INTERACTING EFFECTS OF COVER CROP AND SOIL MICROBIAL COMMUNITY COMPOSITION ON NITROUS OXIDE PRODUCTION IN NO-TILL SOILS

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Nitrous oxide (N_2O) is an atmospheric constituent that contributes to climate warming and stratospheric ozone depletion. A large fraction of the anthropogenic N_2O emission originates from agricultural soils suggesting therefore a strong connection between N_2O accumulation in the atmosphere and agricultural land management. During the last 2-3 decades, no-till (NT) farming and integration of cover crops into crop rotation represent two major developments in agriculture, but much remains to be learned about the impact of these management approaches on N_2O emission and underlying biological soil factors. This dissertation focuses on the contribution of different components of the soil microflora to N_2O production, and how different types of cover crops (legume vs grass) affect the soil microbial community composition, mineral N availability, and N_2O emission in plowed (PT) and NT soils. To address these questions, several laboratory and greenhouse experiments were conducted. Results of these experiments documented soil microbial community responses to cover crop addition and could inform the selection of cover crops most suitable to soils under different tillage practices.

Pierre-Andre Jacinthe, PhD., Chair

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Chapter 1

Introduction

1.1. Nitrous oxide emission

Agricultural and industrial expansion during the past two centuries has resulted, either directly or indirectly, in significant changes in the composition of the earth's atmosphere. Solar radiation in the visible electromagnetic spectrum passes through the atmosphere to warm the earth's surface and is then reflected as infrared thermal radiation. This thermal radiation is then absorbed by greenhouse gases (GHGs) such as nitrous oxide (N_2O) and contributes to the warming of the lower atmosphere (Finlayson-Pitts and Pitts, 2000). In addition to its implication in the energy balance of the earth, N_2O contributes to the depletion of the stratospheric ozone layer through the formation of stratospheric NO (Baggs et al., 2003), and is a long-lived atmospheric constituent with a lifetime of 166 ± 16 years (Baggs et al., 2003). In terms of global warming potential (GWP), N_2O is 12 and 320 times more potent than methane (CH_4) and carbon dioxide (CO_2), respectively, on a 100-year time scale (IPCC, 2007).

Nitrous oxide accounts for 2-4% of total GWP, and this contribution could increase to as much as 10% in the future as a result of increase in atmospheric N_2O concentration (Forster et al., 2007). Current concentration of N_2O in the atmosphere is about 310 ppbv, and is increasing at a rate of 0.6-0.9 ppbv yr^{-1} (Baggs et al., 2003). This increase in N_2O concentration is due to natural processes and anthropogenic activities including intensive agriculture. Total anthropogenic N_2O emission is estimated at 6.7 Tg nitrogen (N) yr^{-1}, with agriculture being the largest contributor to that total emission (2.8

Tg N) (Montzka et al., 2011). When compared to an earlier assessment (1.5 Tg of N from agriculture; Watson et al., 1992), recent estimates of 6.7 Tg N yr^{-1} (Montzka et al., 2011) indicate that the annual rate of N_2O emission from agricultural ecosystems has nearly doubled during the last 20 years.

1.2. Nitrous oxide production sources and pathways

Nitrous oxide is produced by both natural sources and human activities such as agriculture, fossil fuel combustion and wastewater management (Vantichung, 2011). It has been shown that agricultural activities (tillage practices and fertilizer application) have the largest contribution (about 67%) to the global N_2O emission (Ruser et al., 2006; USEPA, 2009). Montzka et al. (2011) estimated that row-crop agriculture contributes more than 61% of the total anthropogenic N_2O emission. It has been also estimated that agricultural soil management contributes 69% of the overall N_2O emission in the US (US Department of State, 2007). The North Central region, the major corn-producing region of the country (the US corn belt), contributes 25 to 33% of N_2O emission from the US agricultural sector (Mummey et al., 1998). Therefore, because of this large contribution, management of N_2O emission from the agricultural sector is central to any effort aimed at reducing N_2O emission both in the US and around the globe.

In soil ecosystems, N_2O is an important gaseous by-product of N cycle (Robertson et al., 2000). Nitrification and denitrification are the two main pathways of N_2O production (Fig. 1-1). These microbial pathways have been investigated in soils, wastewater treatment plants, sediments and water bodies (Davidson, 2012). Nitrification

is the biological oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) through the activity of both autotroph and heterotroph microorganisms. Denitrification is the dissimilatory reduction of NO_3^- to N_2O/N_2 under anaerobic conditions. Denitrifying microorganisms in soils are mostly facultative anaerobes with the ability to reduce nitrate instead of oxygen during respiration under oxygen-deficient or anaerobic conditions. During denitrification, N_2O is a regular intermediate that is released in significant amounts in low oxygen conditions with adequate supply of nitrate and organic carbon (Zhou et al. 2001).

1.3. No-till farming and soil processes

On a global scale, it has been estimated that agricultural practices such as shifts in soil management from conventional (PT) to conservative and no-till (NT) practices have the potential to reduce greenhouse gases emission by as much as 1.15-3.3 Pg carbon (C) equivalents per year (Cole et al., 1997). Conventional tillage (or moldboard plow), until very recently, 1980s, has been the predominant method for land preparation. Primary justifications for tillage include: (1) preparation of a smooth and suitable seedbed, (2) temporary reduction in soil compaction to promote crop root growth, (3) incorporation of crop residues and agrochemicals into the soil, (4) early soil warming especially in humid and cold climate, (5) following tradition (Feng et al., 2003). However, during the last 3-4 decades, there has been a major shift with the emergence of NT as an alternative land management practice, which could result in the least soil disturbance and benefit soils with higher moisture content, C sequestration and aggregate stability (Horowitz et al.,

2010). In fact, in NT system the land surface is minimally disturbed and only a small groove (3-7 cm) is created to drop the seeds (Jiang et al., 2011).

No-till practice has received significant attention and is currently applied as an alternative agricultural management on over 100 million hectares under different soil and climate conditions around the world (Derpsch et al., 2010). Recent data indicate that NT farming is implemented on approximately 35.5% of US cropland (36 million hectares) (Horowitz et al., 2010). No-till has also gained wide adoption in several other countries (e.g. Australia, Argentina and Brazil) outside North America (Huggins and Reganold, 2008).

Since the land surface is minimally disturbed, NT farming can bring significant changes in the physical and biochemical soil environment. Several studies have investigated the influence of NT on soil aggregate stability and, as a result, decreased soil erosion. The study of Dress et al. (1994) has shown that long-term NT practice can affect both the size (2 to 3-fold increase) and structure (change from platy to granular) of soil aggregates. Other studies also reported the increase in soil aggregate stability with NT farming adoption (West et al., 1992; Vyn and Raimbualt, 1993; Mahboubi et al., 1993). No- till as a soil management system could lead to higher total organic C than does conventional tillage. It has been found that the formation of macroaggregates in NT soils could protect a significant amount of soil organic carbon (SOC) from microbial decomposition (Horowitz et al., 2010). The benefits of NT on SOC sequestration have been observed across various ecoregions of the world. In rain-fed agricultural system in Spain under continuous NT between 5 and 19 years, Lopez et al. (2012) measured SOC pool 55% higher than at sites under PT, and recommended NT as a land management

strategy to increase SOC in that region's cropland. Similarly, Mishra et al. (2010) found significantly larger SOC pool in the top 40 cm of Ohio fields under NT compared to PT, and hypothesized that enhanced SOC sequestration is due to reduced soil disturbance and crop residue decomposition in NT systems. Similar results were reported by other researchers including Spargo et al. (2008) and Gosai et al. (2009), and their findings support the general contention that NT could serve as an effective tool to increase soil SOC sequestration and improve soil quality.

As a result of increased SOC concentration, NT practices can lead to noticeable changes in soil microbial biomass and community composition (Frey et al., 1999). Alvear et al. (2005) investigated the effects of tillage practices on SOC and soil microbial biomass C and N. Their results showed that SOC and total N were significantly higher under NT compared to PT, and the effect on biological activity mostly occurred in the upper soil layers (0-5 cm), where the largest populations of microbes and plant roots are located. Other studies have also reported an enhancement in bacterial and fungal densities in soils under NT management (Locke et al., 2012; Lienhard et al., 2013; Lehman et al., 2014).

1.4. No-till farming and nitrous oxide emission

No-till farming has been advocated as an agricultural practice that could foster both cropland sustainability and GHG emission mitigation (Robertson et al., 2000). The environmental (water conservation, soil health improvement, carbon sequestration) and economic benefits (reduction in fuel and labor costs) associated with NT practice are well

documented, but there is uncertainty with regard to N_2O production in soils under long-term NT (Regina and Alakukku, 2010). The effect of NT on N_2O emission remains highly variable (i.e. NT has been found to increase, decrease or not affect N_2O emission compared with PT). These variable results have motivated several recent studies aimed at gaining a deeper understanding of NT impacts on soil chemical and biological properties controlling N_2O dynamics. Since NT management can alter several physicochemical properties of soils (SOC, moisture, soil structure), it can also lead to increased production of GHG such as N_2O. Results of the study on eastern corn belt Alfisol, under corn (*Zea mays* L.) - soybean (*Glycin max* L.) rotation showed that soybean-corn rotation under NT had higher N_2O emission than PT (Smith et al., 2011). Almaraz et al. (2009) noted in corn fields in southwestern Quebec that, although CO_2 emission was nearly similar under different tillage practices, N_2O emission was significantly higher under NT than PT. Although NT has been promoted as a management approach to increase SOC and improve soil fertility and quality, if the practice leads to increased N_2O emission, its environmental benefits would be neglected (Robertson et al., 2000).

1.5. Nitrogen fertilizer application and N_2O emission

Nitrogen accumulation has increased in many regions of the US as a result of over application of N fertilizers to maintain crop productivity. As N inputs to agricultural fields increase, N loss would also increase through NO_3^- leaching and gaseous emissions (e.g. N_2O) as a result of either nitrification or denitrification (Wallenstein et al., 2006). Mohn et al. (2000) reported higher denitrification N loss in fertilized (2.9 kg N ha^{-1} yr^{-1})

than in unfertilized plots (1.7 kg N ha^{-1} yr^{-1}), and attributed the increased N flux to greater denitrification activity as a result of N fertilizer application. Although only a small portion (less than 3%) of applied N fertilizer is typically emitted as N_2O, these emissions can be considerable on national and global scales given the heavy reliance of intensive modern agriculture on synthetic N fertilizer (Robertson et al., 2000). The positive correlation between N fertilizer application and N_2O emission (Smith et al., 2011) in agroecosystems would suggest that reduction in N fertilizer application rates can be considered as an effective way of mitigating N_2O emission. Since reduction in N fertilizer application rates might not be acceptable to farmers due to the possible decrease in crop yield, N fertilizers needs to be substituted with a new N source. Recently, cover crops have been introduced as green manures for increasing available N in agricultural soils (Drenovsky et al., 2004; Wortman et al., 2013). Cover crop application improves soil quality by providing the growing crops with a source of C and N, increasing the abundance of soil microorganisms as a result of creating a more favorable habitat for soil bacteria and fungi, characterized by greater soil moisture and limited soil disturbance (Elfstrand et al., 2007; Ramos-Zapata et al., 2012). Suppressive impact on weed growth is another benefit of cover crops to agricultural soils (Teasdale and Mohler, 1993).

