle of crop-specific pathogens with less susceptible plants—taking advantage of the natural mortality and antagonistic effects in root-zone microorganisms (Ghorbani et al. 2008); iii) improving yield stability (Berzsenyi et al. 2000); iv) enhancing below-ground community structure and activity (Tiemann et al. 2015); and v) increasing crop nutrient use efficiency (Tilman et al. 2002). Growing crops in rotation also has a strong effect on crop residue decomposition dynamics. Indeed, breaking continuous monoculture patterns has been linked with microbial change in below-ground communities; e.g., different above-ground crop residues may change the composition of below-ground microbial communities by suppling a range of plant input quality, quantity and chemical complexity of decomposable material—positively impacting biodiversity-function relationships and soil aggregation (Tiemann et al. 2015). Under conditions with abundant labile organic matter resources and microbial biomass, crop residue decomposition—even low-quality residue inputs—can be enhanced, which is beneficial for soil nutrient cycling and may result in soil organic matter accrual over time via soil C and N stabilization (McDaniel et al. 2016).

In Ontario, the most frequently used rotations for grain production are corn-soybean-wheat. This crop rotation sequence represents 19.8% of the national seeded area, followed by corn-soybean with 17.9%, while unrotated monoculture for corn accounts for only 5% of the national seeded area (Statistics Canada, 2011). However, wheat acreage (spring and winter varieties) in Canada has declined by 15% in the last 20 years, mainly due to global surpluses and declining crop profitability compared to other crop options (Canadian Wheat Research Priorities Report, 2017; Schewe et al. 2017). Moreover, globally, wheat production is expected to decline by 6% for each degree Celsius of global temperature increase (Asseng et al. 2015). Together, the changing economy and global warming raise concerns that growers will be less likely to implement the more ‘diverse’ crop rotation of corn-soybean-wheat, and that more growers will opt for the relatively less diverse crop rotation of corn-soybean production. Thus, if policy or environmental stewardship programs continue to recommend that growers produce winter wheat in their corn-soybean rotations (despite declining economic incentives for producing wheat), we must obtain a better understanding of the mechanisms that regulate benefits for including winter wheat—such as soil health indices (Congreves et al. 2015) or crop resilience (Gaudin et al. 2013)—and better monetize the ecological benefits of keeping wheat in the rotation. It is possible, however, that winter wheat will have a positive impact on soil-crop N dynamics by improving crop N use, tightening the recycling of crop residue-N, and/or reducing N loss. Characterizing the N dynamics within different rotation configurations of corn, soy, and wheat will contribute to the body of knowledge aimed at better understanding ecosystem services and designing more sustainable agricultural systems (Palm et al. 2014). It is clear that soil ecosystem services may have a special role in producing beneficial soil-plant interactions that benefit other aspects of production, like N use efficiency (Osterholz et al. 2018).

2.3.2. Tillage practice

Conventional tillage has been gradually replaced by the implementation of conservation tillage practices in various parts of the world; with worldwide acreage under conservation tillage increasing from 75 million hectares in 2003 to 105 million hectares in 2009 (Derpsch et al. 2009). In Canada, the implementation of CT dropped by 60% from 1991 to 2006, giving rise to conservation tillage practices such as RT and NT (Statistics Canada, 2007). The province of Ontario had lower rates of NT adoption than the national average (46% of total seeded area) with only 31.2% of total seeded area practicing NT in 2006 (Derpsch et al. 2009), and there was only a very small (1.9%) increase adoption by 2011. Consequently, NT adoption has lagged far behind provinces such as Saskatchewan that had 90.3% of the land prepared for seeding under RT or NT (Statistics Canada, 2011). These adoption differences might be driven by distinct rainfall regimes between east and western Canada.

Conservation tillage practices have the potential to sequester C (Six et al. 2002). Though, in light of the global literature suggesting that these practices sequestrate C only under specific soil and climate conditions—mainly in areas with drier soil conditions—this assertion needs to be considered carefully (Palm et al. 2014). Other authors have identified sampling methodology as playing an important role in C sequestration studies that compared CT with conservation tillage practices (Baker et al. 2006). Another important aspect is the depth of soil that is considered when CT and NT are compared; e.g., NT may facilitate SOC redistribution rather than C accrual, largely due to the presence of a plough layer where texture and drainage may alter sub-surface C dynamics

(Angers and Eriksen-Hamel 2008). Whether or not conservation tillage is the driving factor for C accrual, other ecosystem benefits—including nutrient cycling—should also be considered when comparing tillage systems.

No-till plays an important role in maintaining cooler soil temperatures on warmer days (Johnson and Lowery 1985; Licht and Al-Kaisi 2005) compared to CT and RT practices. Also, NT has been shown to produce significant changes in total microbial biomass and biomass functional groups, probably related to differences in soil aggregation between conservation and conventional tillage practices (Helgason et al. 2010). Other benefits of RT/NT implementation include water conservation and erosion reduction (Six et al. 2002). Research has suggested that NT contributes to slower ammonium (NH_4^+) oxidation into nitrate (NO_3^-) by as much as an order of magnitude, thus contributing to reduced leaching (Laine et al. 2018). All these changes may impact the cycling of reactive N, which can ultimately influence N use efficiency (Grandy et al. 2006).

Reduced and NT practices might play an important role in reducing N_2O emissions in the long-run, and have been considered to significantly minimize N_2O emissions when compared to CT after 10 or more years (van Kessel et al. 2013). Van Kessel et al. (2013) also found that fertilizer N placement (surface *vs.* incorporated) is crucial in explaining N_2O flux differences between CT and RT/NT systems. They found that N_2O emissions in conservation tillage were lower when fertilizer N was placed in equal or lower than 5 cm depth, which suggests that there may be important N cycling changes when conservation tillage practices are implemented.

2.3.3. Crop rotation and tillage system interaction

Combining crop rotations with conventional or conservation tillage practices can help minimize yield reductions that are often observed when NT is implemented alone (i.e., without a diverse crop rotation) (Pittelkow et al. 2015). Also, the combination of diverse rotations with NT may improve soil organic C and N levels (Havlin et al. 1990) and benefit ecosystem services when compared to monoculture and CT (Osterholz et al. 2018). On the other hand, the benefits of combining NT with crop rotation are not always observed; e.g., NT alone has been shown to improve soil C levels and increase soil microbial activity (Balota et al. 2004). These inconsistencies among studies are likely related to differences in site conditions—indicating that region-specific research is required before widespread adoption of these practices can be recommended.

Another variable that often impact crop yields is weed seed bank reduction. In a 6-year tillage-crop rotation study, Murphy et al. (2006) found that the weed seed bank declined in NT under diversified rotation compared to CT under monocultures. This demonstrates that interactions between tillage system and rotation likely influences multiple factors as opposed to just a single factor such as nutrient availability. Many soil ecosystem services are likely to impact yield changes, and I believe that nutrient cycling—or, more appropriately, *soil biogeochemistry*—rather than simply nutrient availability at any one point in time, should be looked at more closely.

2.3.4. The role of wheat and red clover in crop rotations

The use of non-growing season cover crops represents an important means of diversifying crop rotations in Canadian cropping systems, though to date its adoption has been limited to only 13.7% of Canadian farms (Statistics Canada, 2016). Schipanski et al. (2014) suggested based on a multiple cover crops analysis, that introducing cover crops would increase eight out of eleven ecosystem services when introduced to row crop rotations, including N supply, biomass production, soil C storage, NO_3 retention, weed suppression, beneficial insect conservation, and soil fungi colonization. The inclusion of cover crops also can contribute to changes in soil microbial communities , soil aggregate stability, and soil moisture, which are linked with soil organic C and total N accrual and, consequently, are particularly important in low-input agricultural scenarios where N inputs are limited (Tiemann et al. 2015).

Red clover (*Trifolium pratense*), as with other legume crops, is usually under-seeded between the rows of a winter wheat crop. Legume combinations may improve subsequent cropping yields in the short- (2–3 year) and long-term (20 years or more) (Fischer et al. 2002; Berzsenyi 2000; Meyer-Aurich et al. 2006). Another important benefit of including red clover in crop rotations is related to the resiliency of the subsequent cereal crop, which can result in higher yield stability (Gaudin et al. 2013). The introduction of red clover has been demonstrated to provide many benefits for crop production, such as lowering the need for N inputs in both the winter wheat phase of the rotation (Gaudin et al. 2014) and in the subsequent cereal phase (Gaudin et al. 2015), as well as promoting greater N fertilizer-induced soil organic C and total N gains (Congreves et al. 2017). With these benefits, including cover crops such as under-seeded red clover in winter wheat should be considered an appropriate management strategy in the context of food security and soil sustainability (Tiemann et al. 2015). Although red clover may provide a series of benefits for crop rotations, the competition for nutrients where it is under seeded should not be underestimated (Fischer et al. 2002).

2.4. Long-term trials

Conservation practices can influence crop yields and ecosystem services if they are implemented continuously over the long-term (Pittelkow et al. 2015). Indeed, many years of conservation practice are necessary to stabilize certain ecosystem services, which then can be lost with a single year of conventional operation such as fallow management (Triplett and Dick 2008). Furthermore, changes in some soil properties (e.g., SOC) can only be confirmed and quantified after several years, and so the approximate 600 long-term trials being conducted world-wide may help provide information for these changes that may be only apparent in the long run (Körschens 2006). And, as ecological responses to a changing climate will present increased variation and inconsistencies across geographies (Walther et al. 2002), confirming these changes will be even more challenging in the face of a warming climate. Thus, long-term trials (>20 years) have great importance (Poulton 1995).

2.5. Nitrogen cycling at the soil-plant level in agroecosystems

Nitrogen can exist in various forms and its cycling in agroecosystems is strongly influenced by farming practice and environmental conditions (Nesheim et al. 2015). Biogeochemical N cycling processes can be divided into external processes that add or remove N from the ecosystem, and internal processes that convert one chemical N form into another or transfer N between soil pools (Bottomley et al. 1994). One example of the external processes affecting N inputs in agroecosystems is N fixation in which leguminous plants obtain N via biological N_2 fixation (Peoples and Herridge 1995). Globally, biological (managed) fixation is responsible for about 20 Tg N yr^{-1} and is an important source of N in agronomic systems (Smil 1999). Another external process that can result in N inputs in agroecosystems is the application of N-fertilizers, which are then susceptible to transformations in the soil (Slemr and Seller 1984).

Nitrogen mineralization is defined as the transformation of organic N into inorganic forms such as nitrate (NO_3^-) or ammonium (NH_4^+) and ammonia (NH_3) (Stevenson et al. 1982). The rate and quantity of mineralization depends on various factors such as the total N, water soluble N, lignin, and cellulose content, and C/N ratio of the decomposing material, as well as soil microbial respiration, microbial biomass, and microbial N content (Bengtsson et al. 2003). Nitrogen immobilization goes hand in hand with mineralization and is the process by which mineral N is converted into organic forms. Immobilization is primarily performed by microorganisms and their activity is positively correlated to factors such as plant root biomass (Laungani and Knops 2012), soil mineral N availability (Jenkinson et al. 1985), microbial density/activity and stoichiometry (Bengtsson et al. 2003, Buchkowski et al. 2015). Soil ammonia compounds can be converted to nitrate via a two-step nitrification process: i) the conversion of ammonia and ammonium into nitrite (NO_2^-), followed by ii) the conversion of nitrite into nitrate (Barth 1970). Denitrification occurs under anaerobic conditions where microorganisms reduce nitrogen compounds instead of oxygen in a stepwise manner: electrons from organic matter, molecular nitrogen or oxidized sulfur compounds are transferred to nitrogen oxidation, hence building up a proton motive force that is used for ATP regeneration, mainly producing N_2 (while nitrogenous gases are formed as intermediates in low concentration) (Schmidt et al. 2003). Understanding how conservation agriculture techniques impact nitrogen transformation processes is one of the steps—along with a strong farm extension program—to support the introduction of newer technologies to farming communities.

2.5.1. Stable isotopes as a technique to identify the nitrogen fate

The use of stables isotopes in research represents a powerful technique to help answer hypotheses in countless studies. In agricultural studies, ^{13}C and ^{15}N are the primary stable isotopes utilized to reconstruct past agricultural conditions (Aguilera et al. 2008), characterize biological pathways in food webs (Handley and Raven 1992), and compare fertilizer-N recovery rates under different management systems (Kramer et al. 2002). The first use of N isotopes in agricultural studies was performed by Norman and Wekman (1945), who introduced a technique that enabled researchers to better understand N transformations and its cycling in plants—including N use efficiency (Bottomley et al. 1994; Peterson and Fry 1987). These isotopes played a key role in helping researchers identify the role of N in enhancing soil priming for inorganic and organic inputs (Jenkinson et al. 1985; Dittert et al. 1998), distinguishing how agricultural practices influence N plant uptake (Malhi and Nyborg 1991), determining soil N rates for microbial processes (Davidson et al. 1991), and estimating soil N losses (Sebilo et al. 2013). Nitrogen isotopes are also used as a tool to determine N_2O fluxes from specific emission pathways using enriched (Mathieu et al. 2006) or natural abundance isotope fractionation (Wrage et al. 2004). This powerful technique may be used to determine the influence of agricultural practices such as crop rotations and tillage systems in plant residue nutrient recycling, where residue-^{15}N decomposition dynamics is traced in soil, plant, and gaseous components.

2.6. Research objectives

A. Evaluate the legacy influence of including wheat under-seeded with red clover in corn-soybean rotations on N cycling by tracing the fate of crop residue derived-^{15}N.

B. Determine the influence of winter wheat and red-clover on N cycling in different crop phases by tracing the fate of crop residue derived-^{15}N.

C. Characterize – in detail – the influence of wheat under-seeded with red clover on soil N processing of above- *vs.* below-ground crop residue.

2.7. Research hypotheses

i. Soil N cycling is regulated by the legacy of long-term crop rotation and tillage.

ii. Including winter wheat and red clover in a corn-soy based rotation will benefit key soil ecosystem services, such as, i) reduced potential N losses (N_2O), and ii) increased crop residue-N turnover, supplying more N for the next crop.

3. TRACING THE SUPPLY OF CROP RESIDUE-DERIVED NITROGEN INTO SUBSEQUENT CROP

3.1. INTRODUCTION

Previous research demonstrated that including winter wheat and red clover in corn-soybean based rotations improved corn N yields (Gaudin et al. 2015), crop yield stability (Gaudin et al. 2013), and soil overall health ("capacity of soils to function to sustain biological productivity") indices (Congreves et al. 2015). However, the question remains: *why* does including this particular crop phase into a corn-soybean system result in these benefits? Further research is needed to better understand the ecosystem mechanisms that may regulate such benefits. It is possible that the legacy of including winter wheat and red clover in a corn-soybean rotation influences crop residue decomposition dynamics and N turnover, supplying more N to the subsequent crop, which might explain the improved crop N use and higher yields.

The research trial described in this Chapter was conducted to address the following hypotheses:

i. soil N cycling is regulated by the legacy of long-term crop rotation and tillage;
ii. including winter wheat and red clover in a corn-soybean rotation will benefit key soil ecosystem services such as the ability to increase crop residue-N turnover, thus supplying more N for the next crop; and
iii. that crop residue and its decomposability are linked to subsequent crop N uptake.

3.2. MATERIALS AND METHODS

3.2.1. Long-term field trial

This research was conducted at the Elora Research Station (43°38'25.6"N; 80°24'36.4"W), near Guelph, Ontario where a long-term field trial has been maintained since 1980 (Gaudin et al. 2015). Soil at the site is a Woolwich silt loam, classified as a Grey Brown Luvisol or Albic Luvisol. The field trial is arranged as a split-plot randomized complete block design, with four replications. The main effect is crop rotation, and the split effect is tillage. The main plot dimensions are 16.76 m by 6.10 m, with a 10.67 m pathway between replicates (Fig. 3.1).

Figure 3.1. Satellite image of the long-term field trial as recorded on April of 2016. Source: Google Earth.

The main effect consists of seven long-term crop rotations, each with a four-year sequence (Table 3.1). The split-effect consists of two different tillage systems: conventional tillage (CT) and no-till (NT). Conventional tillage was performed to a depth of 15–20 cm using a moldboard plow in the fall, and field cultivation (two passes) prior to seeding each spring (Gaudin et al. 2015). Plots were maintained to ensure that weed and pest pressure were similar between plots and that they were carefully supressed to avoid productivity to be altered by those factors (Gaudin et al. 2015). No-till plots were maintained by leaving crop residues on the soil after each harvest. Crop varieties were changed throughout the years to ensure that the trial represented commercial cropping practices (Gaudin et al. 2015). Historically, corn crops received 160 to 180 kg N ha^{-1} annually; soybean received only 8 kg N ha^{-1} (due to its ability to fix atmospheric N); and winter wheat received 110 kg N ha^{-1} (Gaudin et al. 2015). These rates were equal to, or greater than recommended crop rates (OMAFRA 2013) ensuring that nutrient outputs or losses were replenished every year according to soil tests.

For this thesis, the research was conducted over the 2017 and 2018 growing seasons, focusing on four crop rotations chosen to evaluate the influence of a ‘simple’ corn-soybean rotation compared to a relatively more ‘diverse’ rotation which included winter wheat and red clover (Table 3.1). The crops were harvested mechanically, and the red clover was terminated at early spring.

The field management from 2010–2018 for the selected rotations in 2017 and 2018 is shown in Table 3.2.

Table 3.1. Crop rotations analyzed for the 2017-2018 study.

Rotations [a]	**2017 phase**	**2018 phase**
corn-corn-soybean-soybean (C**CS**S)	Corn	Soybean
soybean-soybean-corn-corn (S**SC**C)	Soybean	Corn
corn-corn-soybean-wheat/red clover (C**CS**Wrc)	Corn	Soybean
soybean-wheat/red clover-corn-corn (S**WrcC**C)	Wheat/red clover	Corn

[a] Bolded font indicates the crop phases present in 2017 and 2018.