1.6. Research objectives

Considering the positive effects of NT practice on soil properties and also the need to mitigate N_2O emission emphasize the need for new N management practices in order to reduce N_2O emission from NT agroecosystem. Variable effects of NT on N_2O

emission could be linked to temporal changes in biochemical soil properties with NT duration. Therefore, there is a need to characterize the structure and composition of the microbial community, and better understand N cycling processes in soils under NT for varying lengths of time. To achieve this goal, throughout my doctoral research I conducted three main projects corresponding to the following three chapters of my dissertation. The specific objectives of each of these projects were as follow:

A. To determine the contribution of fungi and bacteria to N_2O production and identify the dominant producer of N_2O in soils under different tillage practices. For this project, I examined N_2O production in soils under PT, mid- and long-term NT managements. I used selective inhibition tests to find the optimum level and type of biocides with the most inhibitory effect on N_2O production and assess N_2O production from each group of soil microorganisms (bacteria or fungi), separately.

B. To investigate the decomposition pattern of different cover crops in soils under different tillage managements, and determine the fate of N released from cover crop residues. To address this research objective, I conducted a four-month greenhouse experiment to study mineral N release and N_2O emission from PT and mid-term NT soils. Corn was grown on PT and NT soil treated with either hairy vetch (*Vicia villosa*) or rye (*Secale cereale*), carbon:nitrogen ratio of 11 and 82, respectively.

C. To investigate the effects of cover crops of different chemical compositions on the size and diversity of microbial communities in soils under different tillage practices. To achieve this research objective, I conducted a five-week laboratory

experiment to study bacterial and fungal biomass and diversity in PT and mid-term NT soils amended with hairy vetch or rye of different carbon:nitrogen ratios.

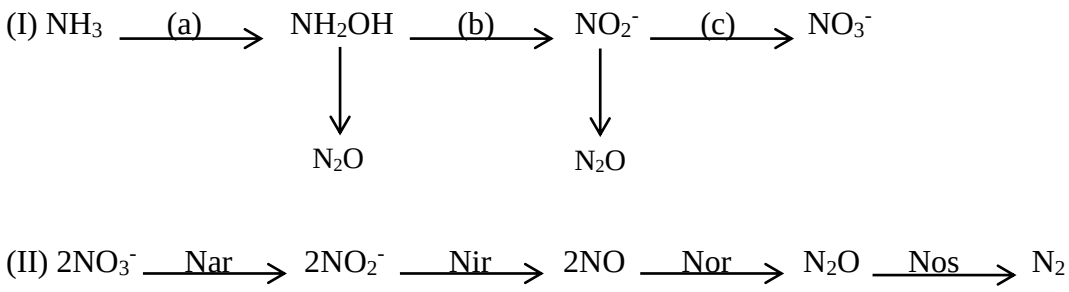

Fig. 1-1. (I) Nitrification: pathway and enzymes involved: (a. Ammonia monooxygenase, b. Hydroxylamine oxidoreductase, c. Nitrite oxidoreductase), (II) Denitrification process (including intermediates and enzymes involved; Nar: nitrate reductase, Nir: nitrite reductase, Nor: nitric oxide reductase, Nos: nitrous oxide reductase).

Appendix. Previous studies on the effects of NT practice on N_2O emission

Authors, Year	Location	Soil type	NT duration	Results
Elder and Lal, 2008	North Central Ohio	Histosol	1 year	Decreasing N_2O emission
Wang et al., 2011	Queensland, Australia	Vertisol	Long-term	Decreasing N_2O emission
Alvear et al., 2005	Southern Chile	Ultisol	NM	Increasing N_2O emission
Spargo et al., 2008	Virginia coastal plain	Ultisol	0-14 years	Increasing N_2O emission
Gosai et al., 2009	Northeast India	Vertisol	Long-term	Increasing N_2O emission
Regina and Alakukku, 2010	Northern Europe	Molisol	5-7 years	Increasing N_2O emission
Metay et al., 2007	Cerrados, Brazil	Oxisol	5 years	No significant difference
Harada et al., 2007	Northern Japan	Paddy soil	NM	No significant difference

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Chapter 2

Evaluation of potential inhibitors of bacterial and fungal denitrification in soils under different tillage practices

2.1. Abstract

Nitrous oxide (N_2O) is an atmospheric constituent that has been implicated in climate warming and stratospheric ozone depletion. Among anthropogenic activities, agriculture is the largest contributor to global N_2O emission. Therefore, agricultural land management decisions could influence the atmospheric N_2O burden. During the last several decades, no-till (NT) farming has been introduced as a better tillage practice compared to conventional plowing (PT), but the net effect of NT on N_2O emission and underlying soil factors are not well understood. In this study, the N_2O production potential and the contribution of bacteria/fungi to that production in soils under long-term NT (11 to 50 years), PT and woodlot were investigated. First, a selective inhibition technique using different biocides was developed to determine the type and level of biocides resulting in greater inhibition of N_2O production. Results showed that streptomycin and cycloheximide, the biocides most commonly used in previous studies, were not effective inhibitors of N_2O production compared to bronopol and captan. Results also revealed that the soils with longer duration of NT (50 years) had higher fungi:bacteria ratio compared to other soils. Our results support that bacteria are the dominant N_2O producers in the soils investigated, regardless of tillage management and land uses.

2.2. Introduction

As a gaseous by-product of the soil N cycle, nitrous oxide (N_2O) production is an important form of nitrogen (N) loss from soil ecosystems (Robertson 2000). In addition, N_2O has been implicated in the accelerated greenhouse effect and stratospheric ozone depletion. Considerable amounts of N_2O can be emitted from agricultural soils as a result of N fertilizer application and tillage operations. Among anthropogenic activities, agriculture has been identified as the largest contributor to global N_2O emission (Ruser et al. 2006).

Although N_2O production in agricultural soils originates from nitrification process as a byproduct, denitrification has been shown as the dominant process responsible for N_2O production (Alvear et al. 2005; Ball et al. 2008; Davidson 2012). Denitrification is the dissimilatory reduction of nitrate (NO_3^-) to N_2O/N_2 under anaerobic conditions. Denitrifying microorganisms in soils are mostly facultative anaerobes that have the ability to reduce NO_3^- as a substitute for oxygen during respiration under oxygen-deficient or anaerobic conditions (Bremner 1997). During denitrification, N_2O is a regular intermediate that is released in significant amounts in low oxygen conditions with adequate supply of NO_3^- and metabolizable organic carbon (Zhou et al. 2001).

No-till (NT) is a farming practice that has gained wide acceptance in recent decades. In many regards, NT is an attractive alternative to the conventional plow-till (PT) practice. In contrast to PT, the land surface remains relatively undisturbed under NT, and the current year's crop seeds are planted directly into the residue left by the previous crop. The impact of NT on N_2O production and associated soil processes has not

been fully elucidated. Some studies reported higher N_2O emission from NT systems (Alvear et al. 2005; Regina and Alakukku 2010) whereas others reported lower N_2O emission under NT practice (Elder and Lal 2008; Wang et al. 2011). Some studies have shown no significant changes in N_2O emission with NT adoption (Metay et al. 2007; Harada et al. 2007).

No-till farming has well documented effects on soil moisture, soil temperature and organic carbon availability (Venterea 2011), as well as the size (biomass) and composition of the soil microbial community (Frey et al. 1999). Linn and Doran (1984) studied three agricultural sites in the US Midwest and found that the population of facultative anaerobes and potential denitrification activity were higher in the surface layers of soils under NT compared to PT. Likewise, Groffman (1985) reported higher denitrification activity under NT than PT in the topsoil layer of agricultural fields in Georgia. At the present it is unclear if NT could induce a shift in soil microbial population and composition, and ultimately the contribution of various groups of microorganisms to N_2O production in agricultural soils.

Both fungi and bacteria participate in N_2O production in soils (Crenshaw et al. 2008), but the contribution of fungi and bacteria to N_2O production could be affected by tillage practices (Hayatsu et al. 2008). No-till farming has been shown to result in increased soil fungi:bacteria ratios (Bailey et al. 2003). Studies such as Frey et al. (1999), Holland and Coleman (1987), Herold et al. (2012) and Lienhard et al. (2013) have shown that the soil microbial community tends to be dominated by fungi under NT and by bacteria in PT systems. Frey et al. (1999) studied the effect of NT and PT management on bacterial and fungal abundance in soils, and reported a larger fungal

community under NT management compared to PT. These results are in accord with those of Holland and Coleman (1987) who reported 144% higher fungal biomass in NT compared to PT soils. Also, a study on soils in northeastern Laos revealed that bacterial and fungal densities were five times higher under NT than PT (Lienhard et al. 2013). It has been suggested that litter mixing with soil (and thus direct contact between the soil bacteria and substrate) as a result of plowing creates conditions favorable to bacterial growth under PT. However, in NT systems where there is a spatial separation between microorganisms and decomposing litter on the soil surface, the presence of hyphae confers an ecological advantage to fungi (Beare et al. 1992). The ratio of soil fungi:bacteria can be used to assess the response of soils to change in management intensity (Bailey et al. 2003), and could reflect overall soil conditions as determined by agricultural management, edaphic and climatic factors (Hayatsu et al. 2008).

Kladivko (2001) suggested that increased soil water under NT management may contribute to fungal dominance in the soil microflora. This shift in fungal dominance has implication for SOC accretion as studies have shown that soil management that favors fungal community generally results in enhanced SOC storage (Frey et al. 1999; Guggenberger et al. 1999). Bailey et al. (2002) measured SOC pool, and fungal and bacterial biomass in soils across different ecosystems. Their results revealed that, in general, the soils with higher fungal activity had higher SOC, and that PT soils had both low fungal activity and low SOC content. Fungal cell walls are rich in melanin and chitin, polymers that are resistant to degradation, whereas the main component of bacterial cell membranes is phospholipids which are rich in energy and readily decomposable (Bailey et al. 2002).

Since N_2O is primarily produced through biological pathways, it seems necessary to assess shifts in soil microbial communities with no-till (NT) adoption in order to better understand the variable effects of NT farming on N_2O emission from agroecosystems. Several studies have evaluated the effect of tillage systems, such as NT and plow-till (PT) on N_2O flux but, to our knowledge, no research has explicitly linked the impact of tillage on the soil microbial community responsible for N_2O production. Here, in order to determine the effects of NT on N_2O emission, the contribution of the dominant groups of microorganisms responsible for N_2O production is examined. Thus, the main objective of this study is to assess the N_2O production potential and the relative contribution of fungi and bacteria to that production in soils under PT and NT practices (both medium- and long-term). Since soil organic carbon (SOC) pool increases with NT and denitrifiers are generally heterotrophs, it is hypothesized that N_2O production potential will be greater under NT compared to PT. It is also hypothesized that, as the soil microbial community becomes fungal-dominated with longer duration of NT, there will be a parallel increase in the relative contribution of fungi to N_2O production in agricultural soils.

To determine the ratio of soil fungi:bacteria, as an indicator of the soil microbial community composition, different approaches can be applied, but the selective inhibition (SI) has by far been the most widely-used technique (Bailey et al. 2003). The technique was first introduced by Anderson and Domsch (1973) and then was evaluated and modified for agricultural and forest soils (Anderson and Domsch, 1975). Different biocides such as bactericides (e.g. streptomycin sulphate, bronopol and oxytetracycline) and fungicides (e.g. captan, cycloheximide, ketoconazole, benomyl and nystatin) have been employed in past studies (Alphei et al. 1995; Lin and Brookes 1999; Bailey et al.