3.2.2. ^{15}N tracer study establishment and sample collection

On June 15-16, 2017 two micro-plots were established within each of the selected crop rotation plots (Table 3.1) under CT and NT. The 1 m^2 micro-plots (1.5 m wide and 0.67 m long) were centred on crop rows and spaced 2 to 3.2 m apart and 1.5 to 2.0 m from the plot edges. The micro-plots were defined using a rectangular wood frame. One set of micro-plots received conventional (^{15}N natural abundance) urea fertilizer at 5 kg N ha^{-1}, while the other set received ^{15}N enriched urea at 5 kg N ha^{-1}. The ^{15}N enrichment varied depending on the crop (Table 3.3) to reflect differences in crop N uptake, typical plant N concentrations, and crop biomass. The urea was dissolved in 4 L of water, and evenly distributed to the micro-plot surface using a watering can; an additional 2 L of water was applied to ensure that the tracer moved into the soil.

Table 3.2. Historical nitrogen fertilizer applications (kg N ha^{-1}) to the selected crop rotations from 2010 to 2018 during the crop season.

Crop Year	C**CS**S (kg N ha^{-1})[a]	S**S**C**C** (kg N ha^{-1})[b]	C**CS**Wrc (kg N ha^{-1})[c]	S**Wrc**C**C** (kg N ha^{-1})[d]
2010	0	136	0	136
2011	0	136	95	136
2012	190	0	190	0
2013	151	0	151	102
2014	0	151	0	151
2015	0	142	102	142
2016	136	0	136	0
2017	166	19	166	193
2018	0	151	0	151
Total	644	736	841	1013

Bolded letters indicate the crop phases present during the period of study, in 2017 and 2018.

[a] Soybean (S) phase begins in 2010.

[b] Corn (C) phase begins in 2010

[c] Soybean phase begins in 2010

[d] Corn phase begins in 2010

Table 3.3. ^{15}N enrichment levels applied to the soil on June 15-16[th] of 2017.

Legacy rotation treatments	**Crop**	**^{15}N atom% applied**	**mg ^{15}N m^{-2}**	**kg N ha^{-1}**
C**C**SS and C**C**SWrc	Corn	80	400	5
S**S**CC	Soybean	40	200	5
S**Wrc**CC	Winter wheat/red clover	40	200	5

Bolded rotation phases acronyms letters represent planted crop phases in 2017.

Plant samples were collected from the micro-plots on October 10, 2017, with the plants cut at 3–5 cm from the soil surface. The seed and residues were manually separated, and fresh weights recorded. Plant samples were dried for 48 hours at 60°C, and dry weights recorded. Once dry, crop residues were shredded into smaller fragments (<10 cm), simulating the residue size produced by a combine harvester. Sub-samples (~100 g) were collected for further nutrient analyses and shipped to Saskatoon in paper bags. The remaining above-ground crop residues were temporarily stored in a dry area until main-plot harvest was completed, and the field was prepared for the crop residue exchange procedure. The crop residue exchange between micro-plots was performed on October 18, 2017; dried ^{15}N-enriched above-ground plant residue from the ^{15}N enriched micro-plot was placed onto the natural abundance micro-plot, and vice-versa (Fig. 3.2). Equivalent amounts of dry crop residues were evenly spread by hand across the ^{15}N natural abundance and ^{15}N enriched micro-plot surfaces. For the CT system, any soil movement caused by tillage was measured to account for any lateral movement of the micro-plot position. A plastic net screen (2 mm mesh diameter) was secured overtop the residues to ensure it would not blow away during the non-growing season.

Figure 3.2. Depiction of the crop residue exchange procedure where enriched ^{15}N above-ground residue is placed onto the natural abundance micro-plot, and vice-versa. This procedure isolates the above- and below-ground ^{15}N enriched pools.

Soil samples from 0-15, 15-30, and 30-45 cm depths were collected from four sampling points within the micro-plots using a push probe (19 mm diameter) on October 16th 2017; the samples were then composited for each soil depth. Soil samples were kept in a cooler while field work was being completed, frozen at -20°C and shipped to the University of Saskatchewan for nutrient analysis.

Prior to seeding the next phase of the rotation (May 18, 2018), soil samples were collected in the same manner as in 2017, frozen and shipped to the University of Saskatchewan. Soil sampling also was performed for bulk density estimates by randomly collecting soil samples from within the main plots using an 86.7 cm^3 volume steal ring that was used to extract a top layer section; the soil samples were oven dried for 48 hours at 105°C and dry weights were recorded.

In the following fall, on September 24, 2018, plant samples and soil samples from micro-plots were collected for harvest data – in the same manner as described for the two previous seasons.

3.2.3. Plant tissue and soil analyses

Grain and crop residue samples were ground (< 2 mm) using a Wiley mill (Thomas Model 4, 800 rpm, using 2 mm metal screen). A sub-sample of the ground material (~10 g) was ground to a powder using a Retsch ball grinder (Mixer Mill MM 200, shaking at 25 Hz for 2 minutes). The plant tissue powder (*ca*. 1 to 3 mg) was weighed into aluminum tin capsules to measure %N, %C and bulk ^{15}N abundances using gas chromatography-isotope ratio mass spectrometry (GC-IRMS – Thermo Scientific Delta V mass spectrometer coupled with Costech ECS4010 elemental analyzer). All stable isotope analyses were carried out at the Stable Isotope Facility in the Department of Soil Science, University of Saskatchewan.

Soil samples were thawed at 22°C ± 2 for 4 hours, sieved (< 2 mm), and a sub-sample (~5 g) analyzed for inorganic N (Maynard et al. 2007). Briefly, soil inorganic N was extracted using a 2M KCl solution (1g:5 mL soil: KCl), shaken for 30 minutes at 160 rpm, filtered using Whatman filters No. 42 and stored at -10°C. For analysis, the extracts were thawed and a sub-sample (~1 mL) analyzed for NH_4^+ and NO_3^- concentrations (based on soil bulk density), using an air segmented, continuous flow colorimetric method with a SEAL AA3 HR chemistry analyzer (SEAL analytical Kitchener Ontario). Total soil N and bulk ^{15}N abundance were determined using a sub-sample of air-dried and sieved soil (~10 g) that was powdered using a Retsch ball grinder (Mixer Mill MM 200, shaking at 25 Hz for 2 minutes) and weighed (*ca*. 1 to 3 mg) into aluminum tin capsules for GC-IRMS analysis as previously described for plant tissue samples.

3.2.4. ^{15}N calculations

The equations and terminology used in this section were based on the ^{15}N calculation guide produced by the International Atomic Energy Agency of Vienna (IAE, A. 1983). The recoveries of ^{15}N-labelled urea fertilizer or ^{15}N-labeled crop residues in the tissues (i.e., grain, stover, roots) of the subsequent crop in rotation and in the soil (0-15, 15-30, 30-45 cm depths) at harvest were calculated using Equations 3.1–3.4:

$$Ndff = \frac{^{15}N\ atom\%\ excess\ in\ plant\ tissue\ or\ soil\ pool}{^{15}N\ atom\%\ excess\ in\ urea} \times 100 \quad (3.1)$$

$$TNdff = \frac{Ndff}{100} \times urea - N\ applied \quad (3.2)$$

$$Ndfr = \frac{^{15}N\ atom\%\ excess\ in\ plant\ tissue}{^{15}N\ atom\%\ excess\ in\ the\ crop\ residue} \times 100 \quad (3.3)$$

$$TNdfr = \frac{Ndfr}{100} \times residue\ N\ returned\ to\ soil \quad (3.4)$$

where *Ndff* is the proportion of N derived from fertilizer (%); *TNdff* is the total amount of fertilizer-N recovered in the plant tissue or soil (mg); *Ndfr* is the proportion of N derived from the crop residue (%); and *TNdfr* is the total amount of fertilizer-N recovered in the plant tissue or soil (mg).

Equations 3.3 and 3.4 were used to estimate the N contributions from both the above- and below-ground residues. However, whereas determinations of above-ground residue biomass were straightforward (i.e., the harvested biomass was weighed before being returned to the soil), below-ground (root) biomass could only be estimated. For the present study, root biomass was estimated using root-to-shoot ratios from the published literature: 0.17 for corn (Diaz 2012), 0.26 for soybeans (Diaz 2012), 0.15 for wheat (Williams et al. 2013), and 0.43 for red clover (Skuodienė and Tomchuk 2015). In addition, below-ground N includes contributions from root exudates and residual fertilizer-N; consequently, below-ground ^{15}N (expressed as ^{15}N atom% excess) was defined as the ^{15}N in the roots (recovered at harvest) plus ^{15}N in the soil.

3.2.5. Statistical analyses

Statistical analyses were performed using the PROC MIXED method in SAS (SAS Institute, SAS release 9.4, Cary, NC, USA). Crop yields, inorganic N, total N, and residue-^{15}N derived data were subjected to analysis of variance and Tukey's multiple means, where significant effects were noted at $\alpha = 0.05$, but also at the 0.10 level if P-values were greater than 0.05 but still less than 0.10. Fixed effects were rotation and tillage, and rotation × tillage interaction. The random effect was replication. The assumption for such analysis was that the residuals were normally distributed, homogenous and centered around zero, and these were assessed using PROC UNIVARIATE and a Shapiro-Wilk test (Shapiro and Wilk 1965).

3.3. RESULTS

3.3.1. Crop yields and residue at harvest

In general, crop biomass obtained from the micro-plots was considered equivalent to that of commercial production; i.e., the yields equated to 7.50 to 11.64 Mg ha^{-1} for corn; 1.71 to 3.13 Mg ha^{-1} for soybean; about 3.9 Mg ha^{-1} for winter wheat (Table 3.4). Moreover, whereas these results indicate that, for a given phase of the rotation, the 'diversified' rotation generally produced numerically higher crop yields compared to the correspondent 'simple' rotation—though the differences were not always significant. For instance, in 2017 corn yields were 22.2% and 6.3% higher from the 'diverse' C**C**SWrc rotation compared to the 'simple' C**C**SS rotation under CT (P=0.033) and NT (P=0.342), respectively. Likewise, the soybean phase in 2018 yielded 16.5% and 13.1% more from the diversified rotation than the simple rotation under CT (P=0.074) and NT (P=0.033), respectively. Also, in 2018, however, corn yields were only marginally greater for the diversified rotation compared to the simple rotation under both CT and NT (i.e., 5.1% [P=0.924] and 3.3% [P=0.964], respectively) (Table 3.4).

Table 3.4. Crop grain yields and residue biomass (leaves plus stalk) at harvest in 2017 and 2018.

	-------------------2017 -------------------			-------------------2018 ------------------		
Tillage system	**Rotation and phase**	**Yield (Mg ha^{-1})**	**Crop residue (Mg ha^{-1})**	**Rotation and phase**	**Yield (Mg ha^{-1})**	**Crop residue (Mg ha^{-1})**
CT	C**C**SS	7.51 (0.50)	6.23 (0.37)	CC**S**S	2.27 (0.09)	1.54 (0.08)
NT	C**C**SS	8.56 (0.32)	5.85 (0.24)	CC**S**S	2.72 (0.13)	1.74 (0.09)
CT	C**C**SWrc	9.65 (0.73)	6.72 (0.37)	CC**S**Wrc	2.72 (0.13)	1.94 (0.10)
NT	C**C**SWrc	9.14 (0.49)	5.85 (0.25)	CC**S**Wrc	3.13 (0.08)	2.16 (0.07)
CT	S**Wrc**CC	3.97 (0.33)	2.95 (0.23)[a]	SWrc**C**C	11.64 (0.80)	5.38 (0.32)
NT	S**Wrc**CC	3.84 (0.21)	2.98 (0.14)[b]	SWrc**C**C	10.61 (0.71)	5.14 (0.39)
CT	S**S**CC	1.71 (0.10)	1.67 (0.13)	SS**C**C	11.05 (0.73)	4.74 (0.42)
NT	S**S**CC	2.01 (0.13)	1.91 (0.12)	SS**C**C	10.26 (0.72)	4.48 (0.34)

[a] Mean values calculated using the harvested biomass from the natural abundance and ^{15}N-labeled micro-plots; values in parentheses are the standard errors of the mean.
[b] Data applies to wheat; the red clover yielded an additional 1.78 (0.06) Mg ha^{-1} of biomass.
[c] Data applies to wheat; the red clover yielded an additional 2.06 (0.09) Mg ha^{-1} of biomass.

3.3.2. Nitrogen-15 enrichment levels sufficient to trace crop residue-N

At harvest in 2017, I found that 51.3 and 68.3% of ^{15}N-enriched fertilizer was recovered in the grain, crop residue, and soil (0–45 cm bgs) for corn production in the C**C**SS and C**C**SWrc rotations, respectively (Fig. 3.3A). Similarly, 65.6% and 68.8% of the ^{15}N fertilizer was recovered from the S**S**CC and S**Wrc**CC systems at harvest (Fig. 3.3B). For these rotations, the majority (30.3% to 50.3%) of the ^{15}N fertilizer remained below-ground in the soil (0-45 cm depth)—either in the fine root material, root exudates, or residual fertilizer ^{15}N (Fig 3.3). The unaccounted for ^{15}N was assumed either to have moved below the 0-45 cm depth or was lost from the system. Regardless, the ^{15}N atom% excess in the above- and below-ground crop residues ranged from 0.0215 to 0.9659 (Table 3.5 to Table 3.7), which was considered sufficient for tracing the residue-^{15}N into the subsequent crop in rotation. Residual ^{15}N values found in the soil after 2017 harvest are listed on Tables 3.8 and 3.9. Above-ground crop residue ^{15}N recovery rates were observed to be higher for corn relative to soybean crops.

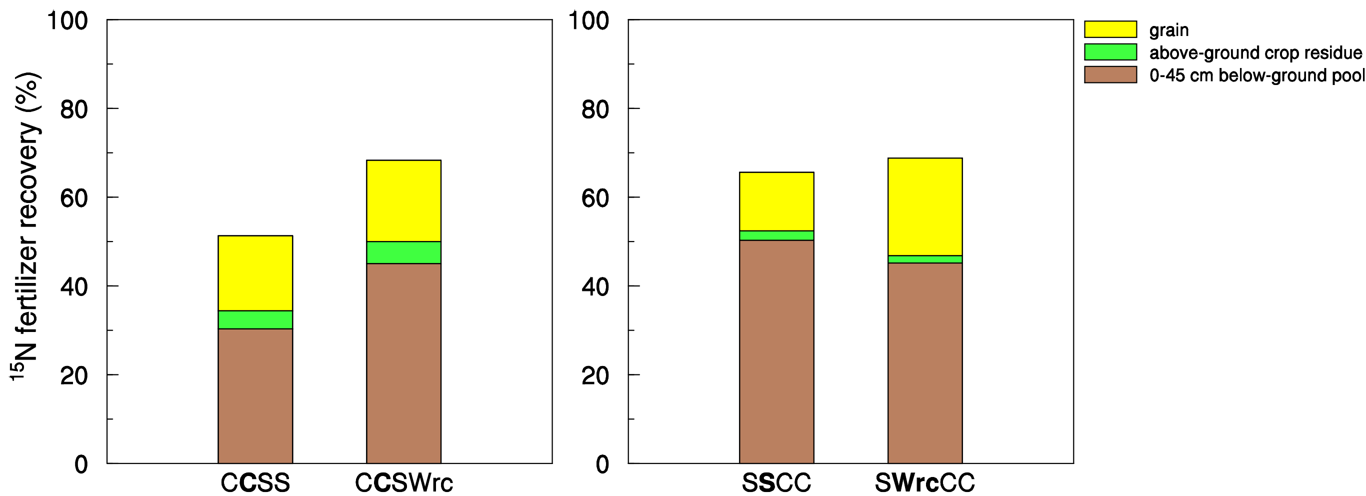

Figure 3.3. Recovery of applied ^{15}N-enriched urea in (A) the corn micro-plots (C**C**SS and C**C**SWrc legacy rotations) and (B) soybean or wheat micro-plots (S**S**CC and S**Wrc**CC, respectively). N in the below-ground pool was measure in the 0-45 cm profile. Bolded letters indicate crop phase at 2017 harvest.

Table 3.5. Average %N, %C, C:N ratio, and ^{15}N atom % excess (with standard error in parentheses) in the above-ground corn crop tissues at harvest in 2017, from C**C**SS and C**C**SWrc rotations under conventional (CT) and no-till (NT) tillage systems. Bolded crop rotation letters indicate the crop present in 2017.

Crop / Rotation	Plant tissue	Tillage practice	%N	%C	C:N ratio	^{15}N atom% excess
Corn C**C**SS	Grain	CT	1.10 (0.02)	42.4 (0.3)	38.5	0.3752 (0.1346)
	Grain	NT	1.10 (0.02)	42.2 (0.5)	38.4	0.3678 (0.1411)
	Residue	CT	0.39 (0.03)	45.0 (1.0)	115.4	0.4030 (0.1447)
	Residue	NT	0.37 (0.02)	43.9 (0.3)	118.7	0.3928 (0.1520)
Corn C**C**SWrc	Grain	CT	1.16 (0.02)	42.6 (0.3)	36.7	0.4090 (0.1516)
	Grain	NT	1.14 (0.03)	42.4 (0.3)	37.2	0.3596 (0.1400)
	Residue	CT	0.56 (0.09)	43.8 (0.4)	78.2	0.3269 (0.1350)
	Residue	NT	0.54 (0.09)	43.2 (0.8)	80.0	0.3694 (0.1228)

Table 3.6. Average %N, %C, C:N ratio, and ^{15}N atom % excess (with standard error in parentheses) in the above-ground corn crop tissues at harvest in 2017, from S**S**CC and S**Wrc**CC rotations under conventional (CT) and no-till (NT) tillage systems. Bolded crop rotation letters indicate the crop present in 2017.

Crop / Rotation	Plant tissue	Tillage practice	%N	%C	C:N ratio	^{15}N atom% excess
Soybean S**S**CC	Grain	CT	5.69 (0.31)	47.5 (2.7)	8.3	0.1919 (0.0403)
	Grain	NT	5.80 (0.07)	48.9 (0.7)	8.4	0.1642 (0.0364)
	Residue	CT	0.64 (0.06)	42.7 (0.3)	66.7	0.1869 (0.0767)
	Residue	NT	0.66 (0.06)	42.9 (0.3)	65.0	0.1664 (0.0674)
Wheat S**W**rcCC	Grain	CT	2.37 (0.05)	42.2 (0.3)	17.8	0.2279 (0.0870)
	Grain	NT	2.47 (0.06)	42.1 (0.4)	17.0	0.2206 (0.0851)
	Residue	CT	0.63 (0.03)	43.9 (0.4)	69.7	0.1453 (0.0577)
	Residue	NT	0.58 (0.04)	43.6 (0.4)	75.2	0.1483 (0.0640)
Red clover SW**rc**CC	Biomass	CT	2.02 (0.15)	42.7 (0.3)	21.1	0.0313 (0.0133)
	Biomass	NT	2.20 (0.12)	42.6 (0.5)	19.4	0.0215 (0.0088)

Table 3.7. Average %N, %C, C:N ratio, and ^{15}N atom % excess (with standard error in parentheses) in the below-ground crop tissues removed from intact soil cores at harvest 2017.

Crop / Rotation	**Plant tissue**	**Tillage practice**	**%N**	**%C**	**C:N ratio**	**^{15}N atom% excess**
Corn	Root	CT	0.48 (0.01)	37.6 (1.1)	78.3	0.9659 (0.0124)
C**C**SS	Root	NT	0.51 (0.01)	26.4 (1.6)	51.8	0.7864 (0.0182)
Corn	Root	CT	0.45 (0.03)	25.7 (1.2)	57.1	0.8161 (0.0084)
C**C**SWrc	Root	NT	0.47 (0.03)	25.3 (2.4)	53.8	0.8421 (0.0351)
Soybeans	Root	CT	0.31 (0.02)	10.5 (0.6)	33.9	0.4083 (0.0312)
S**S**CC	Root	NT	0.70 (0.11)	25.2 (6.9)	36.0	0.8720 (0.0215)
Wheat and red clover	Root	CT	0.99 (0.38)	30.6 (4.7)	30.9	0.2734 (0.3647)
S**Wrc**CC	Root	NT	1.10 (0.02)	27.2 (0.8)	24.7	0.0842 (0.0100)

Table 3.8. Average soil total %N, %C, and ^{15}N atom % excess (with standard error in parentheses), representing the residual soil N at harvest 2017 in ^{15}N enriched micro-plots from C**C**SS and C**C**SWrc rotations. Bolded crop rotation letters represent the harvested crop phase in 2017.

Crop/ Rotation	**Soil depth**	**Tillage**	**%N**	**%C**	**^{15}N atom% excess**
Corn C**C**SS	0-15 cm	CT	0.19 (0.01)	2.2 (0.1)	0.0196 (0.0047)
	15 – 30 cm	CT	0.13 (0.01)	1.6 (0.2)	0.0084 (0.0031)
	30 – 45 cm	CT	0.06 (0.01)	2.2 (0.6)	0.0087 (0.0017)
	0-15 cm	NT	0.18 (0.04)	2.1 (0.5)	0.0218 (0.0053)
	15 – 30 cm	NT	0.11 (0.04)	1.3 (0.4)	0.0111 (0.0030)
	30 – 45 cm	NT	0.05 (0.01)	1.7 (0.2)	0.0166 (0.0027)
Corn C**C**SWrc	0-15 cm	CT	0.21 (0.01)	2.3 (0.1)	0.0214 (0.0053)
	15 – 30 cm	CT	0.13 (0.01)	2.0 (0.4)	0.0127 (0.0028)
	30 – 45 cm	CT	0.08 (0.01)	1.6 (0.1)	0.0180 (0.0038)
	0-15 cm	NT	0.23 (0.02)	2.6 (0.2)	0.0310 (0.0053)
	15 – 30 cm	NT	0.12 (0.03)	1.5 (0.4)	0.0081 (0.0016)
	30 – 45 cm	NT	0.07 (0.01)	2.1 (0.7)	0.0384 (0.0068)

Table 3.9. Average soil total %N, %C, and ^{15}N atom % excess (with standard error in parentheses), representing the residual soil N at harvest in 2017 in ^{15}N enriched micro-plots from S**S**CC and S**Wrc**CC rotations. Bolded crop rotation letters represent the harvested crop phase in 2017.

Crop / Rotation	Soil depth	Tillage practice	%N	%C	^{15}N atom% excess
Soybean S**S**CC	0-15 cm	CT	0.20 (0.02)	2.2 (0.2)	0.0163 (0.0034)
	15 – 30 cm	CT	0.14 (0.03)	1.5 (0.3)	0.0061 (0.0018)
	30 – 45 cm	CT	0.08 (0.02)	1.1 (0.2)	0.0069 (0.0017)
	0-15 cm	NT	0.22 (0.02)	2.4 (0.2)	0.0175 (0.0026)
	15 – 30 cm	NT	0.16 (0.04)	1.6 (0.3)	0.0064 (0.0007)
	30 – 45 cm	NT	0.08 (0.02)	2.1 (0.5)	0.0126 (0.0059)
Wheat and red clover S**Wrc**CC	0-15 cm	CT	0.21 (0.02)	2.3 (0.1)	0.0147 (0.0013)
	15 – 30 cm	CT	0.12 (0.03)	1.6 (0.3)	0.0040 (0.0011)
	30 – 45 cm	CT	0.09 (0.03)	2.6 (0.7)	0.0093 (0.0017)
	0-15 cm	NT	0.24 (0.01)	2.7 (0.1)	0.0148 (0.0020)
	15 – 30 cm	NT	0.14 (0.03)	1.7 (0.3)	0.0048 (0.0013)
	30 – 45 cm	NT	0.08 (0.02)	2.0 (0.1)	0.0088 (0.0028)

3.3.3. Soil inorganic and total N dynamics

Soil inorganic N was concentrated in the top 0-15 cm of the soil profile at each sampling time, while relatively lower quantities were found in the deeper depth increments (Fig 3.4). In 2017, differences in soil inorganic N levels between the 'simple' C**C**SS and 'diverse' C**C**SWrc rotations were not observed at any depth increment in the corn plots (Fig. 3.4, leftmost panels). Prior to seeding the plots with soybean in 2018—and compared to the simple rotation—the 'diverse' rotation had higher inorganic N levels in the top 15 cm under both NT (P=0.041) and CT (P=0.086) (Fig. 3.4, middle panels), despite having received similar amounts of N fertilizer in the previous year. At soybean harvest in 2018, more inorganic N remained in the 15–30 cm depth for the diverse *vs*. simple rotation under NT only (P=0.022); no differences were observed for the other soil depth increments, or for the 0–45 cm profile (P=0.695 and P=0.273 for CT and NT, respectively) (Fig. 3.4, rightmost panels). Overall, these results provide evidence that the 'diverse' rotation increased soil inorganic N in the upper portion (0–15 or 15–30 cm bgs) of the soil profile during the transition from corn to soybean production, with a more pronounced effect under NT relative to CT.

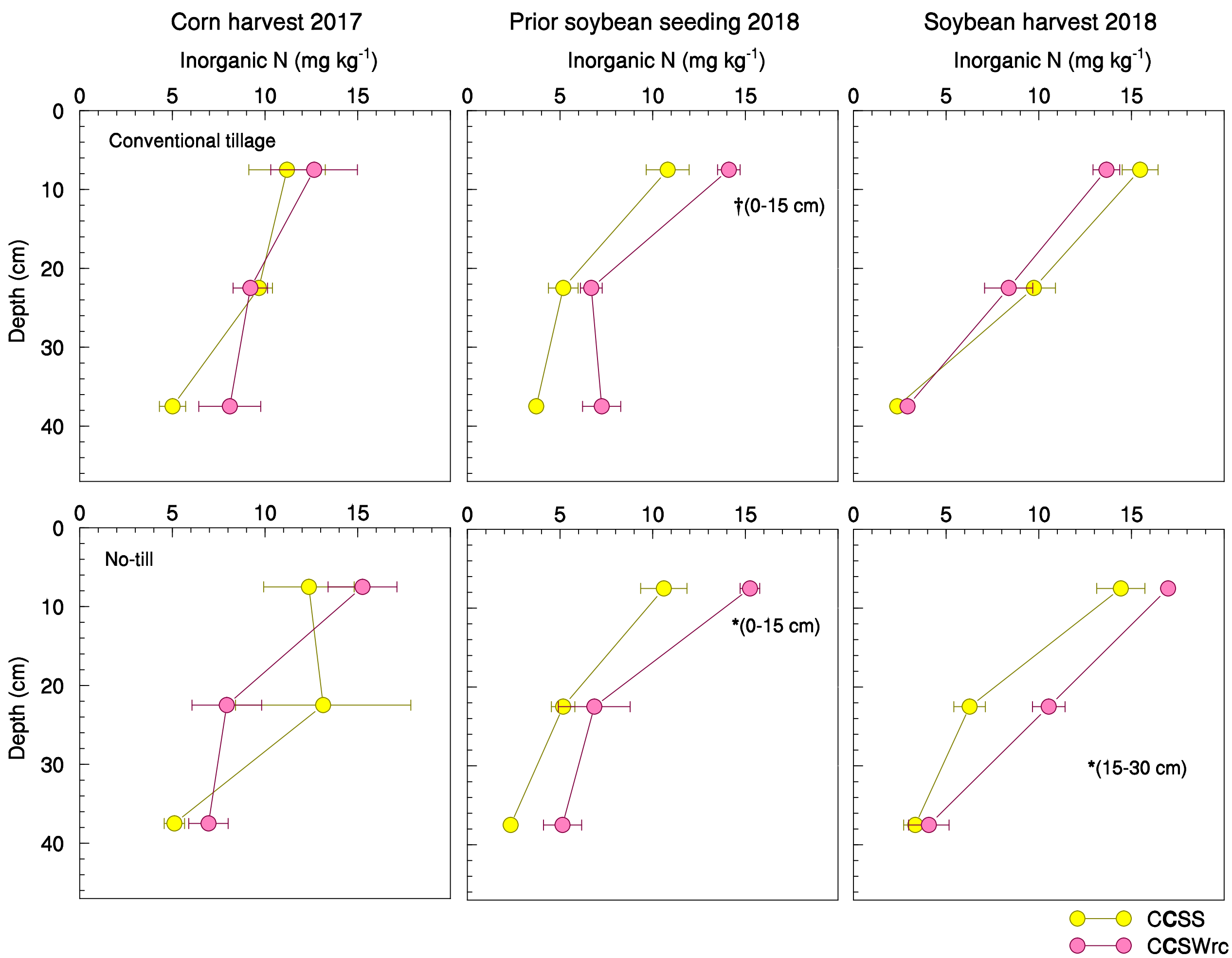

Figure 3.4. Soil inorganic N (sum of ammonium and nitrate levels) at 0-15, 15-30, and 30-45 cm depths, from C**C**SS or C**C**SWrc rotations, under conventional (CT) or no-till (NT) tillage systems. The bolded phase in the legend represents the crop grown in 2017. The * and † symbols denote statistical differences using Tukey-Kramer method at α = 0.05 and 0.10, respectively.

Total soil N levels tracked with those of the inorganic N; i.e., total N concentration in the 0–15 cm depth increment averaged 0.22% for the C**C**SWrc rotation, which was 13.6% higher than the average in the C**C**SS rotation (0.19%). However, for total soil N, the difference was not significant for either tillage system (P=0.487 and P=0.424 for CT and NT, respectively).

In the non-corn phase of the ‘simple’ *vs.* ‘diverse’ rotation (S**S**CC *vs*. S**Wrc**CC) in 2017, no differences were observed for soil inorganic N levels at harvest, regardless of tillage system (Fig. 3.5, leftmost panels). However, at corn planting in 2018, the diverse rotation had higher inorganic N levels in the 0–15 cm depth increment under CT (P=0.044) and NT (P=0.025) (Fig. 3.5, middle panels). This finding is similar to that observed for the CC**SS** *vs.* CC**S**Wrc comparison described above. By 2018 corn harvest, higher soil inorganic N remained in the diverse *vs*. simple rotation in the 0-15 cm (P=0.023) and 15-30 cm (P=0.003) depths under NT, but not under CT (Fig. 3.5, rightmost panels). This was explained by presence of actively growing red clover plants that persisted through to corn harvest under NT but not under CT.

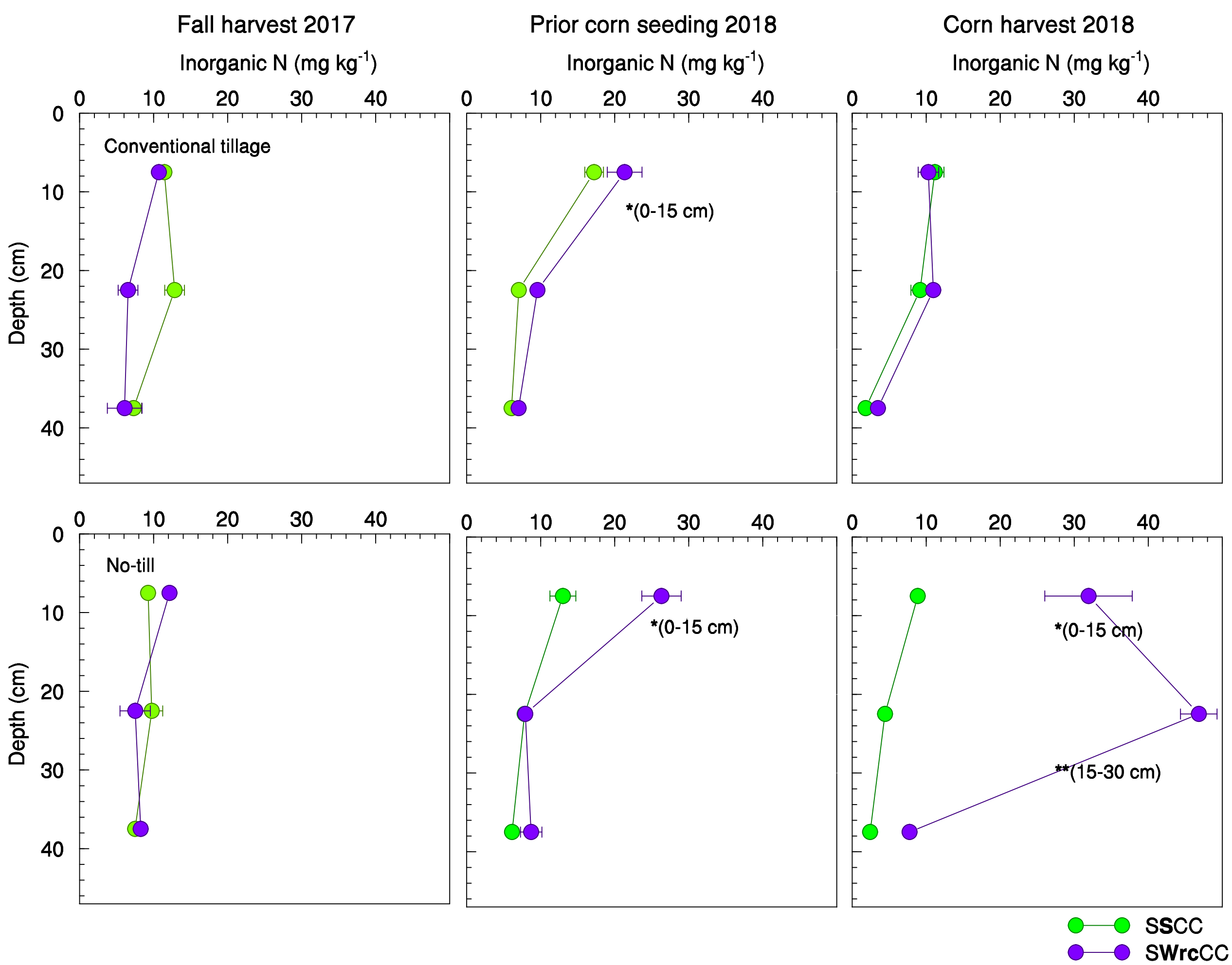

Figure 3.5. Soil inorganic N (sum of ammonium and nitrate levels) at 0-15, 15-30, and 30-45 cm depths, from S**S**CC or S**Wrc**CC rotations, under conventional (CT) or no-till (NT) tillage systems for samples collected at 2017 fall, 2018 spring, and 2018 fall. Bolded crop rotation phases represent the planted crop in

2017. Note: denotes statistical differences (Tukey-Kramer) at $\alpha = 0.05$ (*) and $\alpha = 0.01$ (**).

No difference in total soil N in the top 15 cm was found for the S**Wrc**CC (0.22%) and S**S**CC (0.21%) rotations (P=0.406).

Overall, the soil inorganic N results demonstrate that cropping system management produced a legacy of greater inorganic N availability in the surface soil of the ‘diverse’ rotation compared to ‘simple’ rotation—especially during key periods, such as at seeding—with a more pronounced difference under NT relative to CT. This indicates that the legacy of rotation and tillage management is producing changes in inorganic N availability when transitioning between distinct crop phases.