2002; Bailey et al. 2003; Rousk et al. 2009; Sassi et al. 2012). The SI technique has also been applied in several studies investigating denitrification process (Bailey et al. 2003; Yanai et al. 2007; Laughlin et al. 2009; Ananyeva et al. 2010) in which different types and levels of biocides have been used, and variable results have been obtained. Through analysis of available results, it was not possible to identify one type of biocide (and optimum concentration) that has the most inhibitory effect on N_2O production in soils. Therefore, in this study, a standardized method is developed to determine the relative contribution of fungi and bacteria to N_2O production in agricultural soils under different tillage management practices.

2.3. Materials and methods

2.3.1. Soil samples collection

This study was conducted with soil samples (0-10 cm) collected from agricultural fields in Indiana (39°51′ 49″N, 86°21′31″W) and Ohio (39°51′ 48″N, 83°40′20″W) (USA). Management practices at these sites included conventional tillage (plow-till, PT), medium-term (11 years) and long -term (50 years) no-till (NT). Soil samples were also collected from a nearby deciduous forest (woodlot, WL) for comparison. At the sampling sites, soils are classified as Crosby (fine-silt loamy mesic aeric Epiaqualfs) and Brookston (fine-silt loamy mesic typic Epiaqualfs). Soil samples were transported to the laboratory in plastic bags, sieved (2 mm) and kept in a refrigerator (4 ^{o}C) until used in the experiments described below. A portion of each soil sample was air-dried and used for determination of chemical properties (Table 2-1).

2.3.2. Selection of fungal and bacterial denitrification inhibitors

The selective inhibition (SI) technique (Anderson and Domsch, 1975) was used with adaptation. First, a series of assays was conducted to identify the biocides (bactericide and fungicide), and application rates that yield maximum inhibition of denitrification. The tested biocides included some of the most commonly reported in the literature as well as some novel products. This evaluation was conducted using soil samples collected from the long term (50 years) NT site near South Charleston, OH.

Field-moist (0.16 g water g^{-1} soil) soil samples were left overnight at room temperature (22 ^{o}C) in the laboratory for acclimation. Then, 10 g of soil subsamples were placed in serum bottles (250 mL) and amended with 1.44 ml of denitrification enzyme activity (DEA) media (100 mg NO_3-N kg^{-1}, and 40 mg dextrose-C kg^{-1}). Bottles were divided into three groups with one group receiving no treatment (control) and the other two groups treated either with a bactericide or a fungicide. The bactericides evaluated in this study were streptomycin sulfate ($C_{42}H_{78}N_{14}O_{24}\cdot 3H_2SO_4$) and bronopol ($C_3H_6BrNO_4$) obtained from Fisher Scientific (catalog numbers: AC61224-0500 and MFCD00007390, respectively). The fungicides used in this study included cycloheximide ($C_{15}H_{23}NO_4$) and captan ($C_9H_8Cl_3NO_2S$) also from Fisher Scientific (MFCD00082346 and 801175030348, respectively). Biocides, received in dry powder formulation, were used to make biocide solution. Biocides were applied at concentrations ranging between 1 and 16 mg g^{-1} soil, and the final volume of suspension (DEA media and dissolved biocide) in each serum bottle was adjusted with deionized water as needed to reach a final volume of 20 mL. Each treatment was applied in triplicate.

Serum bottles were stoppered, successively evacuated and flushed with ultra-high purity (UHP) N_2 at least 3 times, then injected with acetylene (C_2H_2) for a partial pressure of 10 kPa) to stop the conversion of N_2O to N_2 (Bailey et al. 2003). Then, serum bottles were incubated at 25 ^oC. Gas samples were taken from bottles headspace after 3, 8, 24 and 48 hours of incubation, and stored in evacuated glass vials. Concentration of N_2O was measured using a Varian CP-3800 gas chromatograph interface with a Combipal headspace auto-sampler (CTC Analytics) and equipped with a thermal conductivity detector. The stationary phase consisted of a pre-column (length: 0.3 m; i.d.: 2 mm) and an analytical column (length: 1.8 m; i.d.: 2 mm) filled with Porapak Q (80–100 mesh). The carrier gas was UHP He (60 mL min^{-1}), and oven temperature was 90°C (Jacinthe et al. 2015). The gas chromatograph was calibrated with N_2O standards obtained from Alltech. Based on the results of this first SI test, additional assays were conducted using only the two most effective inhibitors, but increasing their application rate to 32 mg g^{-1} soil.

2.3.3. Assessing fungal and bacterial denitrification as related to tillage practices

Based on the previous results, the selective inhibition technique was applied to soils under different tillage practices to determine the relative contribution of fungal and bacterial microflora to denitrification. Soils used in this assessment were collected from plow-till (PT_1), long-term no-till (NT_1, 50 years) and woodlot (WL) sites in Ohio and from plow-till (PT_2) and medium-term no-till (NT_2, 11 years) fields in Indiana (Table 2-1). Field moist (10 g) soil subsamples were placed in serum bottles and amended with

DEA media as described before. The following biocide treatments were applied: control (no biocide), bactericide (bronopol, 16 mg g^{-1} soil), fungicide (captan, 16 mg g^{-1} soil) and BroCap (mixture of bronopol and captan, each at 16 mg g^{-1} soil). Each treatment was applied in triplicate. The final volume (DEA media and dissolved biocide) of solution in each serum bottle was 20 mL. Serum bottles were evacuated, flushed with UHP N_2, injected with C_2H_2 (10 kPa) and incubated at 25 ^oC. Gas samples were taken from bottles headspace after 3, 8, 24, 48, 72, 96, 120, 144 and 168 hours of incubation. Concentration of N_2O and CO_2 in the bottles headspace was measured by Varian CP-3800 gas chromatograph.

2.3.4. Analytical methods

Soil pH was measured using a soil suspension (1:2 soil to water) and an Accumet model 25 pH/ion meter (22 ^oC) calibrated with pH 4 and 7 buffers. Particle size analysis (texture) of soil samples was conducted using the hydrometer method, in which soil samples were treated with sodium hexametaphosphate ($Na_6P_6O_{18}$, 5%) as a dispersing agent and the density of the suspension measured after 40 sec and 7 hours. Total carbon and nitrogen in soil samples were measured using Vario-Cube analyzer (Elementar Americas). For analysis, finely-ground (150 µm) dried sub-samples (6-9 mg) were placed in tin capsules. Gas samples taken from the headspace of glass vials were measured for N_2O and CO_2 using Varian CP-3800 gas chromatograph. All data collected in this study were analyzed using Microsoft Excel 2013 and graphs were created using Sigma Plot 12.5 (Systat Software, Sane Jose, CA).

2.3.5. Computations

The percentage (%) inhibition caused by a biocide was computed through comparison of gas production in biocide-treated bottles with the control (same soil) using the equation:

$$Inhibition\ \% = (X_i - X_{Control}/X_{Control}) \times 100$$

Where, X_i and $X_{Control}$ represent respectively the amount of N_2O (or CO_2) produced during the incubation in biocide-treated bottles and control, respectively. The ratio of fungi to bacteria (F:B) was calculated based on CO_2 and N_2O production in the control relative to gaseous production in soils subjected to biocide treatments. A similar computational procedure was used in several past studies (West 1986, Wardle and Parkinson 1990, Bear et al. 1990, Scheu and Parkinson 1994, Alphei et al. 1995; Chen et al. 2015). The ratio was calculated as:

$$F{:}B = (A - B)/(A - C)$$

Where, A= respiration measured (as cumulative CO_2 concentration evolved) in the absence of inhibitors; B= respiration in the presence of the fungicide; and C= respiration in the presence of the bactericide (Bailey et al. 2003).

Since some biocides can affect non-target microorganisms, an inhibitor additivity ratio (IAR) was calculated to account for overlap and antagonistic effects:

$$IAR = [(A - B) + (A - C)/(A - D)]$$

Where, A, B and C are cumulative CO_2 concentrations as described above and D=respiration in the presence of both biocides (fungicide and bactericide) (Bailey et al.

2003; Seo and Delaune 2010). An IAR of 1.0 indicates no overlap in the antibiotic actions on non-target organisms and no antagonistic effect of one antibiotic on the other. An overlap is identified by an IAR>1.0 and an antagonistic effect by an IAR<1.0 (Bailey et al. 2003).

2.3.6. Statistical analyses

Statistical significance in this study was determined at $\alpha=0.05$. First, data obtained in this study were tested for normality using Sigma Plot normality test. Since most data obtained were not normally distributed, Kruskal-Wallis test was used to determine the significance of the experimental factors (soil tillage, biocide type and level) on N_2O and CO_2 production. The Kruskal-Wallis test was followed and compared by Mann-Whitney pairwise test (P <0.05) when a significant difference was detected. Statistical tests were conducted using PAST software (ver. 2.17c) downloaded from http://nhm2.uio.no/norlex/past/download.html (University of Oslo).

2.4. Results

2.4.1. Selection of optimum biocides and inhibitory concentrations

The production of N_2O was observed in all treatments, but the rate of production varied with treatments (Figs. 2-1 and 2-2). Streptomycin, which is a bactericide, not only did not inhibit N_2O production at any level of application, but instead stimulated N_2O production (Fig. 2-1a). In contrast, bronopol, another bactericide used in this study,

decreased N_2O production at all application levels, with the most inhibitory effect observed at the application rate of 16 mg g^{-1} soil (Fig. 2-1b). With regard to the fungicides, cycloheximide (Fig. 2-2a) resulted in less inhibition of N_2O production compared to captan (Fig. 2-2b) and the highest inhibition was observed in the level of 16 mg g^{-1} soil. At the 16 mg g^{-1} application rate, the cumulative amount of N_2O produced was significantly ($P < 0.05$) less with bronopol (bactericide) and captan (fungicide) compared to the control (Figs. 2-1b, 2-2b).

Since the most inhibitory effect of bronopol and captan was observed at the highest biocide application rate (16 mg g^{-1} soil) used in the initial assays, additional tests were conducted by extending biocide application to 32 mg g^{-1} soil to determine whether a higher degree of inhibition can be achieved. Incubation was conducted with the same NT_1 soil amended with bronopol or captan (32 mg g^{-1} soil). Gas production was monitored during a 72-hour period. Results showed that both biocides decreased N_2O production compared to controls (Fig. 2-3). The cumulative amount of N_2O produced after 72 hours was statistically similar ($P > 0.05$), in bottles treated with 16 and 32 mg g^{-1} soil (Fig. 2-3). Thus, the two highest application rates resulted in almost the same degree of inhibition of N_2O production.

2.4.2. Nitrous oxide production in soils under different tillage practices

Since the two highest levels (16 mg g^{-1} and 32 mg g^{-1} soil) of bronopol and captan produced the same degree of inhibition in N_2O production (Fig. 2-3), the lower level (16 mg g^{-1} soil), was applied in subsequent tests to determine the effect of tillage

management on the relative contribution of bacterial vs fungal denitrifiers to N_2O production in agricultural soils (Fig. 2-4).