3.3.4. Soil organic carbon

The CC**S**Wrc rotation system had 12% more soil organic C in the top 15 cm relative to the CC**SS** rotation (P=0.004; Fig. 3.6), though there was no effect of tillage (P=0.622) or tillage × rotation interaction (P=0.314). Nor were differences found between the rotation systems in the sub-surface soils (15–30 cm bgs) when comparing these rotation systems.

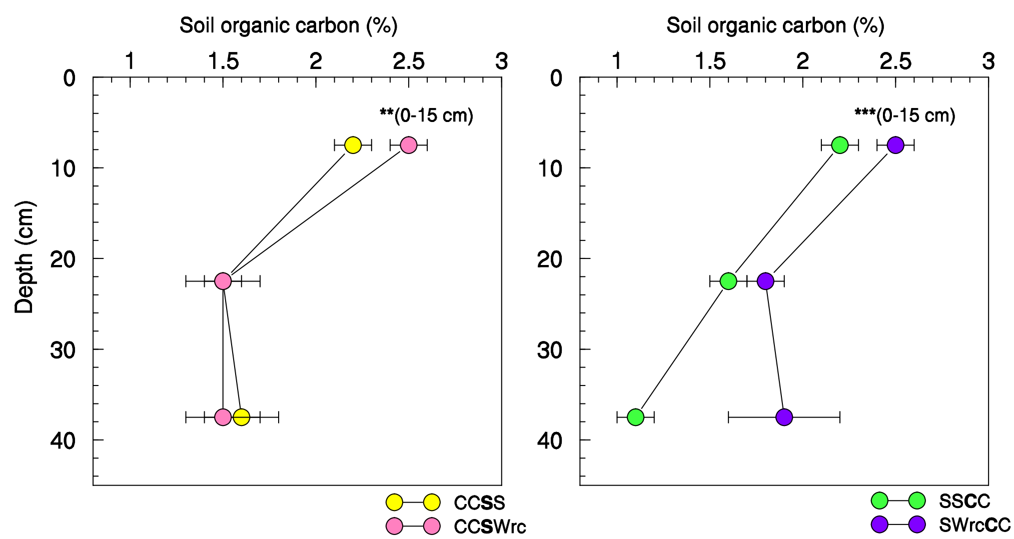

Figure 3.6. Soil organic carbon at 2018 harvest from CC**SS**, CC**S**Wrc, SS**C**C, and SWrc**C**C rotations with conventional and no-till tillage pooled data. Bolded crop rotations letters indicate crop rotation phase present in 2018.

The surface soil (0–15 cm) from the corn phase of the rotations exhibited a strong rotation effect (P=0.001), wherein the SWrc**C**C had 19.9% higher soil organic C levels than the SS**C**C (Fig. 3.6). For this depth increment, the tillage effect also was significant (P<0.001) where NT produced 11.7% more C soil levels than CT.

3.3.5. The use of nitrogen derived from crop residues and other sources by the subsequent crop

The ^{15}N tracer technique was used to track the fate of corn-residue N into 2018 soybean (i.e., CC**S**S and CC**S**Wrc), soybean-residue N into corn (i.e., SS**C**C), and winter wheat/red clover residue N into corn (i.e., SWrc**C**C). When a looking at the N allocated from these residues into the subsequent crops, there was no significant crop rotation effect (P>0.1) (Table 3.10). When looking at the tillage effect on residue N allocation to the subsequent crop, in the soybean phase NT only resulted in more N than CT for above-ground (P=0.021) and below-ground (P=0.033) contributions (Table 3.10), but lower contribution (P=0.016) from 'other' sources (fixed N or indigenous soil N).

Below-ground residual N contributed 3.9 to 10.8 times more N to subsequent crops than the above-ground crop residues, in all cases (Table 3.10). While ≤ 3.4% of grain N was attributed to the above-ground crop residue, between 6.2 and 27 % of grain N was sourced from the below-ground residual N pool (Table 3.10), indicating the relevance of below-ground amendments to subsequent crop N uptake. The percentage of grain N that was derived from fertilizer was less than 15.1%, while the majority of grain N came from other sources (indigenous soil N or fixed N) (Table 3.10).

Overall, 'diversifying' a corn-soybean rotation with wheat and red clover did not produce any effect on residue N uptake into the subsequent crop, while results show a clear tillage effect on subsequent crop N uptake when adding similar corn residue N amounts for above- and below-ground residues, where NT promoted corn residue N uptake compared to CT.

Table 3.10 Nitrogen derived from above- and below-ground residues (Ndfa and Ndfb, respectively), fertilizer (Ndff) and other sources (Ndfo, indigenous soil N or fixed N) in simple (SSCC and CCSS) and diversified (CCSWrc and SWrcCC) rotation systems under conventional (CT) and no-till (NT) tillage systems.

		Corn 2018				
Rotation	**Preceding Crop**	**Grain N ($g\ m^2$)**	***Ndfr*$_a$ (%)**	***Ndfr*$_b$ (%)**	***Ndff* (%)**	***Ndfo* (%)**
Simple (SS**C**C)	Soybean	9.6	3.4	23.0	13.8	59.8
Diverse (SWrc**C**C)	Wheat/red clover	11.4	2.5	27.0	12.8	55.8
		P = 0.272	*P* = 0.426	*P* = 0.974	*P* = 0.687	*P* = 0.842
Tillage practice						
No-till (NT)		10.2	2.8	24.3	12.6	60.4
Conventional (CT)		10.8	1.8	19.0	14.1	65.1
		P = 0.640	*P* = 0.357	*P* = 0.305	*P* =0.783	*P* = 0.414

		Soybean 2018				
Rotation	**Preceding Crop**	**Grain N ($g\ m^2$)**	***Ndfr*$_a$ (%)**	***Ndfr*$_b$ (%)**	***Ndff* (%)**	***Ndfo* (%)**
Simple (CC**S**S)	Corn	14.2	1.5	11.6	12.8	74.1
Diverse (CC**S**Wrc)	Corn	15.3	2.0	7.7	15.1	75.3
		P = 0.582	*P* = 0.309	*P* = 0.114	*P* = 0.280	*P* = 0.716
Tillage practice						
No-till (NT)		16.8 a	2.4 a	13.0 a	14.8	69.7 b
Conventional (CT)		12.2 b	1.0 b	6.2 b	13.0	79.8 a
		P = 0.006	***P = 0.021***	***P = 0.033***	*P = 0.185*	***P = 0.016***

The amounts of residual ^{15}N that remained in the soil were similar between the 'diverse' and 'simple' rotations at each depth increment for the soybean crop in 2018 (Fig 3.7), as well as the corn crop in 2018 (Fig 3.8).

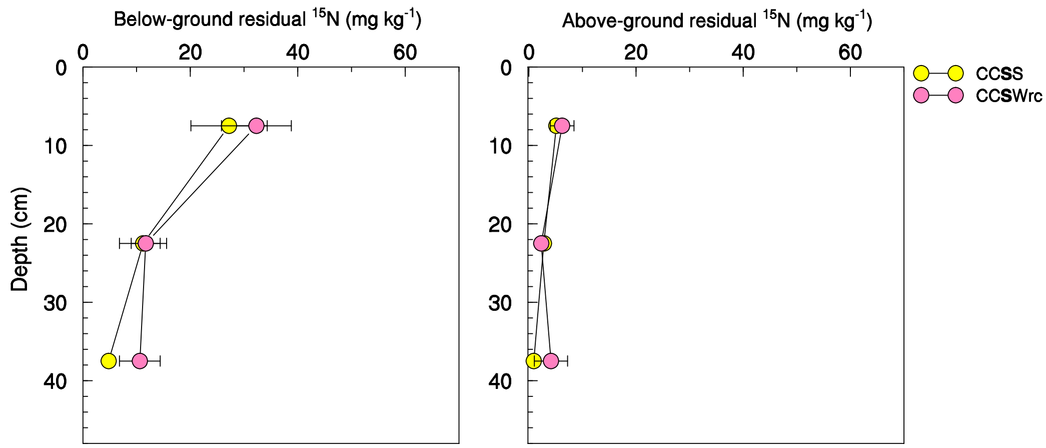

Figure 3.7. The amount of below- and above-ground residual ^{15}N (mg kg^{-1} soil) that remained in the soil at the 0–15, 15–30, and 30–45 cm depths, when soybean was harvested from the CC**S**S and CC**S**Wrc rotations in 2018. Bolded crop rotations letters indicate the rotation phase present in 2018.

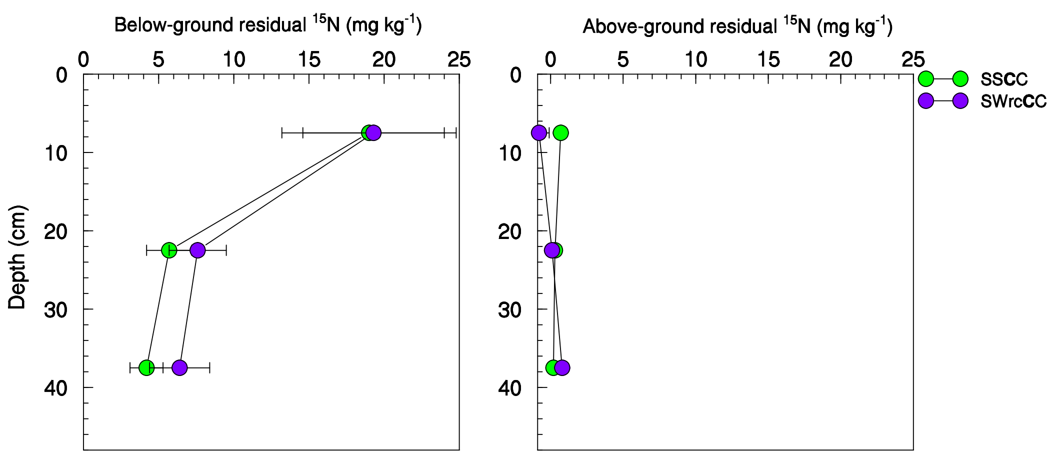

Figure 3.8. The amount of below- and above-ground residual ^{15}N (mg kg^{-1} soil) that remained in the soil at the 0-15, 15-30, and 30-45 cm depths, when corn was harvested from SS**C**C and SWrc**C**C rotations in 2018. Bolded crop rotations letters indicate crop rotation phase present in 2018.

3.4. DISCUSSION

3.4.1. Nitrogen cycling with the introduction of winter wheat and red clover in corn-soybean based systems

With the results from the previous section I can infer that corn-soybean rotations that included wheat with under seeded red clover i) produced greater overall yields, ii) had higher soil N availability at spring; iii) produced no effect on overall subsequent crop N uptake; when compared to ‘simple’ corn-soybean rotations. Crop yields in the ‘diversified’ rotation were greater than those in the ‘simple’ rotation, which supports previous findings from the same long-term trial (Gaudin et al. 2015). Likewise, in Iowa USA, Osterholz et al. (2018) found that yields in a similar diverse long-term corn-soybean rotation that included oats/red clover were greater than those in a simple corn-soybean rotation. Whereas Osterholz et al. (2018) originally hypothesized that yield gains in their diversified rotation were due to N cycling, it was also determined that yield gains were not related to soil inorganic N pool sizes or N availability in the short term. These findings parallel those of my study, which found little evidence to support the hypothesis that the ‘diversified’ system increased crop residue-N turnover—at least over the two-year duration of my study.

My results suggest that the indigenous soil N (resulting from the legacy of long-term management) may contribute to most of the subsequent crop N uptake in ‘simple’ and ‘diverse’ systems, rather than the added crop residue derived N from the immediate previous crop phase. I propose that the legacy of annual fertilizer N contributions is driving this effect, rather than the crop ‘diversification effect’ (Table 3.10). At the long-term Elora plots, the previous yield-based and crop NUE benefits shown by Gaudin et al. (2015) might be attributed to the build-up of soil indigenous N in the ‘diverse’ system (Gaudin et al. 2015).

Here, I explore mechanisms that may have contributed to the build-up of soil N and its enhanced availability for crop production in the ‘diversified’ rotation. One possible explanation is that there could be a distinct residue-N cycling mechanism in the ‘diverse’ rotation. My study also found that the soil inorganic N pools *increased* during key periods in rotation systems including wheat and red clover (Fig. 3.4 and Fig. 3.5), suggesting that it is more important to consider the temporal dynamics of crop residue-N mineralization, immobilization, and the accrual of soil N reserves. It is possible that residue-derived N may be immobilized at greater rates under ‘diverse’ rotations relative to ‘simple’ ones. In this way, more residue-derived N inputs in the ‘diverse’ systems could be transformed into soil organic N over the long-run and, in turn, could contribute to higher proportions of mineralizable-N during key periods such as during crop seeding, in the ‘diverse’ system. Support for this explanation is provided by higher soil organic C concentration in the surface soil (0–15 cm) in the ‘diverse’ rotation compared to the ‘simple’ one (Fig. 3.6). Similarly, other researchers concluded that a large portion of the crop N demand can be satisfied by inorganic N that is mineralized from soil organic matter (Murphy et al. 2017). For example, Osterholz et al. (2017) reported gross ammonification rates from soil organic N pools that were 3.4 to 4.5 times greater than corn N uptake.

Another possible explanation for increased soil N pools in the ‘diverse’ rotation is that during the last 10 years this rotation has received about 26% more fertilizer N than the ‘simple’ rotation (Table 3.4)—reflecting the additional N fertilizer applied during the winter wheat phase. In addition to the fertilizer N input, the red clover also supplies soil N via biological N-fixation. Indeed, red clover was shown to provide an average of 57 kg N ha^{-1} yr^{-1} when under-seeded in winter cereals (Schipanski and Drinkwater 2011) and, according to OMAFRA (2001), ‘plowdown’ red clover alone contributes 45 kg N ha^{-1}per year. It seems certain that the build-up of soil-N reserves in the ‘diverse’ rotation is a result of the greater N inputs.

Based on the results reported herein, it can be argued that N cycling and crop N use was regulated by the legacy of long-term management, though is unrealistic to claim that the *winter wheat and red clover* crop phase alone is influencing N cycling. It is most likely that the additional N inputs (N fertilization and the N fixation by the intercropped red clover) are driving the changes in N cycling and crop N utilization. It can also be inferred that the crop N demand is not satisfied by the above-ground N supplied by the immediately preceding crop, but rather by N provided from other pools; e.g., mineralization of the indigenous soil organic N—refuting my initial hypothesis. Regardless, it is clear that past management impacts how N is cycled and used by crops in the present-day.

3.4.2. Nitrogen cycling under no-till *vs.* conventional tillage

My results demonstrated that the amount of crop residue N (i.e., above- or below-ground residual N) utilized by the subsequent crop was greater for the NT system compared to the CT system (Table 3.10). This effect was most pronounced for the ‘diverse’ rotation and is likely resulted from a greater synchrony between N release from the crop residue and crop N uptake, what can be related to reduced N leaching as observed by Laine et al. (2018). Another possible explanation is that the crop residue in NT stayed closer to the root zone for subsequent crops, whereas in CT it might had slightly moved away from the root zone. A synergic combination of a 'diverse' rotation with NT also was identified by Gaudin et al. (2015), who suggested that this combination of practices can benefit nutrient demand and crop yields.

3.4.3. Contributions of above- versus below-ground residual N to subsequent crop

My results demonstrated that below-ground N contributions to subsequent crops were *higher* than above-ground crop residues, a finding which matches those of Arcand et al. (2014) where below-ground pea and canola residues contributed more than above-ground residues to a subsequent wheat crop. In their study, below-ground residues contributed at least twice as much as above-ground residues for N allocated to the subsequent crop. In the present study, below-ground residues contributed up to 10.8 times as much N as above-ground residues. This difference may be attributed to the different decomposition dynamics when comparing these residues types in the short term, due to the fact that below-ground residues are incorporated in the soil and are more susceptible for microbial mineralization. Together, these results demonstrate the importance of below-ground residues—including N rhizodeposition and root turnover during crop growth—to subsequent cropping systems (Arcand et al. 2014).

4. NITROUS OXIDE PRODUCTION AS INFLUENCED BY THE LEGACY OF CROP ROTATION AND TILLAGE SYSTEM

4.1. INTRODUCTION

Nitrous oxide is a potent greenhouse gas, with a global warming potential 298-times greater than that of CO_2 (IPCC 2007). Agricultural soils represent the largest source of anthropogenic N_2O emissions, contributing up to 66% of total N_2O emissions in Canada (Environment and Climate Change Canada 2019). Thus, there is a clear need to develop agricultural practices that reduce N_2O emissions.

Past research inferred that crop rotation 'diversification' (Meyer-Aurich et al. 2006) and NT (Wagner-riddle et al. 2007) have the potential to lower N_2O emissions from agricultural soils. Crop rotation diversification and NT could benefit the functioning of certain soil ecosystem services; e.g., by supporting a 'tighter' N cycle that is less susceptible to N_2O loss. For corn-soybean based rotations, it is possible that adopting NT, or including winter wheat and red clover in the rotation, can reduce N_2O production/emission. To test this, I conducted a soil incubation study aimed addressing the following hypotheses:

i. soil N cycling is regulated by the legacy of long-term crop rotation and tillage; and
ii. including winter wheat and red clover in a corn-soy based rotation will benefit key soil ecosystem services, such as, reduced potential N_2O production.

4.2. MATERIALS AND METHODS

4.2.1. Soil and plant sample collection and preparation

Soils from the same field trial and micro-plots described in Chapter 3 were used in an incubation study to examine the legacy effect of long-term crop management on soil-derived N_2O emissions. Soil under long-term conventional (CT) and no-till (NT) tillage systems were evaluated from the following long-term (37 year) crop rotations (with the 2017 crop phase bolded, when soils samples were extracted from the field): i) Corn-**corn**-soybean-soybean (C**C**SS), ii) Corn-**corn**-soybean-winter wheat under-seeded with red clover (C**C**SWrc), iii) Corn-corn-soybean-**soybean** (S**S**CC), and iv) Corn-corn-soybean-winter **wheat under-seeded with red clover** (S**Wrc**CC).

Three intact soil cores (15-cm in diameter by 10-cm deep) were collected from each micro-plot on October 16, 2017. For each ^{15}N natural abundance (A) and enriched (B) micro-plot, one soil core was collected within a crop row, one between rows, and another directly over a recently harvested plant. All soil cores were kept in a cooler while in the field, transported to a freezer and stored at -20°C while waiting to be shipped to the University of Saskatchewan (Saskatoon, SK) where they were kept frozen at -40°C until they were processed for analyses. On January 16, 2018 the soil cores were thawed over a 24-hour period at 22± 2°C, sieved (< 2 mm), and air dried for 48 h at 22 ± 2°C. All visible roots were carefully removed; fresh and dry root weights were recorded. For each selected crop phase, tillage, and rotation, the ^{15}N natural abundance soil cores were mixed to create a homogeneous composite sample representative of bulk soil in the proximity of the plant root. Nitrogen-15 enriched soils were used to extract enriched below-ground root material. Nitrogen-15 enriched and natural abundance plant materials were collected at harvest in 2017, oven dried at 65 °C for 48 hours, and ground to pass a 2-mm screen (see Chapter 3).

4.2.2. Incubation experiment

Soil microcosms were established by weighing 50 g of air-dry soil into 4.7 cm (i.d.) plastic dram vials, adjusting the gravimetric soil water content to 23% (equivalent to 70% water filled pore space) by adding 11.7 mL of deionized water, and mixing thoroughly. The soil microcosms were packed to a density of 1.4 g cm^{-3} to approximate bulk density in the field, covered with parafilm to allow gas exchange while preventing water loss, and pre-incubated for seven days at 22 ± 2°C. For each rotation/tillage combination, treatments were established by amending the soil with crop residues collected at harvest in 2017. The treatments were: (i) an unamended control, (ii) ^{15}N enriched above-ground crop residue, (iii) ^{15}N enriched below-ground crop residue, (iv) ^{15}N natural abundance above-ground crop residue, and (v) ^{15}N natural abundance below-ground crop residue. Above-ground crop residues were applied by mixing 1± 0.01 g of dried and ground plant material, with the winter wheat/red clover treatments receiving 0.5± 0.01 g wheat residue plus 0.5± 0.01 g red clover residue. The crop residues were gently moistened by adding 0.5 mL of deionized water before thoroughly mixing the residues with the soil inside the vial. The crop residues were first moistened to minimize their impact on the targeted soil moisture content (i.e., 70% water filled soil pore space). For below-ground crop residue treatments, 0.2± 0.01 g root material was applied in the same manner. Immediately after mixing, the soil microcosms were re-packed to 1.4 cm^{-3} bulk density, placed inside a 1L glass jar and flushed with 'ultra-zero air' (AI 0.0UZ), and immediately sealed with a lid that was fitted with a rubber septum for gas sampling. Deionized water (1 mL) was added to the base of each jar to ensure a high relative humidity and minimize soil water loss throughout the experiment. The jars were placed in a dark chamber at 22 ± 2°C and incubated for 14 days. Replicate (n = 4) microcosms were prepared for each treatment and were arranged in a completely random design inside the incubation chamber. In addition, the unamended control included an extra microcosm that was frozen and subsequently used as a destructive soil incubation start sample (i.e., at time-zero) and analyzed to quantify the initial soil mineral N content of the soils.

4.2.3. Gas sampling and analysis

Gas samples were collected from the headspace of the jars at 2, 6, 10, 24, 34, 52, 100, 196, 268, and 339 h after the start of the incubation. Syringes were flushed twice with 'ultra-zero air' (UZA) and filled with this gas prior to collecting 50 mL of gas sample (by removing one 20 mL sample followed by one 30 mL sample). To ensure that the introduced UZA and pre-existing air in the jar were well mixed, the syringe was pumped twice while inserted in the rubber septa. The additional air inserted into the jars (50 mL) was noted and accounted to correct gas concentration calculations. These gas samples (20 and 30 mL) were transferred with a syringe to two pre-evacuated 12 mL Exetainer® vials (absolute pressure of about 1-2 kPa) and time was recorded. After the gas samples were collected at 10, 34, 52, 100, 196, 268 and 339 h, each jar was flushed with UZA. For the earlier events (2 and 6 h) the jars were simply placed back in the dark chamber without flushing due to operational constraints. During all events the jars were checked to determine any loss of soil moisture; however, water additions were not necessary during the incubation. Exetainer® vials with 20 mL were used to measure N_2O, CO_2 and O_2 concentrations using a gas chromatograph (Scion 456-GC). The 30 mL samples were used to determine ^{15}N-N_2O concentrations via cavity ring down spectroscopy (i.e. using a Picarro G5131-*i* isotopic and gas concentration analyzer) which was attached to an automated arm sampler (OpenAutoSampler; custom-designed Arduino-based hardware). After the last gas sampling event, all microcosms were removed from the sealed jars and transferred to a freezer at -10°C where they were stored until they could be analysed to determine inorganic N, mineralized residue-^{15}N, and active C.

4.2.4. Soil inorganic N and residue ^{15}N mineralization analysis

Soils from the destructively sampled microcosms (i.e., collected on 'day zero' and 'day 14') were removed from the freezer, thawed at 22 ± 2°C for 4 h, and sub-sampled (~5 g) to determine inorganic N (Maynard et al. 2007). Briefly, soil inorganic N was extracted by adding 25 mL of 2M KCl solution to an Erlenmeyer flask, mixing it with the soil sub-sample. The soil:KCl suspensions were shaken for 30 min at 160 rpm, then filtered using Whatman filters No. 42, and stored at -10°C until they could be analysed. For analysis, the extracts were thawed and brought to room temperature, and a 1-mL aliquot analyzed to determine total extractable inorganic N based, adjusting calculations using soil moisture values. Concentrations of NH_4^+ and NO_3^- were determined colorimetrically using an air-segmented continuous flow analyser (SEAL AA3 HR chemistry analyzer; SEAL analytical, Kitchener, ON).

Concentrations of $^{15}NO_3^-$ and $^{15}NH_4^+$ at the start and end of the incubation were determined using an acid diffusion method adapted from Brooks et al. (1989). Briefly, a 3-mL aliquot of the KCl extract was mixed with 1M NaOH and 40 mg of Devarda's Alloy inside a 12-mL Exetainer® vial. The vial was sealed with rubber cap equipped with a metal hook from which a 0.8-mm diameter disk cut from Whatman No. 42 filter paper—and infused with 10 µL of 0.25 N $KHSO_4$—was suspended. The disks were collected after 24 h, encapsulated in aluminum tin capsules, and analysed using GC-IRMS. Rates of residue-^{15}N mineralization (Ndfr) were calculated using Equation 4.1.

$$Ndfr = \frac{Atom\%\ ^{15}N \text{ excess of inorganic N}}{Atom\%\ ^{15}N \text{ excess of amendment}} \times Inorganic\ N\ in\ microcosm \tag{4.1}$$

4.2.5. Soil ^{15}N abundances and active C analysis

Sub-samples of the soils from each incubation treatment (i.e., the composite soils) were analysed to determine the concentrations of total N and ^{15}N, total C and ^{13}C using GC-IRMS. Briefly, 10-g soil were powdered using a Retsch ball grinder (Mixer Mill MM 200, shaking at 25 Hz for 2 min), and a 1- to 3-mg sub-sample weighed into aluminum tin capsules.

For active C, soils from the microcosms destructively sampled on 'day 14' were removed from the freezer, thawed for 24 hours at 22 ± 2°C, thoroughly mixed and air-dried for 48 h at 22 ± 2°C, and active C determined using the method of Weil et al. (2003). Briefly, a 2.5 g sub-sample of the thawed soil was weighed into a 50-mL centrifuge tube containing 18 mL of deionized water and 2 mL of 0.2M potassium permanganate. The suspension was mixed for 2 min at 120 rpm and then allowed to settle for additional 10 min. A 0.5 mL aliquot of the supernatant solution was transferred to another 50-mL tube containing 49.5 mL of deionized water, sealed, and hand-shaken for 10 seconds. A 4-mL aliquot of the dilute extract was transferred to a transparent plastic cuvette that was then placed in a colorimeter (Halo SB-10 UV-VIS single beam spectrophotometer) to determine absorbance at 550 nm.

4.2.6. Incubation ^{15}N calculations

Nitrous oxide derived from the crop residues were determined using the Equations 4.2 and 4.3:

$$F_{N2O}dfr = \frac{N_2O{-}^{15}N\ atom\%\ excess}{crop\ residue\ ^{15}N\ atom\%\ exces} \times 100 \qquad (4.2)$$

$$T_{N2O}dfr = \frac{F_{N2O}dfr}{100} \times [N_2O]_T \qquad (4.3)$$

where F_{N2O} *dfr* is the proportion of above- or below-ground crop residue ^{15}N emitted as N_2O-^{15}N; T_{N2O} *dfr* is the total amount of N_2O derived from crop residue; and $[N_2O]_T$ is the total concentration of N_2O emitted from the residue-amended microcosm.

Total cumulative N-N_2O emissions were calculated by summing N-N_2O fluxes from each gas sampling event (10 events), which were obtained by the subtracting treatment N-N_2O flux by the respective control N-N_2O flux.

4.2.7. Statistical analysis

The cumulative N_2O and CO_2 data were subjected to analysis of variance and Tukey's multiple means testing using PROC MIXED in SAS (version 9.4), where significant effects were noted at $\alpha = 0.05$. Fixed effects were rotation (diversified *vs*. simple), tillage (NT *vs*. CT), incubation time, rotation × tillage, rotation × incubation time, tillage × incubation time, and rotation × tillage × incubation time. The random effect was replication.

For the inorganic N and active C data I used PROC MIXED and identified significant effects at $\alpha = 0.05$. Fixed effects were rotation, tillage, sampling time, rotation × tillage interaction, rotation × sampling time, tillage × sampling time, and rotation × tillage × sampling time. This analysis assumes that the residuals are normally distributed, which was verified using the Shapiro-Wilk test (Shapiro and Wilk 1965).

4.3. RESULTS

4.3.1. Soil N_2O and CO_2 production from a rotation legacy of CCSS and CCSWrc

Here I present the data for cumulative total N_2O and CO_2 emissions from soils amended with corn residues (stover or roots) from the simple (C**C**SS) and diversified (C**C**SWrc) rotations. In general, cumulative N_2O emissions peaked during the first 48 hours after corn stover or root was mixed with the soil, whereas cumulative CO_2 emissions did not peak until 14 days after the residues were amended to the soil.

For microcosms that received above-ground corn crop residues, cumulative N_2O emissions exhibited a significant rotation effect (P=0.054) but no tillage effect (P=0.606). The C**C**SWrc rotation resulted in 77% greater N_2O emissions compared to C**C**SS, regardless of tillage system (P=0.485) (Fig. 4.1). Cumulative soil CO_2 production largely paralleled that of N_2O, with 22% greater production from the C**C**SWrc compared to the C**C**SS (P=0.031). In the case of CO_2, however, there was a weak impact of tillage (P=0.080), but no tillage × rotation interaction (P=0.244) (Fig. 4.2).

When corn roots were applied to soils, cumulative N_2O production was not influenced by rotation (P=0.218), tillage (P=0.161), or tillage by rotation (P=0.326) (Fig. 4.1). For cumulative CO_2 emissions, however, there was a significant crop rotation effect (P=0.010), where the C**C**SWrc soil produced 39% greater cumulative CO_2 than the C**C**SS soil (Fig. 4.2). Unlike with the corn stover additions, however, there was no significant tillage (P=0.666) or tillage × rotation interaction (P=0.522) effect on cumulative CO_2 (Fig 4.2).

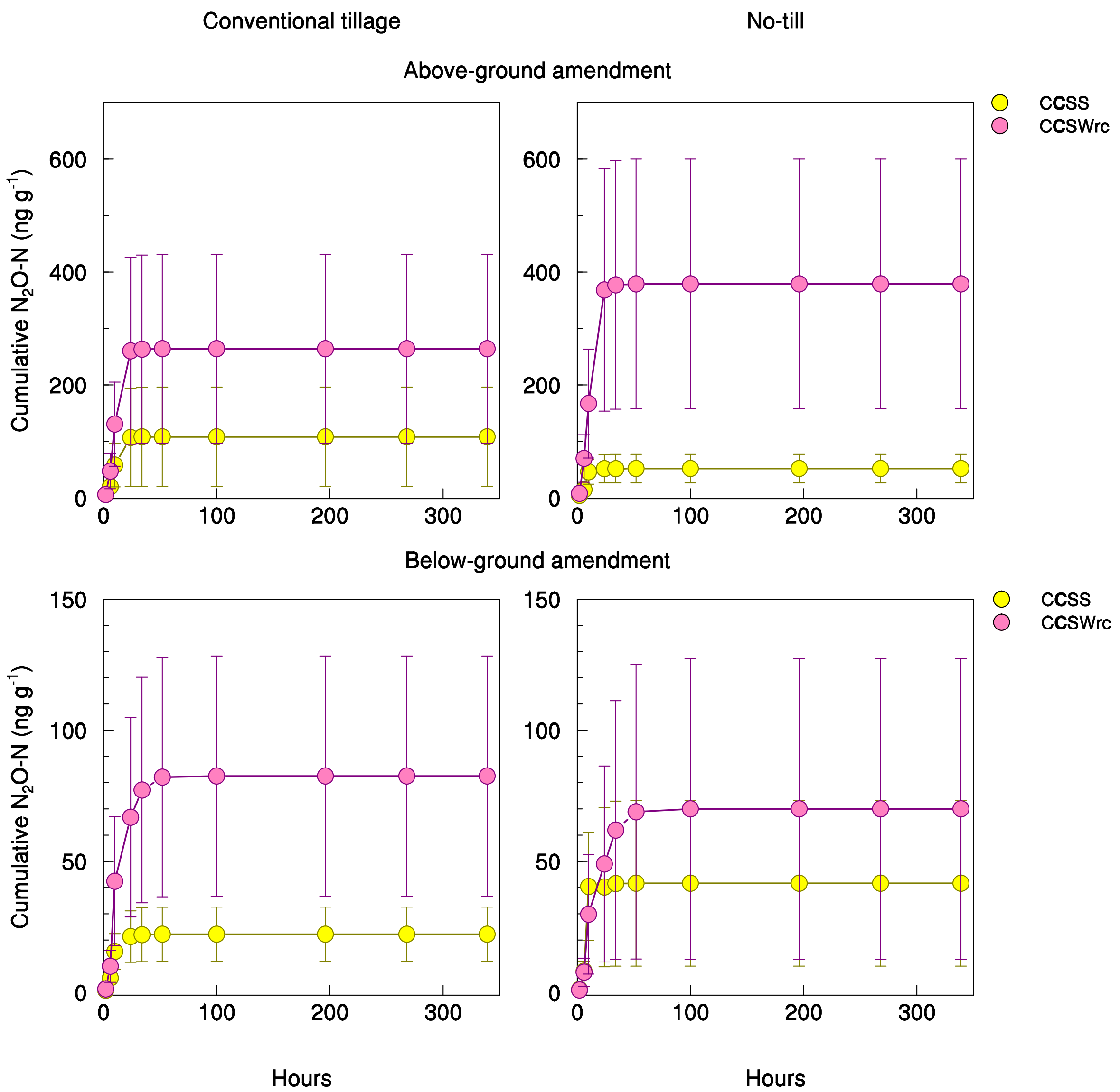

Figure 4.1. Total N_2O-N cumulative emissions during the entire incubation length, after above- (stover) and below-ground corn residue (root) was amended to microcosms containing soils that had a rotation legacy of C**C**SS or C**C**SWrc under conventional or no-till tillage systems. Bolded letters indicate the crop phase present at 2017 soil sample collection.