In general, the same pattern in N_2O concentrations was observed in all soil types, i.e. control had the highest N_2O concentration followed by soils treated with captan, Bronopol and BroCap resulted in the lowest N_2O concentrations in all sampling times. There was an increase in N_2O concentration in all treatments over time, and this was clearly observed in controls (Fig. 2-4). Although adding biocides resulted in lower concentrations of N_2O compared to controls, only bronopol- and BroCap-treated soils showed significantly lower N_2O production ($P < 0.05$) in the all soils investigated. With captan, a significant decrease in N_2O production was only observed in the PT_1 soil (Fig. 2-4).

Comparing N_2O production inhibition in biocide-treated soils (Fig. 2-6) revealed no significant difference ($P < 0.05$) between bronopol and BroCap treatments, indicating that bacteria are the dominant microbial group responsible for N_2O production in these agricultural soils. Greater inhibition of N_2O production was generally observed with bronopol compared to captan treatments (Fig. 2-6). These results indicate bacteria as the major group of denitrifiers in soil microflora.

2.4.3. Respiratory response of soils to biocide treatments

A steady accumulation of CO_2 was observed during the 7-day incubation period (Fig. 2-5). A slight increase in CO_2 production rate was noted after day 4 in most treatments. As expected, the highest concentration of CO_2 was observed in the controls

and the lowest in the biocide-treated soils. The concentration of CO_2 increased with time irrespective of soils and biocide treatments but, among all the treatments, the NT_1 soil exhibited the highest accumulated CO_2 concentration after 168 hours (Fig. 2-5b). Results of respiration inhibition (Fig. 2-7) varied, and were not significantly different ($P > 0.05$) and did not show similar pattern as N_2O inhibition.

2.4.4. Fungi:Bacteria ratio

Fungi:bacteria ratios (F:B) were calculated using both the reduction in respiration rate and in N_2O production in biocide-treated soils relative to the controls. For all the soil and biocide treatment combinations, F:B values were < 1, suggesting that bacteria were the dominant group of microorganisms responsible for CO_2 and N_2O production in the soils tested (Table 2-2). This dominance could either be due to the greater abundance or higher metabolic activity of bacteria compared to fungi. The highest and lowest F:B values were observed in the NT_1 and PT_2 soils, respectively. Although no significant difference was detected among soils with respect to F:B ratios ($P > 0.05$), the observed trend suggests an increase in fungal contribution to respiration in these cropland soils with greater duration of NT.

2.4.5. Inhibitor additivity ratio

Data from the treatment involving the combination of bronopol and captan (both at 16 mg g^{-1} soil; BroCap) was used to calculate IAR. Results showed that the IAR

was > 1 in almost all the soils tested, indicating an overlap in the effect of the applied biocides (Table 2-3). In general, IAR values tended to be the highest in the NT soils. The lowest values for IAR were obtained with the PT soils.

2.5. Discussion

2.5.1. Biocides efficiency in inhibiting bacterial and fungal activity

Based on the results of this study and the results reported by others from previous studies (Laughlin and Stevens 2002; Bailey et al. 2003; Rousk et al. 2009; Sassi et al. 2012) there seems to be no single concentration of biocides that could lead to maximum inhibition of N_2O production in all types of soils. The optimum concentration of biocides used in selective inhibition tests varies greatly (Appendix), with optimum concentration ranging between 1 and 16 mg biocide g^{-1} soil reported in the literature (Alphei et al. 1993; Bailey et al. 2002; Laughlin and Stevens, 2002; Bailey et al. 2003; Rousk et al. 2009; Herold et al. 2010). The optimum concentration (16 mg biocide g^{-1} soil) found in the present study was in the upper end of that range. It has been suggested (Alphei et al. 1993) that high clay content can reduce the efficiency of biocides and that higher concentrations are needed to obtain significant reduction in respiratory activity. Given the texture of the soils used in this study (Table 2-1), this reasoning would be consistent with the results. Therefore, as it was done in the present study, preliminary tests must be first conducted to determine optimum concentration of inhibitors for each new set of soils under investigation.

In this study, different types and levels of biocides were used to find the most effective products against denitrification and respiratory activity mediated by bacteria and fungi. These products include inhibitors that were tested in some of the pioneering work to develop the selective inhibition procedure (Anderson and Domsch, 1973; West and Sparling, 1986) as well as some inhibitors introduced more recently in the literature to distinguish fungal and bacterial activity (Bailey et al. 2002; Bailey et al. 2003; Boyle et al. 2008; Rousk et al. 2009; Sassi et al. 2012). Among the biocides tested in the present study, streptomycin, the bactericide traditionally used in SI procedure, showed almost no inhibitory effect on the activity of bacterial denitrifiers (Fig. 2-1a). This result was somewhat surprising given the large number of past investigations in which streptomycin was used as a bactericide (Alphei et al. 1993; Johnson et al. 2003; Crenhshaw et al. 2008; Seo and Delaune 2008). However, this result is in agreement with several past studies that have also noted several instances of inefficient inhibition of bacterial growth by streptomycin (Nakamato and Wakahara, 2004; Herold et al. 2012). Boyle et al. (2003) also reported that streptomycin was a much less effective bactericide than bronopol and oxytetracycline-HCl in reducing bacterial activity.

Identifying the factors contributing to streptomycin inefficiency remains a challenge, but soil redox condition is likely not a contributing factor since streptomycin inefficiency has been reported in studies using either water-saturated or unsaturated soils (Laughlin et al. 2009). Cycloheximide is another product that has exhibited surprisingly low biocide efficiency. Cycloheximide was the least efficient of the biocides examined and resulted in higher N_2O production compared to control (Fig. 2-2a). These results contrast with those of other studies in which cycloheximide was found to inhibit fungal

activity at low concentrations (e.g. 1-2 mg g^{-1} soil) (Crenshaw et al. 2008; Herold et al. 2012) and affect prokaryotic growth at higher concentrations (Velvis 1997; Rousk et al. 2009). In the present study no effects of application rate on cycloheximide efficiency was apparent and the literature does not provide much information to explain cycloheximide inefficiency in the present study. Overall, these mixed results from streptomycin and cycloheximide underscore the necessity of testing the efficiency of these biocides for new soils.

In this study, biocides that resulted in the inhibition of N_2O production were bronopol and captan. Several investigators (Ingham 1985; Lin and Brooks. 1999; Bailey et al. 2002; Bailey et al. 2003; Sassi et al. 2012) have also found these products to be effective and successful selective inhibitors in past studies examining bacterial and fungal contribution to N_2O production and respiration.

Although the addition of bronopol and captan resulted in inhibition of N_2O production in comparison to controls, some level of microbial activity was still maintained, leading to the accumulation over time of both N_2O and CO_2 in the incubation bottles (Figs. 2-4 and 2-5). Even in the presence of both bactericide and fungicide (BroCap treatments) some N_2O and CO_2 production was observed. Similar observations were reported in selective inhibition studies involving desert, prairie, forest and agricultural soils (Bailey et al. 2002; Crenshaw et al. 2008). This slight increase in N_2O and CO_2 production in biocide-treated soils could be due to an increase in activity of surviving bacteria and fungi at the expense of metabolizable C and N released from dead microbes in the biocide-treated soils. Moreover, it needs to be noted that denitrifiers are only a group of soil microorganisms and even with inhibiting N_2O producers there are

still other active microorganisms that are functioning. Additional factors that may contribute to these results include: (i) constitutive enzymes, which get degraded slowly, were likely to be present and active during the experiment, and new enzymes were not synthesized due to the biocides (Heilmann et al. 1995), (ii) antibiotic-resistant bacterial and fungal strains that are capable of maintaining respiratory and N_2O production activity in the presence of biocides (Heilmann et al. 1995), (iii) antibiotics themselves might serve as substrates for non-target organisms (Alphei et al. 1995).

2.5.2. Bacteria and fungi contribution to N_2O production in agricultural soils

In this study, the lowest rates of N_2O production were recorded in the bronopol-treated (bactericide) soils compared to the controls and captan (fungicide) treatments (Fig. 2-4). These results suggested that bacteria were the main group of microorganisms contributing to N_2O production in the soils investigated, irrespective of tillage management. This statement is further approved by the computed F:B ratios based on N_2O concentrations (Table 2-2). A study by Herold et al. (2012) using arable soils also reported similar results with fungi and bacteria contributing 18% and 54% respectively of the total N_2O production. This finding is also consistent with the results of Seo and DeLaune (2010) who found that bacteria was responsible for the bulk of the denitrification activity in soils under strongly-reducing and anaerobic conditions, whereas fungi-mediated denitrification was of greater significance under aerobic or moderately reducing conditions. However, some studies have shown that fungi are the dominant organisms responsible for N_2O production in aerobic grassland soil (Laughlin et al.

2009). In this current study, results of F:B based on respiration (Table 2-2) indicated higher bacterial activity in the soils tested than fungal activity, which could be the reason for greater N_2O production from bacteria than fungi. It should be noted that this study was conducted under anaerobic condition which is not the naturally setting for agricultural soils and has to be tested on the same soils but under aerobic condition.

2.5.3. Tillage management impacts on soil bacteria and fungi

Under NT management, soils are less disturbed in comparison to PT and are generally covered with crop residue cover. This contributes to higher moisture content in NT, a soil environment that is likely favorable to the proliferation of denitrifying microbes (MacKenzie et al. 1997; Guggenberger et al. 1999; Bateman and Baggs. 2005). In addition, fungi are more likely to succeed in soil systems that are left undisturbed, allowing for the development of fungal hyphae which are in contact with crop residue on the land surface (Frey et al. 1999). In contrast, because of soil mixing and the direct contact between decomposers and substrates in PT soil systems, the microbial community is generally dominated by bacteria (Bailey et al. 2002).

Although bactericide addition resulted in lower concentrations of N_2O compared to controls (Fig. 2-4), captan only showed significant decrease in N_2O production in PT_1 ($P < 0.05$) (Fig. 2-4a). This could indicate that soil under PT management has denitrifier fungi community that is affected more easily by the fungicide compared to NT and WL soils. Since PT_2 (Fig. 2-4d) and NT_2 (Fig. 2-4e) showed the same pattern for biocide treated soils compared to the control, it could show that medium-term NT management

did not make changes in soil microflora responsible for N_2O production. This was also observed while comparing N_2O production in NT_1 and NT_2 production, which showed more inhibition of N_2O production in NT_1 (Fig. 2-4b) than NT_2 (Fig. 2-4e) in captan treatments. This could indicate larger population of denitrifier fungi in soil with longer duration of no-till management (NT_1).

Comparison of the N_2O inhibition data (Fig. 2-6) also showed that captan caused the highest level of inhibition in NT_1 compared to the other soils tested in the study. Significant difference ($P < 0.05$) between bronopol and captan treatments in all soils except NT_1, showed more contribution of bacteria to N_2O production compared to fungi. Although NT_1 with a 50-year duration of NT management had the highest inhibition of N_2O production in captan treatments compared to other captan-treated soils, bacteria still had greater contribution to N_2O production, based on the inhibition obtained from bronopol treatments (Fig. 2-4). This indicated the dominant role of bacteria in N_2O production even in the soils with long-term NT practice, which is not in agreement with the hypothesis of this study regarding fungal dominance of NT soils. Results by Herold et al. (2012) indicated that, although fungi play an important role in denitrification in soils under long-term NT management, the proportion of microbial community changes as a result of NT practice causing bacteria to be main contributors to N_2O production in soils irrespective to tillage practices. It should be noted that, besides tillage practice, other factors can contribute to making changes in soil microbial communities including soil organic carbon, pH, exchangeable base content, quality and quantity of crop residue (Lienhard et al. 2013).

2.5.4. Ratio of fungi to bacteria in soils under different tillage managements

Results of this study showed greater inhibition of N_2O production in soils treated with bronopol (bactericide) compared to captan (fungicide) (Fig. 2-4), which indicated bacterial dominance in N_2O production in the soils tested. Some studies suggested that N_2O production results do not necessarily imply to the microbial community in soils, since the proportion of microflora that are able to denitrify has been estimated to be less than 5% of the total soil microbial community (Henry et al. 2006), whereas, in some other studies N_2O production results have been used to define F:B ratio (Chen et al. 2015). In this current study, F:B ratios computed based on both CO_2 and N_2O (Table 2-2) indicated bacteria as the dominant producers of N_2O. Results of inhibitor additivity ratios (IAR), calculated to test the validity of F:B ratios, were the highest for NT soils compared to PT soils. These values greater than 1 for IARs were obtained by Scheu and Parkinson (1994), Imberger and Chiu (2001), Bailey et al. (2003). Since it has been suggested that the most accurate estimation of F:B are achieved when IAR is close to 1 (Seo and Delaune 2010), F:B ratios in NT and PT soils are of the lower and higher confidence, respectively. It is assumed that higher IARs for NT soils, similar to the results by Bailey et al. (2002), could be due to higher degree of non-target inhibition.

Although the F:B ratios (computed using respiration in controls and biocide-treated soils) showed no significant difference among the soils tested, it is important to note that the largest F:B ratio was measured in soil from the long-term (50 years) no-till site (Table 2-2).This would indicate an increase in F:B ratio with longer duration of NT management. A higher F:B ratio in NT soil compared to PT soils have been generally reported (Frey et al. 1999; Bailey et al. 2002; Jahangir et al. 2011). These studies

collectively suggested that a less intensive tillage system can be favorable to fungal growth. However, Johnson et al. (1999) noted that the sensitivity of the SI procedure is insufficient to detect the effect of land management.

2.6. Conclusion

One of the objectives of this study was to identify the types and levels of biocide leading to optimum inhibition of N_2O production in agricultural soils. Streptomycin and cycloheximide, the biocides most commonly used in selective inhibition assays, hardly resulted in any inhibition of N_2O production. In fact, in some of the soils, streptomycin stimulated the process. The reason for this lack of inhibition remains to be elucidated. Captan and bronopol, however, inhibited N_2O and CO_2 production, with an optimum concentration of 16 mg g^{-1} for both biocides. Fungi:bacteria ratios smaller than 1 were measured in all the soils tested, suggesting that bacteria were the dominant N_2O producers in the soils investigated. Although the difference was not significant, this ratio was at the highest in the long-term NT. Further studies on microorganisms species in agricultural soils with NT or PT management, and also investigation of how soil microbes are affected by the biocides used in this study could be helpful to fully understand the effects of biocides and tillage practices on soil microbial community and biotic control of N_2O production in agroecosystems.

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Table 2-1. Chemical properties of soils (0-10 cm) used in the study.

Site	Tillage	pH	Total C %	Total N %	Soil texture
S. Charleston (Ohio)	PT_1	7.16	1.26±0.10	0.15±0.02	Silt clay loam
	NT_1	6.14	2.12±0.02	0.20±0.05	Silt clay loam
	WL	5.54	3.14±0.12	0.25±0.06	Silt loam
Starkey (Indiana)	PT_2	6.41	1.33±0.23	0.19±0.06	Silt loam
	NT_2	7.42	1.85±0.16	0.17±0.02	Silt loam

Table 2-2. Fungi:Bacteria ratio (F:B) based on the cumulative CO_2 and N_2O concentration during the 168-hour experiment on soils with different tillage managements treated with either bronopol (bactericide) or captan (fungicide). Biocide was applied at a rate of 16 mg g^{-1} soil. Soils used in these assays were from sites under plow-till (PT_1 and PT_2), long-term (50 years, NT_1), and medium-term no-till (11 years, NT_2). Soils from a woodlot (WL) were also incubated for comparison.

Soil tillage	F:B (based on CO_2 concentration)	F:B (based on N_2O concentration)
PT_1	0.50	0.50
NT_1	0.67	0.87
WL	0.45	0.46
PT_2	0.43	0.24
NT_2	0.49	0.22

Table 2-3. Inhibitor additivity ratio based on the cumulative CO_2 concentration during the 168-hour experiment on soils with different tillage managements treated with either bronopol (bactericide), captan (fungicide) or their mixture (BroCap). Biocide was applied at a rate of 16 mg g^{-1} soil. Soils used in these assays were from sites under plow-till (PT_1 and PT_2), long-term (50 years, NT_1), and medium-term no-till (11 years, NT_2). Soils from a woodlot (WL) were also incubated for comparison.

Soil tillage	IAR
PT_1	1.02
NT_1	1.80
WL	1.46
PT_2	1.22
NT_2	1.71

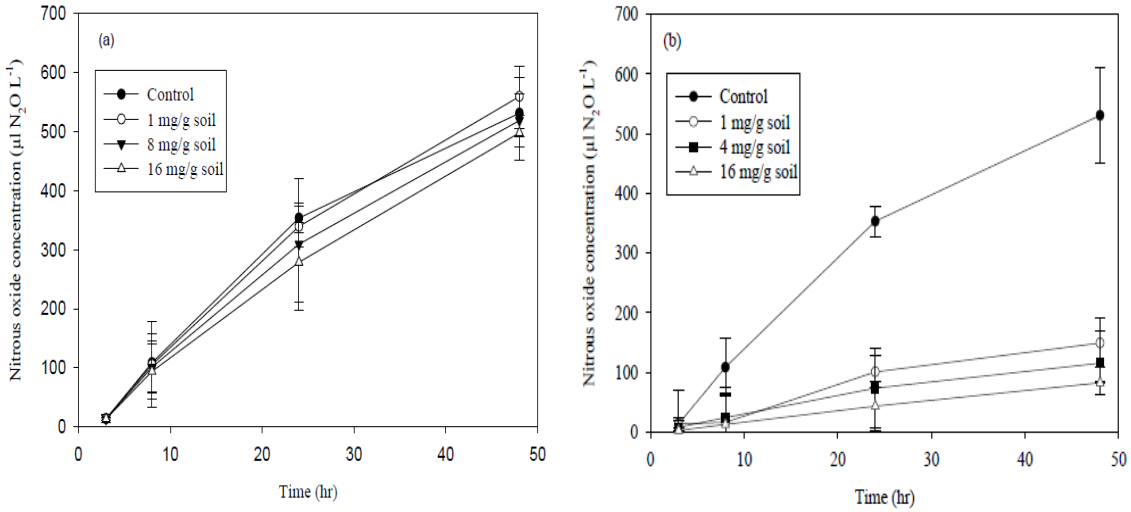

Fig. 2-1. Nitrous oxide production in control and soils treated with different concentrations of bactericide, (a) control vs. streptomycin, (b) control vs. bronopol. Error bars represent standard deviation from a mean of three replicates.

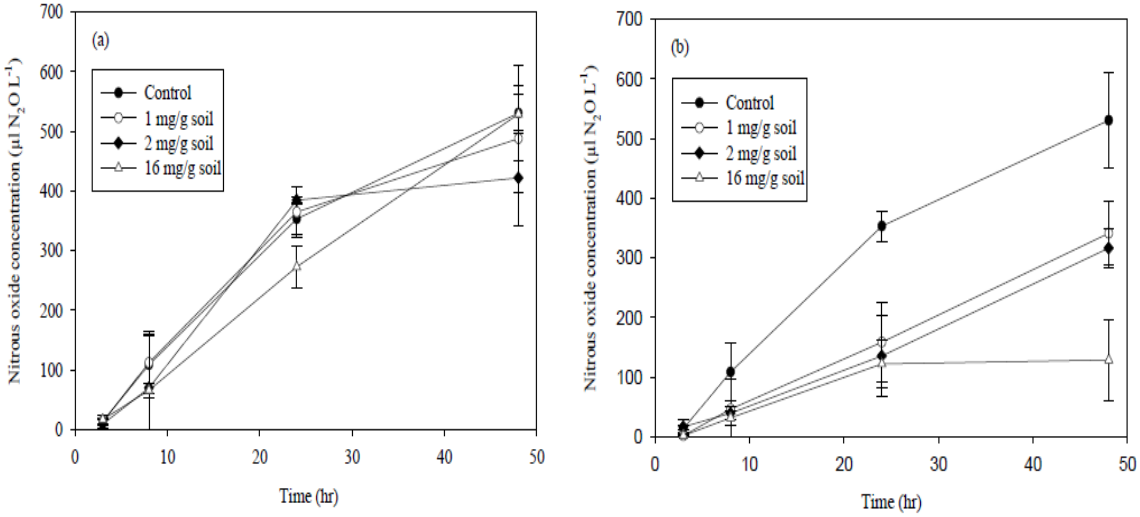

Fig. 2-2. Nitrous oxide production in control and soils treated with the different concentrations of fungicide, (a) control vs. cycloheximide, (b) control vs. captan. Error bars represent standard deviation from a mean of three replicates.

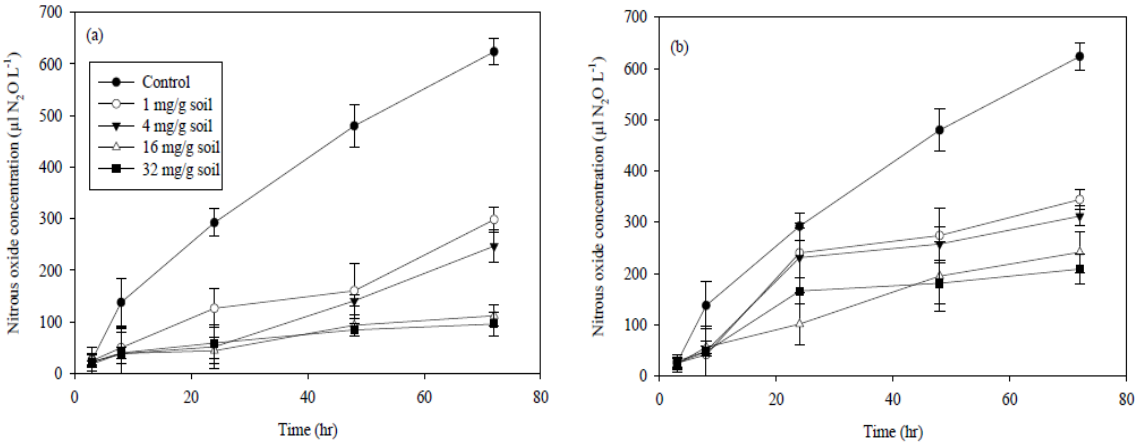

Fig. 2-3. Nitrous oxide production in control and soils treated with different concentrations of biocides, (a) control vs. bronopol, (b) control vs. captan. Error bars represent standard deviation from a mean of three replicates.