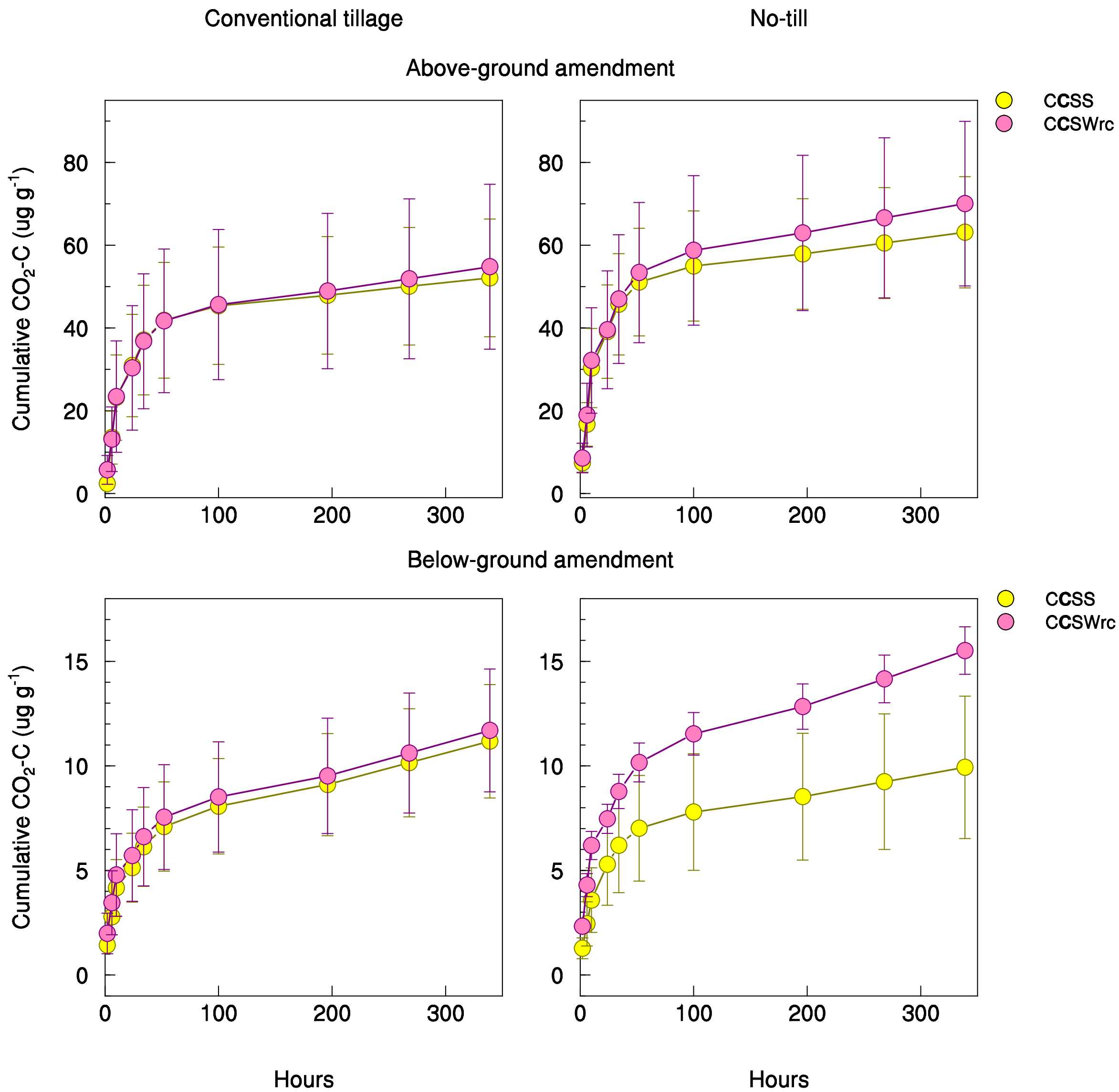

Figure 4.2. Total CO_2-C cumulative emissions during the entire incubation length, after above- (stover) and below-ground corn residue (root) was amended to microcosms containing soils that had a rotation legacy of C**C**SS or C**C**SWrc under conventional or no-till tillage systems. Bolded letters indicate the crop phase present at 2017 soil sample collection.

4.3.2. Soil N_2O and CO_2 production from a rotation legacy SSCC and SWrcCC

For the non-corn phase (S**S**CC and S**Wrc**CC), cumulative total N_2O emissions increased rapidly during the first few hours of the incubation but then plateaued within about 48 to 100 hours of incubation (Fig. 4.3). CO_2 emissions were greater during the same period (100 hours) and after that increased with reduced increments. As a result, statistical comparisons are focused the cumulative emissions after the 100-hours sampling event.

When above-ground crop residues were added to the soil, there was no rotation effect on cumulative N_2O emissions (P=0.191); nor was there a tillage (P=0.652) or rotation × tillage interaction (P=0.768) effect. For cumulative CO_2 emissions, however, the S**Wrc**CC soil produced 2-times more CO_2 than the S**S**CC soil (P<0.001)—though there was no tillage effect (P=0.484) (Fig. 4.4).

The addition of below-ground crop residues to the soils yielded a strong rotation effect on cumulative N_2O production (P<0.001), wherein the S**Wrc**CC soil produced 22-times more N_2O than the S**S**CC soil (Fig. 4.3). Similarly, for cumulative CO_2 emissions, the S**Wrc**CC soil produced 2.4-times more CO_2 than the S**S**CC soil (P<0.001). Conversely, total cumulative N_2O and CO_2 emissions from soils amended with below-ground crop residues were unaffected by tillage (P=0.912 and P=0.6783, respectively), and there was no rotation × tillage effect (P>0.1).

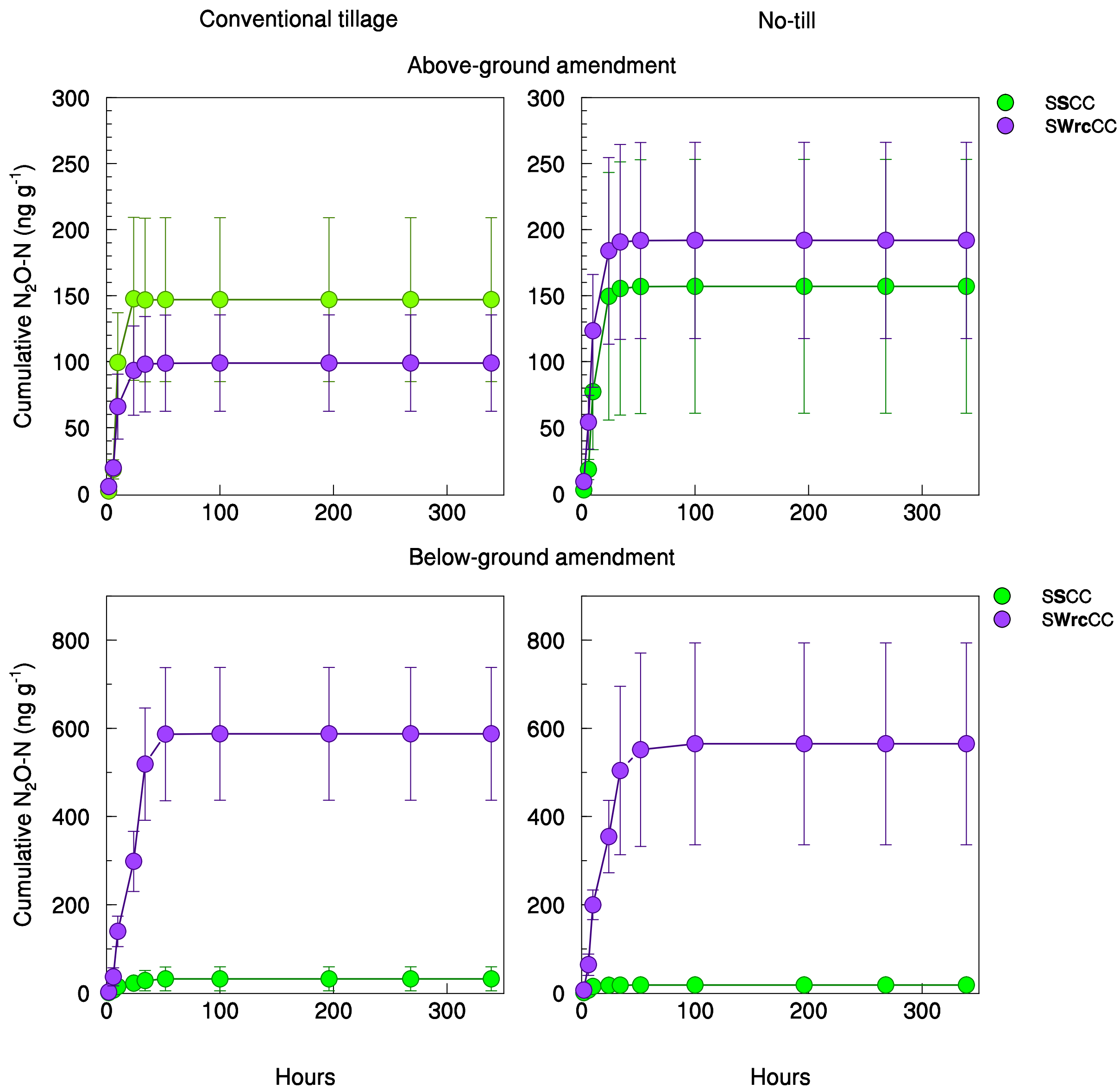

Figure 4.3. Total N_2O-N cumulative emissions during the entire incubation length, after soy or wheat with red clover roots were amended to microcosms containing soils that had a rotation legacy of S**S**CC or S**Wrc**CC under conventional or no-till tillage systems. Bolded crop rotation letters indicate the crop rotation phase at 2017 harvest.

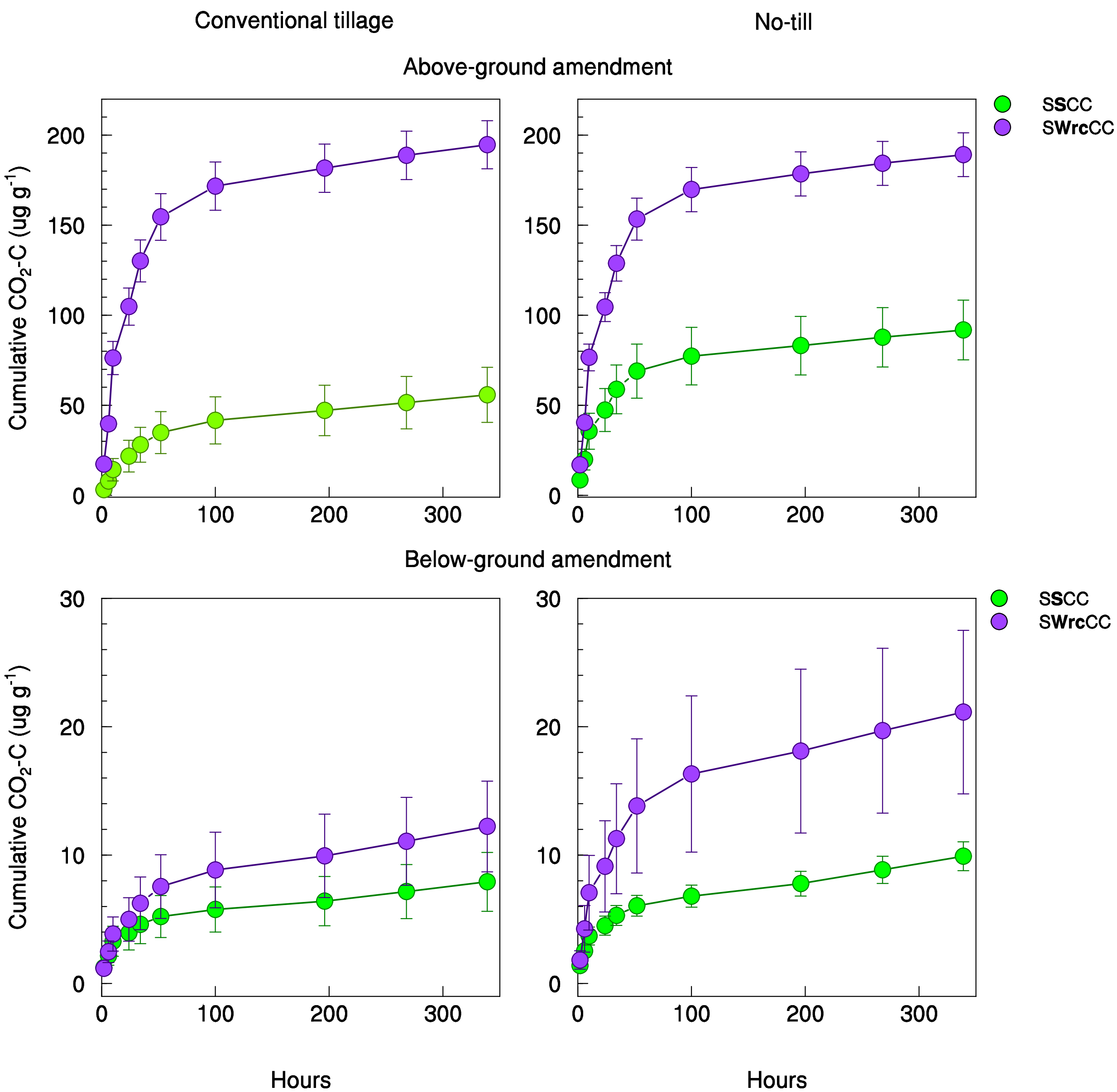

Figure 4.4. Cumulative CO_2-C emissions during the entire incubation period after soy or wheat with red clover roots were amended to microcosms containing soils that had a rotation legacy of S**S**CC or S**Wrc**CC under conventional or no-till tillage systems. Bolded crop rotation letters indicate the crop rotation phase at 2017 harvest.

4.3.3. The source of N_2O production

The presence of the ^{15}N label in the crop residues allowed me to identify the source of any N_2O-^{15}N and quantify the total amount of residue-N lost as N_2O (see Section 4.2.6). For soils amended with the corn crop residues, most of the N_2O was derived from the corn material (Table 4.1). However, when soybean or winter wheat residues were amended to the soils, most of the N_2O was derived from the soil N (Table 4.1).

Table 4.1. Average percent N_2O-N derived from (Ndf) crop residues or soil after 14 incubation days.

	Ndf-aboveground residue (%)	**Ndf-soil (%)**
Corn residue amendments	45.50%	54.50%
Soybean or Winter wheat/red clover amendments	1.70%	98.30%
	Ndf-belowground residue (%)	**Ndf-soil (%)**
Corn residue amendments	2.60%	97.40%
Soybean or Winter wheat/red clover amendments	8.90%	91.70%

4.3.4. Soil inorganic N dynamics and N mineralization from crop residues

The initial levels of soil inorganic N were 74% higher in the C**C**SWrc soil compared to the C**C**SS soil ($P<0.001$), but it did not differ based on tillage system legacy ($P=0.202$) (Fig. 4.5). By the end of the 14-days incubation, soil inorganic N availability had declined in all treatments; however, these declines were greater in soils with corn stover amendments compared to root amendments—with the largest decline occurring in the C**C**SWrc soil (Fig. 4.5). These results help explain the N_2O emissions patterns shown in the previous section, where overall higher N_2O emissions resulted from the C**C**SWrc soils amended with above-ground crop residues.

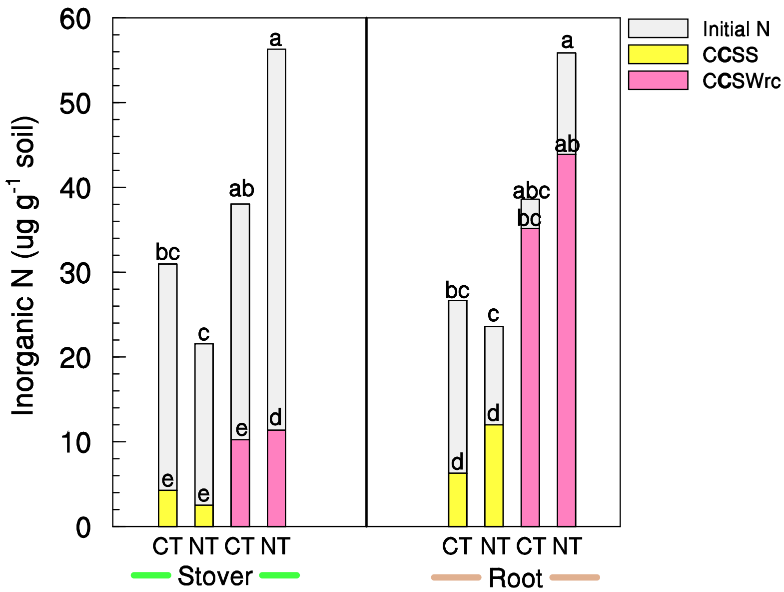

Figure 4.5. Soil inorganic N levels at incubation start (time 0 day – gray bars) and end times (time 14 days – coloured bars) for the C**C**SS and C**C**SWrc rotation legacies under conventional (CT) and no-till (NT) systems after amending above- and below-ground crop residues. Different letters denote statistical differences at alpha=0.05 using Tukey-Kramer method, where incubation initial and end inorganic N values were compared. Bolded crop rotation letters indicate the crop rotation phase at 2017 harvest.

For the non-corn phase of the rotations, the initial levels of soil inorganic N were 43% higher for S**Wrc**CC *vs.* S**S**CC (P<0.001) (Fig. 4.7). Inorganic N levels also were higher in all NT systems compared to CT systems (Fig. 4.6). By the end of the 14-days incubation, inorganic N levels had declined in all treatments except for when soybean roots were added to the S**S**CC soils (Fig. 4.6).

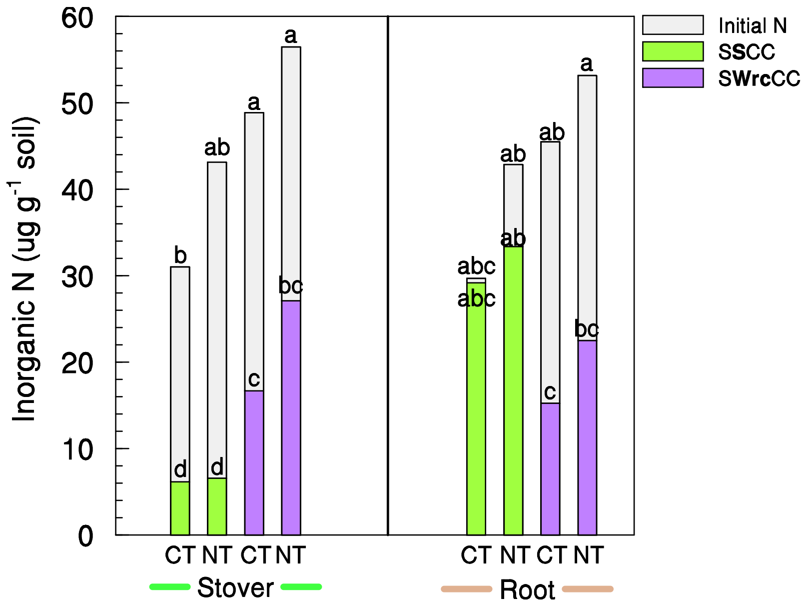

Figure 4.6. Incubation inorganic N levels at incubation start (time 0 day – gray bars) and end times (time 14 days – coloured bars) for S**S**CC, S**Wrc**CC rotations under conventional (CT) and no-till (NT) systems after amending above- and below-ground residues. Different letters denote statistical differences at alpha=0.05 using Tukey-Kramer method, where incubation initial and end inorganic N values were compared. Bolded crop rotation letters indicate the crop rotation phase at 2017 harvest.