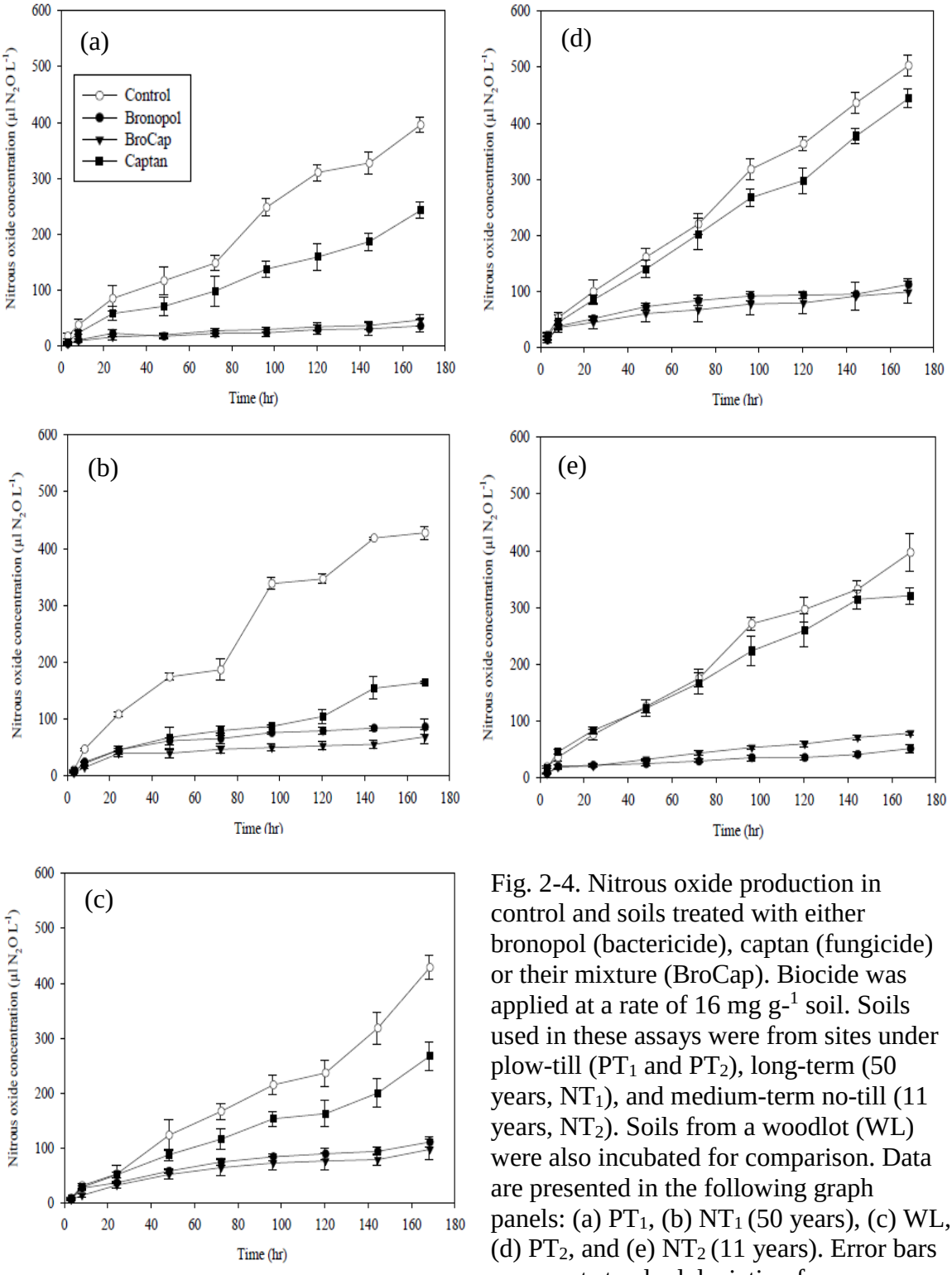

Fig. 2-4. Nitrous oxide production in control and soils treated with either bronopol (bactericide), captan (fungicide) or their mixture (BroCap). Biocide was applied at a rate of 16 mg g^{-1} soil. Soils used in these assays were from sites under plow-till (PT_1 and PT_2), long-term (50 years, NT_1), and medium-term no-till (11 years, NT_2). Soils from a woodlot (WL) were also incubated for comparison. Data are presented in the following graph panels: (a) PT_1, (b) NT_1 (50 years), (c) WL, (d) PT_2, and (e) NT_2 (11 years). Error bars represent standard deviation from a mean of three replicates.

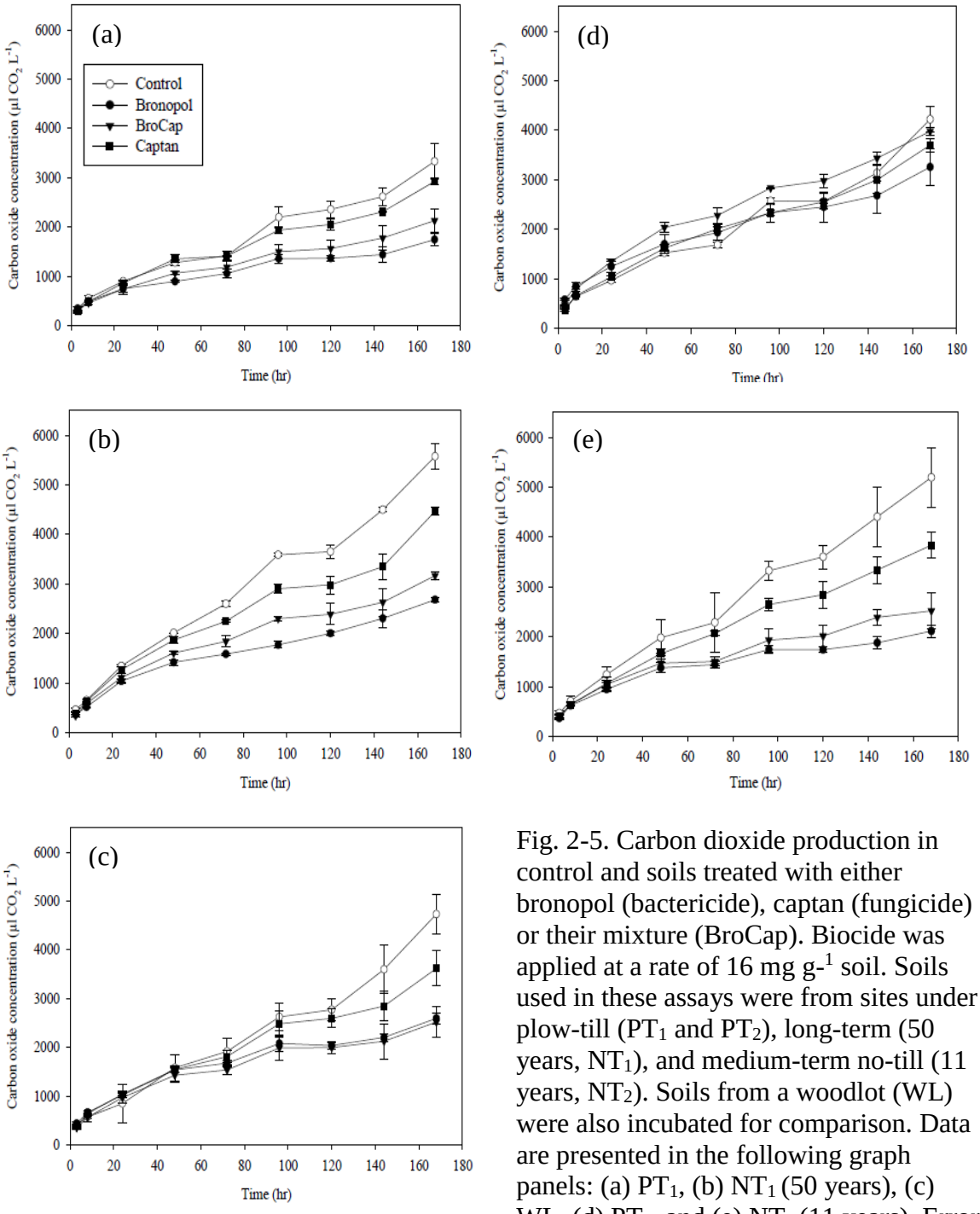

Fig. 2-5. Carbon dioxide production in control and soils treated with either bronopol (bactericide), captan (fungicide) or their mixture (BroCap). Biocide was applied at a rate of 16 mg g^{-1} soil. Soils used in these assays were from sites under plow-till (PT_1 and PT_2), long-term (50 years, NT_1), and medium-term no-till (11 years, NT_2). Soils from a woodlot (WL) were also incubated for comparison. Data are presented in the following graph panels: (a) PT_1, (b) NT_1 (50 years), (c) WL, (d) PT_2, and (e) NT_2 (11 years). Error bars represent standard deviation from a mean of three replicates.

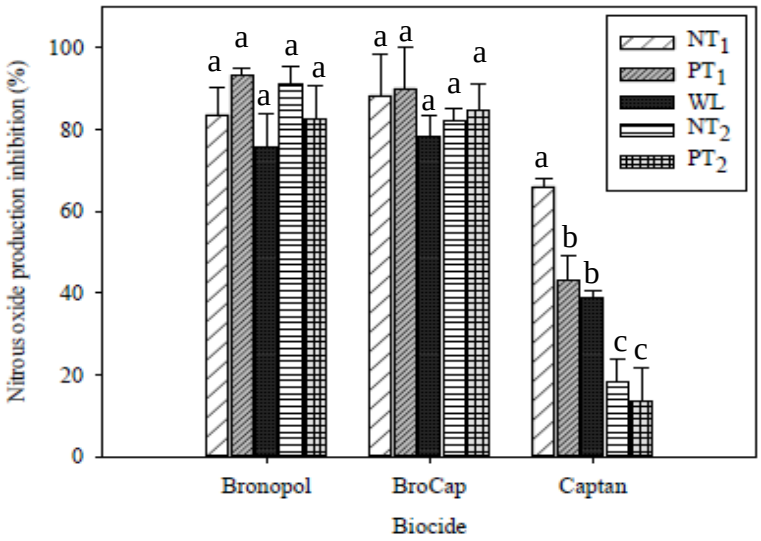

Fig. 2-6. Percent inhibition (%) of nitrous oxide production in soils treated with either bronopol (bactericide), captan (fungicide) or their mixture (BroCap). Biocide was applied at a rate of 16 mg g^{-1} soil. Soils used in these assays were from sites under plow-till (PT_1 and PT_2), long-term (50 years, NT_1), and medium-term no-till (11 years, NT_2). Soils from a woodlot (WL) were also incubated for comparison. Error bars represent standard deviation from a mean of three replicates.