The addition of ^{15}N-labeled crop residues to ^{15}N natural abundance soils (average 0.3686 atom% ^{15}N), allowed me to measure mineralization of the crop residue N (Fig. 4.7 and 4.8). The mineralization of N from corn stover and roots was 5- and 6-fold greater for the C**C**SWrc compared to C**C**SS rotation soils, respectively (Fig. 4.7). The rotation effect was significant for the root comparison (P=0.006) but not for stover (P=0.171). The tillage effect was not significant for soils amended with either the above- (P=0.596) or below-ground (P=0.837) residues (Fig. 4.7). Higher above- and below-ground mineralization rates in C**C**SWrc *vs.* C**C**SS can be linked to the previously described N_2O-N results where I found greater emissions from corn stover applied to soils from the C**C**SWrc rotation relative to soils from the C**C**SS rotation.

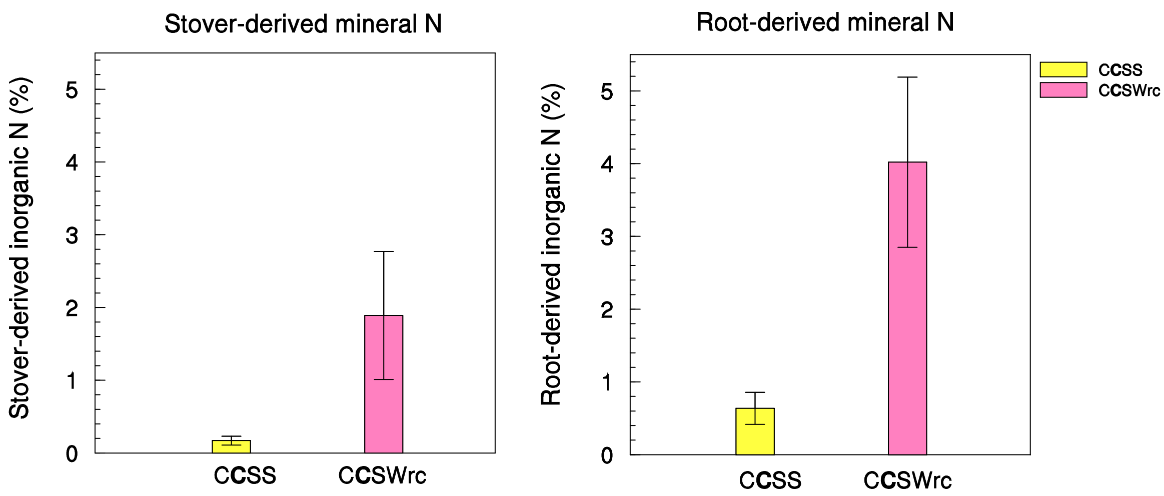

Figure 4.7. Mineralized-N from above- and below-ground residues (corn crops) in microcosms containing legacy soils from C**C**SS and C**C**SWrc rotations under conventional (CT) or no-till (NT) tillage systems after 14 days of incubation. Bolded rotation phases indicate the legacy soil crop phase in 2017 that was used in our incubation study.

In the S**S**CC *vs.* S**Wrc**CC rotation comparison, I found a nearly 5-fold increase in stover derived-N mineralization from the S**Wrc**CC soils relative to S**S**CC soils (P=0.045) (Fig. 4.8). In these soils, the tillage effect was not significant (P=0.534). However, when roots were added to the soils, the opposite result occurred; i.e., there was about a 4-fold increase in root-N mineralization in the 'simple' S**S**CC rotation soil relative to S**Wrc**CC soil (P=0.006). In fact, the root biomass added to the S**Wrc**CC soils resulted in a negative net N mineralizationFor these microcosms, I did not find a significant tillage effect on residue-N mineralization rates (P=0.742), (Fig. 4.8).

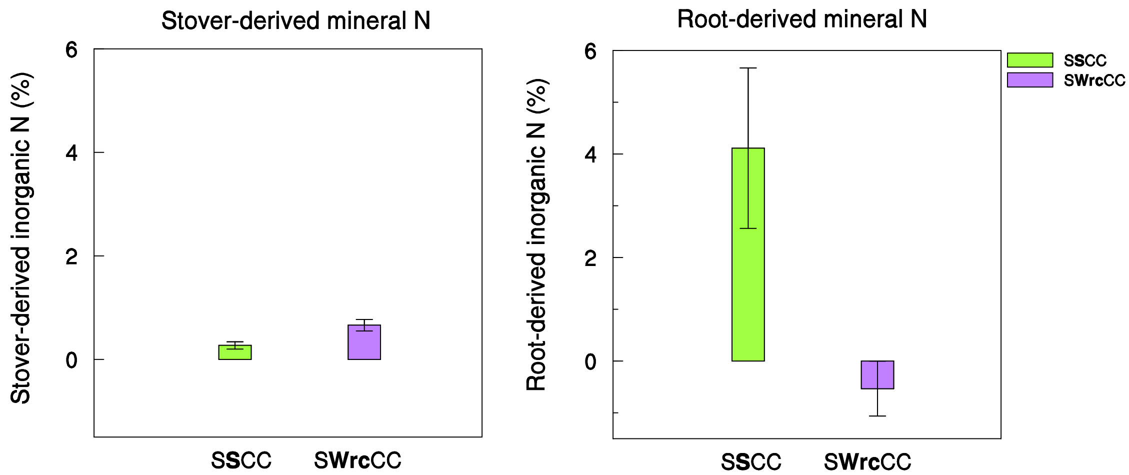

Figure 4.8. Mineralized-N from above- and below-ground residues (soybean or wheat with red clover crops) in microcosms containing legacy soils from S**S**CC and S**Wrc**CC rotations under conventional (CT) or no-till (NT) tillage systems after 14 days of incubation. Bolded rotation phases indicate the legacy soil crop phase in 2017 that was used in the incubation study.

4.3.5. Soil active C dynamics

Soil active C levels were 76% higher in the C**C**SS rotation soils *vs*. the C**C**SWrc soils (P=0.006), but differences between tillage systems were not significant (P=0.0927) and there was no interaction (P=0.263) (Fig. 4.9). Interestingly these results are the opposite compared to those found for inorganic N presented in the preceding section. In the S**S**CC *vs*. S**Wrc**CC rotation comparison, neither the rotation or tillage system legacy (nor their interaction) influenced soil active C levels (P=0.261, P=0.684, and P=0.531, respectively) (Fig. 4.10).

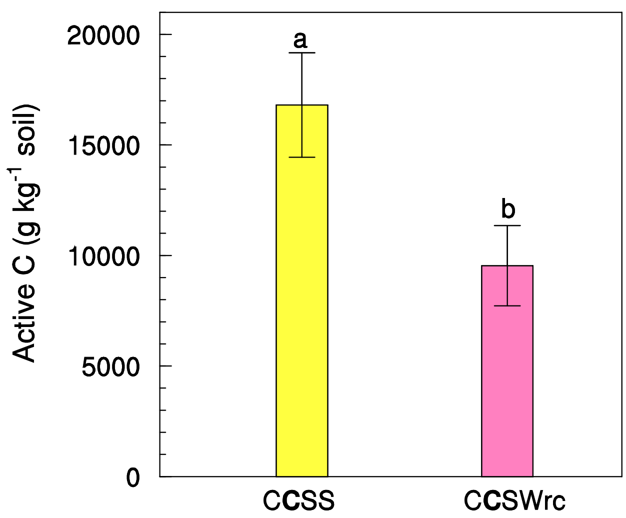

Figure 4.9. Active C after 14 days of incubation in soils that were collected from rotation legacies of C**C**SS and C**C**SWrc. Different letters denote statistical differences at α=0.05 using Tukey-Kramer method. Bolded crop rotation letters indicate the crop rotation phase at 2017 harvest.

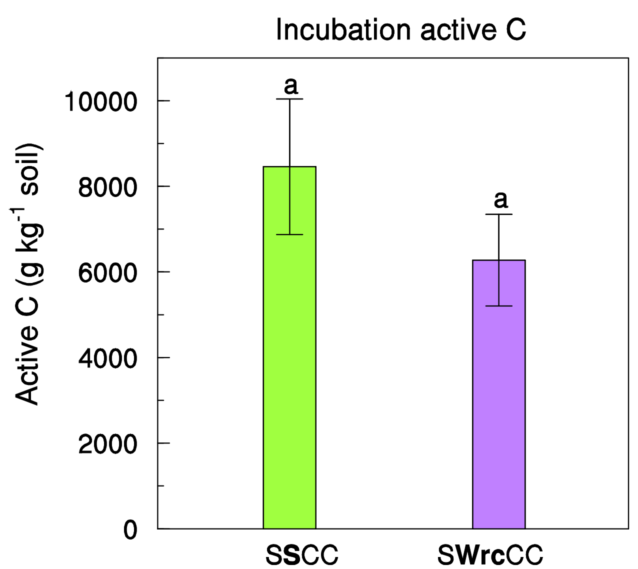

Figure 4.10. Active C after 14 days of incubation in soils that were collected from rotation legacies of S**S**CC and S**Wrc**CC rotations. Different letters denote statistical differences at α=0.05 using Tukey-Kramer method. Bolded crop rotation letters indicate the crop rotation phase at 2017 harvest.

4.4. DISCUSSION

4.4.1. The effect of crop rotation on residue dynamics and N_2O and CO_2 emissions

Promoting crop rotation 'diversification' as a BMP to reduce GHG emissions was suggested by Meyer-Aurich et al. (2006), who made their assumptions based on soil carbon stocks from the same long-term trial analyzed in this study. However, the N_2O and CO_2 results based on my incubation study does not support this claim. In contrast to Meyer-Aurich et al. (2006), my results demonstrate higher levels of N_2O and CO_2 production in soils derived from a 'diverse' rotation, compared to a 'simple' rotation.

Interestingly, my findings suggest that the crop rotation legacy influences N_2O production. For the corn phase of a long-term C**C**SWrc *vs.* C**C**SS rotation, the 'diverse' system produced more i) corn stover-derived N_2O-N and ii) more soil residue induced N_2O-N, than that from the 'simple' rotation. This result is intriguing because the same quantity of corn stover was added to the soils from either rotation, implying that rotation legacy has a role in regulating N_2O production. My results suggest that crop N inputs are processed differently, depending on the long-term management, or that the 'legacy of crop diversification' regulates N cycling—thus supporting my first hypothesis. These changes are likely related to greater crop residue N mineralization in the 'diverse' systems, which (in turn) may be explained by the increased soil N (Deng and Tabatabai 2000). Possible explanations for increased soil N availability in 'diverse' systems include (i) increased crop residue N mineralization-immobilization rates; (ii) greater soil organic matter stocks that improve the capacity for soil nutrient storage; (iii) additional N fertilizer applied to wheat and red clover phase (over 135 kg N ha^{-1} compared to the soybeans phase); and (iv) additional N inputs due to red clover N fixation, possibly adding in average 57 kg N ha^{-1} yr^{-1} when intercropped with a cereal crop (Schipanski and Drinkwater 2011). It is important to note that in most of the N_2O-N standard error values were high, and that could be attributed to the extremely sensitive behaviour of N_2O emissions to differences on soil compaction when creating the soil microcosms, hence slightly changing the water filled pore space contents between microcosm repetitions.

Based on the results presented in this Chapter, it is important to point out that even though 'diverse' rotations may provide beneficial ecosystem services (Gaudin et al. 2015), the disservices related to GHG production should not be ignored. To follow-up on my lab-scale study, it is recommended that GHG production be investigated *in situ* and at a field-scale. One possible strategy to reduce GHG from ‘diverse’ systems would be to reduce N fertilizer based on soil-tests and to better account for N credits supplied by the red clover or other legumes in diversified rotations (Meyer-Aurich et al. 2006).

4.4.2. The effect of tillage on residue dynamics and N_2O and CO_2 emissions

Similar to earlier results from Meyer-Aurich et al. (2006), who used carbon stocks measurements to estimate GHG emissions from tillage systems, my results suggest that the tillage effect on N_2O and CO_2 production is minimal. However, more detailed GHG studies have demonstrated N_2O reduction when NT is combined with other BMPs (Wagner-Riddle et al. 2007). In my study, the soil preparation procedure used to setup the incubation experiment (air drying, homogenization, pre-incubation, and amendment mixing) may have masked N_2O production differences between NT *vs.* CT, due to the changes in soil physical aspects or a shift in microbial communities.

5. GENERAL DISCUSSION AND CONCLUSIONS

Diversifying annual grain cropping systems by including cereals, cover crops or overwintering crops is one strategy to mimic the structure of natural ecosystems, and may contribute to improving soil ecosystem services (Scherr and McNeely 2008). Various studies have demonstrated that diversifying crop rotations results in higher crop yields (Hauggaard-Nielsen et al. 2012) and crop N use efficiency (Smith et al. 2008; Gaudin et al. 2015a), higher tolerance to drought conditions (Gaudin et al. 2015b), improved yield stability (Gaudin et al. 2015b), lower requirements for fertilizer and pesticides (Smith et al. 2008), higher net returns (Meyer-Aurich et al. 2006), improved soil organic matter and microbial activity (Tiemann et al. 2015), and reduced N_2O loss and NO_3^- leaching (Pappa et al. 2011). Although it has been postulated that the benefits of a diversified crop rotation are due to its influence on soil nutrient supply and organic matter (Gaudin et al. 2015b), the underlying mechanisms are largely unknown.

In eastern Canada, the inclusion of winter wheat and red clover in corn-soybean based rotations has been proposed as a method of diversifying these rotations, and is associated with benefits such as improved corn yields, NUE, and soil health (Gaudin et al. 2015a; Gaudin et al. 2015b; Congreves et al. 2017). In the present study, my focus was on understanding how N cycling changes when long-term corn and soybean rotations are 'diversified' by including winter wheat and red clover. To do so, I used ^{15}N tracer techniques to look closely at the N cycling changes over a 2-yr period in the field (Chapter 3) and in incubated soils in the lab (Chapter 4). The two chapters complement one another in the sense that the same soils and residues from the field study were used in the lab-scale incubation. Based on my field research, numerically higher yields were observed for the 'diversified' (CCSWrc or SWrcCC) rotation, compared to the 'simple' (CCSS or SSCC) rotation, which supports past research findings. I originally hypothesized that this yield benefit was related to differences in soil N cycling, with diversified systems providing greater crop residue turnover and N supply to the subsequent crop. However, my research findings demonstrate a more nuanced effect taking place and, in general, do not support this hypothesis (Chapter 3). However, in the lab-scale soil incubation study (Chapter 4) I found that more residue N was mineralized from above-ground crop residue applied to soils from the 'diverse' rotation relative to the 'simple' rotation.

One explanation for my findings may be related to asynchrony between above-ground crop residue-N mineralization and crop N uptake in the field. Further, one must consider that N availability is highly dynamic and influenced by N losses (i.e., the wet spring likely increased the risk of leaching; overwinter freeze-thaw events probably induced N_2O emissions) as well as immobilization/mineralization dynamics. Regardless, the question remains: what is driving the previously documented benefits of diversified rotations? Since I was not able to find greater N levels from the immediate subsequent crop in ‘diverse’ rotations relative to ‘simple’ ones, an alternative hypothesis is that more indigenous soil-N or residue induced N or fixed-N is utilized by crops in diversified rotations, as evidenced by the soybean grain N utilization results in 2018 (Chapter 3), possibly explaining greater N levels and consequently greater crop yields in ‘diverse’ systems. This notion should be explored with future research, and I recommend partitioning the role of fertilizer-N from fixed-N.

Based on my field research (Chapter 3), I observed key differences in the fate of above- *vs.* below-ground residual N, where the below-ground residual N was utilized by the subsequent crop to a much greater degree (up to 10 times more for grain comparisons) than the above-ground crop residue-N, perhaps due a higher C:N ratio observed in the above-ground relative to below-ground residues. This information is important because it points towards the importance of below-ground N pools in building the soil N reserves and supply. Future research should then focus on identifying N turnover characteristics from below-ground pools over a longer period of time, what may help unveil the importance of pre-existing soil N and its interaction with amended plant residue.

It is possible that the legacy of accumulated annual N fertilizer applications over the long-term cropping history at this site is responsible for the soil N reservoir and supply for crop production, explaining any yield benefits observed by ‘diversifying’ the rotation, as opposed to any recently returned crop residues. For example, I consider the fact that the winter wheat and red clover phase receives 135 kg ha^{-1} of fertilizer-N every year it is present, compared to nearly zero (or very little, i.e., 5-8 kg ha^{-1}) of fertilizer-N received in the correspondent soy phase—this N input difference provided 26% more fertilizer-N to the ‘diverse’ rotation compared to the simple rotation over the 2010-2018 period. (However, it must be noted that soybean crops provide some N to the soil, since they are N-fixers). The ‘diverse’ rotations showed higher soil total and mineral N, regardless of the crop phase. A soil N surplus in the diversified rotation may be attributed to the greater accumulated fertilizer-N inputs, as well as higher soil organic matter levels and potentially mineralizable N, or a synchrony between soil mineralized N and crop N uptake (perhaps reducing the risk of total N losses during wet periods, i.e., leaching in the fall or spring). For the ‘diverse’ rotation, either a combination of the accumulated N inputs and a higher soil N reservoir, or the accumulated N inputs alone might be driving the changes that are observed, namely: the higher crop yields, the higher N_2O production, and the increased crop residue mineralization.

Based on the results from Chapter 3 and Chapter 4, it is the clear that the long-term legacy of rotating winter wheat and red clover with corn and soybean crops alters the N dynamics when compared to ‘simple’ corn-soybean rotations (albeit, not in the way originally hypothesized); thus addressing the objectives of this thesis. It is possible that the accumulated legacy of annual N fertilizer applications over the long-term cropping history at this site is responsible for the soil N reservoir for crop production (what might contribute to enhanced soil organic carbon levels), explaining any yield benefits observed by ‘diversifying’ the rotation. At this particular long-term trial, the ‘diverse’ rotation may have higher crop yield, but this may be at the cost of an excessive soil N pool, risking higher N_2O emissions. To develop more environmentally friendly long-term cropping systems, ‘diversifying’ crop rotations should be accompanied with reduced annual N fertilizer applications that account for legume credits or soil-test N levels.

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7. APPENDIX

Table 7.1 Field management information for crop production in 2017.

Date	**Mar 27th**	**April 1st**	**April 1st**	**May 11th**	**May 19th**	**May 19th**	**May 30th**	**Jun 2nd**	**Jun 3rd**	**Jun 12th**	**Aug 14th**	**Oct 17th**	**Nov 21st**
Crop	Wheat	Wheat	Wheat; Corn; Soybean	Corn; Soy	Corn	Soybean	Corn; Soybean	Soybean	Corn; Soybean	Corn; Soybean; Wheat	Wheat	Corn; Soybean	Corn; Soybean; Wheat
Tillage													CT
Seeding					Corn DKC 39-97			Soybean P05T24R					
Fertilizer	136 kg N ha^{-1}	38.8 kg N ha^{-1}	150 kg K ha^{-1}		21.6 kg N/P/K ha^{-1}	21.6 kg N/P/K ha^{-1}	18.7 kg N ha^{-1} / 20.4 kg S ha^{-1}			150 kg N ha^{-1}			
Herbicide				3.7 L ha^{-1}	4.94 L ha^{-1}				3.5L ha^{-1}				
Harvest											harvest	harvest	

Table 7.2. Field management information for crop production in 2018.

Date	**May 14th**	**May 17th**	**May 23rd**	**May 23rd**	**May 25th**	**May 30th**	**Jun 19th**	**Oct 9th**	**Oct 22nd**	**Nov 14th**
Crop	Corn; Soybean; Wheat	Corn; Soybean; Wheat	Corn	Soybean	Soybean	Corn	Corn	Soybean	Corn; Soybean	Corn; Soybean
Tillage	CT	CT								CT
Seeding				Soybean DKB04-41						
Fertilizer							51 kg N ha^{-1}			
Herbicide			0.24 L ha^{-1} 2 L ha^{-1}		2.5 L ha^{-1} 3.4 L ha^{-1}	3 L ha^{-1}		3 L ha^{-1}		
Harvest									harvest	

Table 7.3. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on above-ground 2017 corn residue-N allocation into the subsequent soybean grain crops at 2018 harvest.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	0.35	0.5686[ns]
Tillage system legacy	1	12.27	0.0080**
Crop rotation*tillage system legacies	1	4.00	0.0806*

[a]*numerator degrees of freedom (denominator degrees of freedom is 8) based on proc mixed*

***designates significance at P < 0.01*

** designates significance at P < 0.1*

Table 7.4. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on below-ground 2017 corn residue-N allocation into the subsequent soybean grain crops at 2018 harvest.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	9.34	0.0157*
Tillage system legacy	1	8.84	0.0178*
Crop rotation*tillage system legacies	1	0.07	0.7988

[a]*numerator degrees of freedom (denominator degrees of freedom is 8) based on proc mixed*

** designates significance at P < 0.05*

Table 7.5. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on above-ground 2017 soy residue-N allocation into the subsequent corn grain crops at 2018 harvest.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	3.91	0.0954*
Tillage system legacy	1	0.20	0.6729[ns]
Crop rotation*tillage system legacies	1	0.56	0.4813[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 6) based on proc mixed

** designates significance at P < 0.1*

Table 7.6. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on above-ground 2017 soy residue-N allocation into the subsequent corn residue crops at 2018 harvest.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	3.84	0.0817*
Tillage system legacy	1	0.26	0.6209[ns]
Crop rotation*tillage system legacies	1	0.07	0.8006[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed

** designates significance at P < 0.1*

Table 7.7. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on below-ground 2017 soy residue-N allocation into the subsequent corn grain crops at 2018 harvest.

Fixed Effect	**Num DF**[a]	**F value**	**p-value**
Crop rotation legacy	1	0.19	0.6737[ns]
Tillage system legacy	1	0.71	0.4224[ns]
Crop rotation*tillage system legacies	1	0.06	0.8047[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed

** designates significance at $P < 0.1$*

Table 7.8. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on below-ground 2017 soy residue-N allocation into the subsequent corn residue crops at 2018 harvest.

Fixed Effect	**Num DF**[a]	**F value**	**p-value**
Crop rotation legacy	1	0.13	0.7309[ns]
Tillage system legacy	1	0.69	0.4268[ns]
Crop rotation*tillage system legacies	1	0.13	0.7312[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed

** designates significance at $P < 0.1$*

Table 7.9. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on indigenous soil N corn grain content by 2017 harvest.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	6.87	**0.0153***
Tillage system legacy	1	0.08	0.7801[ns]
Crop rotation*tillage system legacies	1	1.13	0.2927[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 23) based on proc mixed*

** designates significance at P < 0.05*

Table 7.10. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on indigenous soil N soy grain content by 2018 harvest

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	3.26	0.0832
Tillage system legacy	1	20.45	0.0001***
Crop rotation*tillage system legacies	1	1.87	0.1836[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 25) based on proc mixed*

**** designates significance at P < 0.001*

Table 7.11. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on indigenous soil N into grain content by 2017 harvest from the S**S**CC *vs.* S**Wrc**CC rotations.

Fixed Effect	Num DF[a]	F value	p-value
Crop rotation legacy	1	5.54	0.0267*
Tillage system legacy	1	2.78	0.1081[ns]
Crop rotation*tillage system legacies	1	2.04	0.1656[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 25) based on proc mixed

** designates significance at $P < 0.05$*

Table 7.12. Analysis of variance using proc mixed SAS procedure for the single and two-way interaction effects of crop rotation and tillage legacies on indigenous soil N into corn grain content by 2018 harvest from the SS**C**C *vs.* SWrc**C**C rotations.

Fixed Effect	Num DF[a]	F value	p-value
Crop rotation legacy	1	1.72	0.2014[ns]
Tillage system legacy	1	3.15	0.0881[ns]
Crop rotation*tillage system legacies	1	0.17	0.1656[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 25) based on proc mixed

** designates significance at $P < 0.05$*

Table 7.13. Analysis of variance using proc glimmix SAS procedure for the single, two-way, and three-way effects of crop rotation legacy, tillage legacy, and incubation time on peak soil N_2O emissions during the first 34-hrs of incubation after corn stover was amended to the soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	7.41	**0.0075****
Tillage system legacy	1	0.34	0.5584[ns]
Incubation time	4	29.24	**<.0001*****
Crop rotation*tillage system legacies	1	0.18	0.6679[ns]
Crop rotation legacy*incubation time	4	1.79	0.1346[ns]
Tillage system legacy*incubation time	4	0.54	0.7070[ns]
Crop rotation*tillage system legacies*incubation time	4	1.17	0.3285[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 119) based on proc glimmix, Laplace method for negative binomial distribution

*** designates significance at $P < 0.01$*

**** designates significance at $P < 0.001$*

[ns] non-significant, $P > 0.05$

Table 7.14. Analysis of variance using proc glimmix SAS procedure for the single, two-way, and three-way effects of crop rotation legacy, tillage legacy, and incubation time on peak soil N_2O emissions during the first 34-hr of incubation after corn root amendment to the soil microcosms.

Fixed Effect	**Numerator DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	11.70	**0.0009*** **
Tillage system legacy	1	4.30	**0.0403***
Incubation time	4	23.28	**0.0001*** **
Crop rotation*tillage system legacies	1	5.77	**0.0179***
Crop rotation legacy*incubation time	4	1.18	0.3231[ns]
Tillage system legacy*incubation time	4	1.50	0.2063[ns]
Crop rotation*tillage system legacies*incubation time	4	0.76	0.5507[ns]

[a] numerator degrees of freedom (denominator degrees of freedom is 114) based on proc glimmix, Laplace method for negative binomial distribution

** , *** designate significance at P < 0.05 and 0.001, respectively*

[ns] non-significant, P > 0.05

Table 7.15. Analysis of variance using proc glimmix SAS procedure for the single, two-way, and three-way effects of crop rotation legacy, tillage legacy, and incubation time on soil CO_2 emissions during first 34-hrs of incubation after corn stover was amended to the soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	3.15	0.0782[ns]
Tillage system legacy	1	6.50	**0.0119***
Incubation time	4	5.58	**0.0003*****
Crop rotation*tillage system legacies	1	0.12	0.7323[ns]
Crop rotation legacy*incubation time	4	0.61	0.6573[ns]
Tillage system legacy*incubation time	4	1.30	0.2744[ns]
Crop rotation*tillage system legacies*incubation time	4	0.30	0.8762[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 137) based on proc glimmix, Laplace method for negative binomial distribution*

** designates significance at P < 0.05*

**** designates significance at P < 0.001*

[ns] *non-significant, P > 0.05*

Table 7.16. Analysis of variance using proc glimmix SAS procedure for the single, two-way, and three-way effects of crop rotation legacy, tillage legacy, and incubation time on soil CO_2 emissions during first 34-hrs of incubation after corn stover was amended to the soil microcosms.

Fixed Effect	**Num DF**[a]	**F value**	**p-value**
Crop rotation legacy	1	3.15	0.0782^{ns}
Tillage system legacy	1	6.50	**0.0119***
Incubation time	4	5.58	**0.0003*****
Crop rotation*tillage system legacies	1	0.12	0.7323^{ns}
Crop rotation legacy*incubation time	4	0.61	0.6573^{ns}
Tillage system legacy*incubation time	4	1.30	0.2744^{ns}
Crop rotation*tillage system legacies*incubation time	4	0.30	0.8762^{ns}

[a]numerator degrees of freedom (denominator degrees of freedom is 137) based on proc glimmix, Laplace method for negative binomial distribution

** designates significance at $P < 0.05$*

**** designates significance at $P < 0.001$*

^{ns} non-significant, $P > 0.05$

Table 7.17. Analysis of variance using proc glimmix SAS procedure for the single, two-way, and three-way effects of crop rotation legacy, tillage legacy, and incubation time on soil CO_2 emissions during first 34-hrs of incubation after corn root was amended to the soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	24.19	**<0.0001*** **
Tillage system legacy	1	1.03	0.3110[ns]
Incubation time	4	7.15	**<0.0001*** **
Crop rotation*tillage system legacies	1	1.30	0.2562[ns]
Crop rotation legacy*incubation time	4	0.06	0.9927[ns]
Tillage system legacy*incubation time	4	0.01	0.9999[ns]
Crop rotation*tillage system legacies*incubation time	4	0.02	0.9998[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 137) based on proc glimmix, Laplace method for negative binomial distribution*

**** designates significance at P < 0.001*

[ns] *non-significant, P > 0.05*

Table 7.18. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil CO_2 cumulative emissions during 14 days of incubation after corn stover was amended to the soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	8.96	**0.0151***
Tillage system legacy	1	6.66	**0.0297***
Crop rotation*tillage system legacies	1	1.93	0.1985[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimmix, Laplace method for negative binomial distribution

** designates significance at P < 0.05*

[ns] non-significant, P>0.05

Table 7.19. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil CO_2 cumulative emissions during 14 days of incubation after corn root was amended to the soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	12.88	**0.0058****
Tillage system legacy	1	0.05	0.8244[ns]
Crop rotation*tillage system legacies	1	0.82	0.3877[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimmix, Laplace method for negative binomial distribution

*** designates significance at P < 0.01*

[ns] non-significant, P>0.05

Table 7.20. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil N_2O cumulative emissions during 14 days of incubation after corn stover was amended to the C**C**SS and C**C**SWrc soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	4.88	**0.0545***
Tillage system legacy	1	0.29	0.606[ns]
Crop rotation*tillage system legacies	1	0.53	0.4848[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimmix, Laplace method for negative binomial distribution*

** designates significance at P < 0.1*

[ns] *non-significant, P>0.05*

Table 7.21. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil N_2O cumulative emissions during 14 days of incubation after corn root was amended to the C**C**SS and C**C**SWrc soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	1.75	0.2185[ns]
Tillage system legacy	1	2.33	0.1609[ns]
Crop rotation*tillage system legacies	1	1.08	0.3264[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimimix, Laplace method for negative binomial distribution*

[ns] *non-significant, P>0.05*

Table 7.22. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil N_2O cumulative emissions during 14 days of incubation after stover was amended to the SS**C**C and S**Wrc**CC soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	2.00	0.1914[ns]
Tillage system legacy	1	0.22	0.6525[ns]
Crop rotation*tillage system legacies	1	0.09	0.7678[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed

[ns] non-significant, P>0.05

Table 7.23. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil N_2O cumulative emissions during 14 days of incubation after root was amended to the SS**C**C and S**Wrc**CC soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	65.83	<0.0001***
Tillage system legacy	1	0.01	0.9127[ns]
Crop rotation*tillage system legacies	1	1.04	0.3353[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimimix, Laplace method for negative binomial distribution

**** designates significance at P < 0.0001*

[ns] non-significant, P>0.05

Table 7.24. Analysis of variance using proc mixed SAS procedure for the single and two-way effects of crop rotation legacy and tillage legacy on 2018 soil C levels at 0-15 cm depth from CC**S**S and CC**S**Wrc micro-plots.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	10.19	0.0038*
Tillage system legacy	1	0.25	0.6225[ns]
Crop rotation*tillage system legacies	1	1.06	0.3135[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 25) based on proc mixed*

** designates significance at P < 0.05*

[ns] *non-significant, P>0.05*

Table 7.25. Analysis of variance using proc mixed SAS procedure for the single and two-way effects of crop rotation legacy and tillage legacy on 2018 soil C levels at 0-15 cm depth from SS**C**C and SWrc**C**C micro-plots.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	15.35	0.0006**
Tillage system legacy	1	17.74	0.0003**
Crop rotation*tillage system legacies	1	0.68	0.4158[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 25) based on proc mixed*

**** designates significance at P < 0.001*

[ns] *non-significant, P>0.05*

Table 7.26. Analysis of variance using proc mixed SAS procedure for the single and two-way effects of crop rotation legacy and tillage legacy on 2018 soil C levels at 15-30 cm depth from SS**C**C and SWrc**C**C micro-plots.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	15.35	0.1701[ns]
Tillage system legacy	1	17.74	0.9678[ns]
Crop rotation*tillage system legacies	1	0.68	0.0009***

[a]numerator degrees of freedom (denominator degrees of freedom is 25) based on proc mixed

**** designates significance at P < 0.001*

[ns] non-significant, P>0.05

Table 7.27. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil CO_2 cumulative emissions during 14 days of incubation after corn stover was amended to the C**C**SS and C**C**SWrc soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	6.50	**0.0312***
Tillage system legacy	1	3.89	0.0800[ns]
Crop rotation*tillage system legacies	1	1.55	0.2445[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed

** designates significance at P < 0.05*

[ns] non-significant, P>0.05

Table 7.28. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil CO_2 cumulative emissions during 14 days of incubation after corn root was amended to the C**C**SS and C**C**SWrc soil microcosms.

Fixed Effect	**Num DF**[a]	**F value**	**p-value**
Crop rotation legacy	1	10.49	**0.0102***
Tillage system legacy	1	0.20	0.6664[ns]
Crop rotation*tillage system legacies	1	0.44	0.5215[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed.*

** designates significance at P < 0.05*

[ns] *non-significant, P>0.05*

Table 7.29. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil N_2O cumulative emissions during 14 days of incubation after stover was amended to the SS**C**C and S**Wrc**CC soil microcosms.

Fixed Effect	**Num DF**[a]	**F value**	**p-value**
Crop rotation legacy	1	2.00	0.1914[ns]
Tillage system legacy	1	0.22	0.6525[ns]
Crop rotation*tillage system legacies	1	0.09	0.7678[ns]

[a]*numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed*

[ns] *non-significant, P>0.05*

Table 7.30. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil N_2O cumulative emissions during 14 days of incubation after roots were amended to the SS**C**C and S**Wrc**CC soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	65.83	<.0001***
Tillage system legacy	1	0.01	0.9127[ns]
Crop rotation*tillage system legacies	1	1.04	0.3353[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimmix Laplace method for negative binomial distribution

**** designates significance at P < 0.001*

[ns] non-significant, P>0.05

Table 7.31. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil CO_2 cumulative emissions during 14 days of incubation after stover was amended to the SS**C**C and S**Wrc**CC soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	399.62	<.0001***
Tillage system legacy	1	0.53	0.4843[ns]
Crop rotation*tillage system legacies	1	11.95	0.0081[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc glimmix, Laplace method for negative binomial distribution

**** designates significance at P < 0.001*

[ns] non-significant, P>0.05

Table 7.32. Analysis of variance using proc glimmix SAS procedure for the single and two-way effects of crop rotation legacy, tillage legacy on soil CO_2 cumulative emissions during 14 days of incubation after roots were amended to the SS**C**C and S**Wrc**CC soil microcosms.

Fixed Effect	**Num DF[a]**	**F value**	**p-value**
Crop rotation legacy	1	37.05	0.0002***
Tillage system legacy	1	0.18	0.6783[ns]
Crop rotation*tillage system legacies	1	0.00	0.9902[ns]

[a]numerator degrees of freedom (denominator degrees of freedom is 9) based on proc mixed

**** designates significance at P < 0.001*

[ns] non-significant, P>0.05