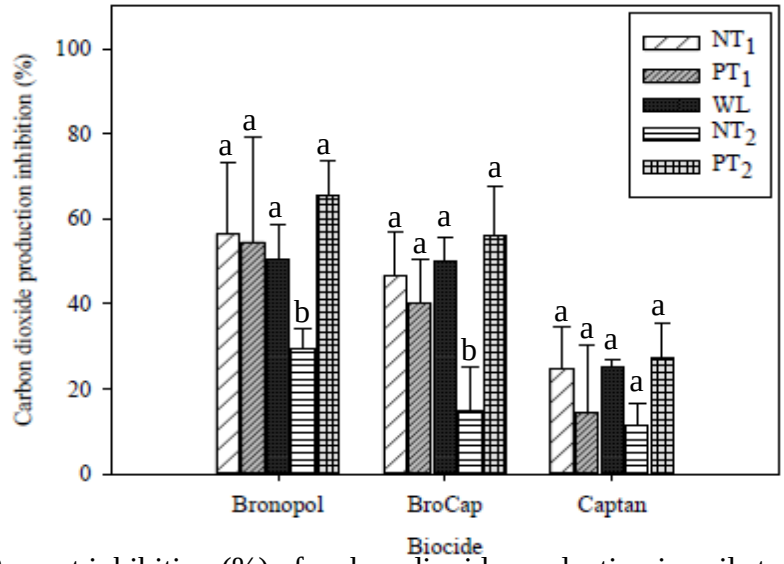

Fig. 2-7. Percent inhibition (%) of carbon dioxide production in soils treated with either bronopol (bactericide), captan (fungicide) or their mixture (BroCap). Biocide was applied at a rate of 16 mg g^{-1} soil. Soils used in these assays were from sites under plow-till (PT_1 and PT_2), long-term (50 years, NT_1), and medium-term no-till (11 years, NT_2). Soils from a woodlot (WL) were also incubated for comparison. Error bars represent standard deviation from a mean of three replicates.

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Chapter 3

New approaches to nitrogen management in agroecosystems under different tillage practice systems influenced by cover crops of different C/N ratios

3.1. Abstract

Heavy application of nitrogen (N) fertilizers in intensive agricultural systems contributes to water pollution through nitrate (NO_3^-) leaching, and emissions of nitrous oxide (N_2O), an atmospheric constituent implicated in the greenhouse effect and stratospheric ozone depletion. The incorporation of cover crop into crop rotation is a management practice that could provide a portion of the N needed by agricultural crops, and thereby would help to reduce N fertilizer input and N loss in agroecosystems. A 10-week greenhouse experiment was conducted to investigate the effects of two cover crops (application rate: 500 kg dry matter ha^{-1}), hairy vetch (*Vicia villosa*, C/N: 11) and rye (*Secale cereal*, C/N: 82), on mineral nitrogen flux, N_2O emission and corn growth in soils under no-till (NT, 11 years) and plow-till (PT). Results indicated that regardless of the tillage practice, the cover crop-amended treatments had higher NO_3^- and N_2O fluxes compared to the non-amended soils. The cover crops resulted in a net cumulative flux of NO_3^- averagely 2.87 kg N ha^{-1} higher than the controls. However, only the vetch treatments resulted in noticeably higher net cumulative N_2O emission (≈ 1.47 kg N ha^{-1} and 2.24 kg N ha^{-1} in PT and NT soil, respectively) than controls at the end of the experiment. Even though no significant difference was observed between rye and vetch with regard to NO_3^- flux, higher N_2O flux was observed in vetch-amended soils than in

rye treatments. In the rye treatment, corn plants were taller (≈ 135.5 cm) and had higher shoot/root biomass (≈ 15.69) compared to plants grown in the control and vetch-amended pots. Based on these results, rye could be suggested as a better cover crop option than vetch regardless of the tillage practice, leading to less N_2O emission and better corn growth in agricultural ecosystems. Even though both rye and vetch improved N availability and uptake compared to controls, they provide only supplemental N in agroecosystems.

3.2. Introduction

In modern agriculture, application of nitrogen fertilizer is needed to maintain crop productivity, but this activity has well documented impacts on water quality and greenhouse gases (GHG) emission (Smith et al., 2007). Although only a small portion (less than 3%) of applied N fertilizer is typically emitted as nitrous oxide (N_2O), these emissions can be considerable on national and global scales given the heavy reliance of intensive modern agriculture on synthetic N fertilizer (Robertson et al., 2000). It has been estimated that agriculture contributes 58% of the total anthropogenic N_2O emission (Smith et al., 2007).

Since N_2O emission has been shown to be positively correlated with N fertilizer application rates (Millar et al., 2010; Smith et al., 2011), reduction in N fertilizer input might be an effective way of mitigating N_2O emission, but this strategy could result in decreased crop yields, and thus not likely to be acceptable to farmers (Millar et al., 2010). There are some alternative practices which could help mitigate N_2O emission without

reducing crop yields, such as optimization of fertilizer source (more efficient slow-release fertilizers; Halvorson et al., 2010) or changing the depth of fertilizer placement (Omonode et al., 2011). The effectiveness of these practices in reducing N_2O emissions varies depending on local conditions and other factors (Martens, 2001).

Halvorson et al. (2010) assessed the ability of four controlled-released N fertilizer formulations to reduce N_2O emission from no-till (NT) corn fields in comparison to dry conventional urea (CU) and liquid urea ammonium nitrate (UAN). Measurements of N_2O fluxes during two growing seasons showed a significant reduction in N_2O emissions with the enhanced-efficiency fertilizers compared to the conventional N fertilizers with no reduction in grain production. Venterea et al. (2005) examined the combined effects of long-term tillage and fertilizer management on N_2O emission. Their results showed that, compared to conventional tillage (PT), no-till (NT) resulted in higher N_2O emission when urea was broadcast-applied (BU). However, when anhydrous ammonia (AA) was used, N_2O emission under NT was lower compared to PT. No difference between tillage practices was observed with surface UAN application.

Another study by Venterea et al. (2011) investigated the effects of long-term (>17 yr) tillage and no tillage practices and different N fertilizer sources on both area-based and yield-scaled N_2O emissions. Results of that study suggested that N_2O emission was higher in NT than PT, although the difference was not significant when results were reported on an area-scaled basis. With regard to fertilizer type, polymer-coated urea (PCU) and urea impregnated with a urease inhibitor (IU) resulted in higher N_2O emission compared to the other N sources. Hernandez-Rarmirez et al. (2009a) studied the effects of two different N fertilizers [urea-ammonium nitrate (UAN) and liquid swine manure

(SM)] on N_2O emissions from soils in the eastern Corn Belt. Results showed that N_2O production was lower with SM compared to UAN. But in another study by Hernandez-Ramirez et al. (2009 b), it was noted that N_2O emission from soils treated with different N sources could be affected by soil moisture content. These results suggest that, regardless of the type of N fertilizer applied, soils that are subject to rewetting events are most likely to produce higher amounts of N_2O.

Emission of N_2O from soils is known to be episodic, with the largest emission occurring during key periods. Several studies have shown that most of the N_2O emission from agricultural fields occurs after N fertilizer application, especially if that coincides with abundant rainfall (Hayatsu et al., 2008). Peaks in N_2O emission have also been observed following freeze-thaw cycles, after harvest and tillage operations (Sehy et al., 2003). Work by Millar et al. (2010) showed a non-linear exponential increase in N_2O emission when fertilizer is applied in excess of crop N demand for optimum production. Therefore, downward adjustments of N fertilizer application rate are needed in order to reduce agricultural N_2O emission. However, reduction in the rate of synthetic N fertilizer application must consider other strategies to providing growing crops with the amount of mineral N they need for optimum yield. Cover crop is an alternative N management strategy that can be combined with tillage practices, in order to reduce the rate of N fertilizer application, and consequently N_2O emission. Cover crop residue deposited on the soil surface provides organic substrate for microbial activity, and therefore controls both C and N cycling in terrestrial ecosystems (Brye et al., 2003). By influencing the amount and location (on surface or incorporated) of crop residue on soil surface, land use

and tillage management practices can influence microbial activity, N mineralization and the fate of mineral N in agro-ecosystems (Jarvis et al., 1996).

No-till practice can affect N transformation through its influence on soil microbial biomass and activity (Martens, 2001). A study on agricultural fields in eastern Montana found that NT practice resulted in larger amounts of soil N content (organic and mineral); however, PT increased mineral N availability (NH_4^+ and NO_3^- concentrations), and this could increase N losses through leaching (Stinner et al., 1984). Long-term NT has also been shown to enhance the efficiency of soil N cycling processes; NT could result in a larger pool of active organic N which may, in turn, lead to a decrease in net N mineralization and increase in net N immobilization (Christensen et al., 1994; Baggs et al., 2003; Hayatsu et al., 2008). Due to its effect on soil biological properties, NT practice could influence the timing and the amount of N release from decomposing cover crop residue (Martens, 2001).

Conventional tillage practices generally resulted in increased mineralization of soil organic matter (Stinner et al., 1984; Tracy et al., 1990) whereas NT practice has been shown to lower N mineralization, and this is thought to be the result of higher soil moisture, lower oxygen and soil temperature in NT soils than PT (Christensen et al., 1994). Immobilization can result in limited N availability for plant uptake (Martens, 2001). If N immobilization under NT practice is significant, N fertilizer application becomes necessary to prevent N deficiency in growing crops, but this would likely increase N_2O emission (Baggs et al., 2003). It is thought that cover crops could be a natural source of mineral N, and thus could help reduce the rate of synthetic N addition. Cover crop is an emerging nutrient management strategy, in which a crop (a grass or a

legume) is used to retain residual nutrients during the dormant season (Lu et al., 2000). At the end of its life cycle, plant nutrients are progressively released from the biomass of the decomposing cover crop biomass. Incorporating cover crop residue into the soil provides plants and microbes with a source of readily available C and N, and consequently could influence CO_2 and N_2O emissions. Bavin et al. (2009) evaluated the impact of reduced tillage (RT) and cover crop [winter rye (*Secale cereale*)] on N_2O emissions in east-central Minnesota. Results showed that N_2O emissions were almost similar under PT and RT but were mainly affected by the application rate and type of N fertilizers.

In addition to tillage practice, the chemical composition of cover crops must also be considered when assessing the impact of that practice on gaseous emissions. The carbon/nitrogen (C/N) ratio and lignin content of plant residue are important variables that could determine N mineralization kinetics in soil ecosystems. Mineralization of plant residues and N_2O emission was shown to be dependent on the C/N ratio of residue (Huang et al., 2004; Gomes et al., 2009). Negative correlations between C/N ratio of crop residues and N_2O emission have been reported (Huang et al., 2004). Greater rates of soil N_2O emissions have been observed when the added crop residue has a low C/N ratio and low lignin:N ratio (Millar and Baggs, 2004; Huang et al., 2004).

Huang et al. (2004) studied the effect of five crop residues with different C/N ratios (rapeseed cake: 8; potato stalk: 37; maize leaf: 57; wheat straw: 63; sugarcane stalk: 118) on N mineralization and consequently N_2O emission. Residue was incubated with or without urea addition. After 21 days of incubation, the results showed the treatment that received residues plus urea had more N_2O emission than the urea-free

treatments. Rapeseed with the lowest C/N ratio had the highest N_2O emission whereas sugarcane, with the highest C/N ratio, resulted in the lowest N_2O emission (Huang et al., 2004). It has also been shown that at C/N ratios in the 20-30 range, the net N immobilization and N mineralization tend to be of similar magnitude. That implies that cover crop residue with C/N ratios lower than 20 could lead to net N mineralization whereas C/N ratios higher than 30 could result in N immobilization (Vigil and Kissel, 1991). Therefore, sugarcane with the C/N ratio higher than 30 resulted in greater N immobilization and subsequently less N_2O emission than the other crop biomass (Ellis et al., 1996; Huang et al., 2004). These laboratory data were later supported by the results of an empirical model (Mu et al., 2009) that predicted less N_2O emission with higher C/N ratios of crop residue.

When an agricultural system includes a combination of NT and cover crops, the amount of mineralized N available for plant uptake needs to be considered in order to determine how the rate of chemical fertilizer application can be adjusted to mitigate agricultural N_2O emission. NT practices (short-term or long-term) could influence N mineralization and immobilization in soils (Tracy et al., 1990; Hayatsu et al., 2008). On the other hand, cover crops with low C/N ratios can lead to enhanced N mineralization whereas cover crops with high C/N ratio can result in N immobilization (Huang et al., 2004). Therefore, in order to implement cover crop as an approach to reduce N_2O emission from agricultural fields, one needs to determine the C/N ratio of the applied cover crop and also know the tillage management history of the field. In other words, the selection of cover crop (grass vs legume) and its management (e.g. when to kill the cover crop before spring planting) must consider both the chemical composition (C/N) of the

cover crop biomass and the N cycling direction (immobilization/mineralization) of the receiving soils. It is of great importance to understand the timing of mineral N release from soil organic matter, especially early in the growing season when plant N needs can be greatest. Since different tillage systems affect how soils and crop residues are mixed, they could affect soil microbial biomass and activity and consequently, the release of plant available N (Hayatsu et al., 2008).

To investigate these questions, a greenhouse experiment was conducted using soils under plow-till (PT) and no-till (NT) practices amended with different cover crops (varying C/N ratios), and the fates of N from cover crop was investigated. The following pathways were considered: gaseous emission (N_2O), N mineralization and assimilation by vegetation. Plant uptake was taken as a measure of N-use efficiency, and thus considered as the most desirable outcome. It is hypothesized that cover crops with low C/N ratios are more suitable for sites under NT, whereas cover crops with high C/N ratio is preferable for PT soils. The overall goal of this study was to identify the best cover crops for fields under NT and PT.

3.3. Materials and methods

3.3.1. Greenhouse experiment design

This experiment was conducted from February to May 2015 in a greenhouse located on the top of the Science Building on the campus of Indiana University- Purdue University Indianapolis, Indiana (USA). Soil (0-25 cm) for this study was collected from agricultural fields in Hendricks county Indiana (39°51′ 49″N, 86°21′31″W). Management

practices at these fields included conventional tillage (plow-till, PT) and no-till (NT, 11 years). Soils at the sampling sites were intergraded between the Crosby (fine-silt loamy mesic aeric Epiaqualfs) and the Brookston (fine-loamy mesic typic Argiaquolls) series. Pots (37-cm height, 49.7-cm width, 49.7-cm diameter) (item# 131415, model# US828102) and saucers (40.6-cm diameter) (item# 485109, model# P13011611286S) used in this experiment were purchased from Lowe's. A layer of 5-cm acid-washed pea gravel was placed at the bottom of each pot before being transported to the field. Pots were filled with either NT or PT surface soils (0-25 cm). The total number of pots was 18 [2 types of soils (NT and PT), 3 cover crops (control, rye and hairy vetch) and 3 replications]. Pots were transported to the greenhouse, set on the saucers, irrigated and left on the bench for 7 days. At the beginning of the experiment, each pot was amended with a solution of potassium nitrate (KNO_3) and dipotassium phosphate (K_2HPO_4) to provide an equivalent 20 kg N ha^{-1} and 10 kg P ha^{-1}. A second application of 40 kg N ha^{-1} as ammonium nitrate (NH_4NO_3) was made 60 days after the beginning of the experiment as the pool of mineral N was depleted and the corn plants began to show signs of nutrient deficiency.

3.3.2. Cover crops collection and corn planting

The cover crops selected for this study are among the most commonly-used cover crops in US Midwest agricultural fields, and included hairy vetch (*Vicia villosa*) (with ≈ 42% and 3.8% C and N content, respectively) and cereal rye (*Secale cereal*) (with ≈ 82% and 1% C and N content, respectively). These cover crops have contrasting chemical

composition, i.e. hairy vetch has a C/N ratio of 11:1 and rye has a C/N ratio of 82:1 (USDA, 1977). Rye was collected from Starkey farm in Brownsburg, Indiana. Vetch was collected from a field maintained by the Indiana Department of Transportation (INDOT) near Lebanon, Indiana. Collected cover crops were transported to the laboratory, air and oven-dried (65 ^{o}C), and shredded. An amount of 10 g of each dried cover crop (equivalent to 500 kg dry matter ha^{-1}) was weighed and spread on the soil surface of each pot. No residue was added to the control pots.

The next day, three seeds of corn (*Zea mays* var. *dwarf*) were planted in each pot. Following corn seeds germination, two plants were removed and only one corn seedling (the most vigorous) was left in each pot. Pots were irrigated once a week during the experiment to keep the gravimetric soil moisture between 25-35%. Soil moisture was measured each time measurements of N_2O and mineral N fluxes were made. The temperature in the greenhouse was between 18-23 ^{o}C during the experiment. Light was provided for 12 hours each day.

3.3.3. Soil mineral nitrogen monitoring

Soil mineral nitrogen (NH_4^+ and NO_3^-) was monitored on a weekly basis in each of the 18 pots using cation and anion PRS^{TM}-probes obtained from Western Ag Innovations Inc, Saskatoon, Canada. Mineral N absorbed by the PRS^{TM}-probes simulates the amount of mineral N that can be taken by plant roots. One anion and one cation PRS^{TM}-probe was labeled and inserted near the corn roots in each pot (≈ 10 cm depth). Every week, PRS^{TM}-probes were removed from the pots and immediately transported to

the laboratory for elution within minutes of their removal. PRS^{TM}-probes were washed with deionized (DI) water to remove soil particles from the membranes. Each washed PRS^{TM}-probe was eluted by adding 35 mL (17.5 mL for each probe, corresponds to 17.5 cm^2 surface area of the PRS^{TM}-probe membrane) of a 0.5 N hydrochloric acid (HCl) solution in a zip lock bag. Air bubbles were pushed away from the membrane surface and removed from the bags as much as possible to ensure PRS^{TM}-probes were completely immersed in the acid solution. After an hour, the eluate from each bag was transferred to a 20-mL clean plastic vial and stored in a freezer until analysis. PRS^{TM}-probes were removed from the elution bags, and prepared for cleaning and regeneration. Used PRS^{TM}-probes were cleaned in a soaking solution of 0.5 N HCl for an hour on a end-over-end shaker.

Clean PRS^{TM}-probes were regenerated by soaking in a 0.5 N sodium bicarbonate ($NaHCO_3$) solution four times at one-hour intervals. Regenerated PRS^{TM}-probes were rinsed with DI and returned to the pots the same day they were removed. During the experiment, three anion and cation PRS^{TM}-probes were kept in a sealed bag containing DI water as blanks in a refrigerator. Samples taken from the PRS^{TM}-probes were measured for NH_4^+ and NO_3^-.

Fluxes of NH_4^+ and NO_3^- required the consideration of a number of factors, including the number and type of (anion or cation) eluted at one time, total ion exchange membrane surface area eluted and the volume of eluent used. Computations were as follows:

$$\frac{\mu g\ nurient}{mL\ of\ eluate} * \frac{17.5\ mL\ eluent}{probe} * \frac{total\ \#\ of\ probes}{bag} * \frac{bag}{\#\ of\ relevant\ probe\ type}$$

$$* \frac{probe}{17.5\ cm^2} * 20 = \frac{\mu g\ nutrient}{cm^2}$$

Soil samples from different depths (0-5, 5-10, >10 cm) were collected from each pot 60 days after the beginning of the experiment. Soil samples were sieved (2-mm sieve), and a subsample (10 g) was weighed and placed in a 50 mL centrifuge tubes and was extracted with 30 mL of 1 M potassium chloride solution (soil:KCl ratio of 1:3). The suspension was shaken for an hour and centrifuged for 5 minutes at 3000 rpm. Supernatants were filtered through a paper filter (Whatman 42) and extracts were stored in 20 mL clean plastic vials in a freezer until analyzed for NH_4^+ and NO_3^-.

3.3.4. Nitrous oxide fluxes monitoring

During the experiment, nitrous oxide fluxes were monitored 2 days after each irrigation events using static chambers. Static chambers (15-cm height, 10-cm diameter) were made of PVC cylinders. After fertilizers application and spreading cover crops on the soil surface, static chambers were inserted into the soil, leaving 10 cm above the soil surface.

During sampling, chambers were covered with PVC lids (11-cm diameter) secured to the base with bungee cords attached to the sides of chambers. The lid was fitted with a gasket at its underside edge to make an air-tight seal and with a butyl rubber septa at its center to form a sampling port. Chamber headspace was sampled 0, 45 and 90 minutes after closure, and air samples (~ 20 mL) were stored in 20-mL evacuated glass

vials fitted with butyl rubber septa. Concentration of N_2O was measured by gas chromatography (electron detector capture, ECD). Daily fluxes of N_2O (F) (μg N_2O cm^{-2} $hour^{-1}$) were computed as:

$$F = \frac{dC}{dt}\frac{V}{A}k$$

where dC/dt is the N_2O concentration change in chamber headspace (μg N_2O-N m^{-3} min^{-1}), V is the chamber volume (m^3), A is the area of soil circumscribed by chamber (m^2), and k is the time conversion factor (1440 min day^{-1}) (Fisher et al., 2014).

3.3.5. Analytical methods

Soil samples were collected in October 2014 at the study sites to determine soil properties. Soil samples were dried, and sieved (2-mm sieve) for determination of chemical properties. Soil pH was measured with an Accumet model 25 pH/ion meter (soil/water ratio of 1:2 w/v), calibrated using pH 4 and 7 buffers. Particle size was determined by the hydrometer method after dispersion of soil with sodium hexametaphosphate ($Na_6P_6O_{18}$) (50 g L^{-1}) (Table 3-1).

After seven weeks of growth, corn height was measured. At the end of the experiment, corn height was measured again before the corn biomass harvested. Shoots and roots were separated, washed, oven-dried and weighed. Subsamples of leaf/root tissues were crushed using a rolling grinder. To determine total carbon and nitrogen in soil and plant samples an aliquot of each dried and crushed soil or plant sample that

passed through a 150-μm sieve was weighed (soil samples 7-9 mg, plant samples 2-3 mg) and analyzed for C and N contents by dry combustion (960 ^{o}C) using a Vario-Cube analyzer (Elementar Americas, New Jersey, USA). Eluates from the PRS-probes and soil KCl extracts were analyzed for NH_4^+ and NO_3^- using US Environmental Protection Agency (EPA) method 350.2 (for NH_4^+) and 353.1 (for NO_3^-) on a Konelab Aquakem analyzer.

3.3.6. Statistical Analysis

Data were first tested for normality using the normality test available in the Sigma Plot software (Systat, San Jose, CA). Since most of the data were not normally distributed, Kruskal-Wallis test was used to determine the significance of the experimental factors (soil tillage and cover crop) on N_2O and mineral nitrogen (NH_4^+ and NO_3^-) fluxes and corn growth during phase 1 (corresponding to the first 60 days) and phase 2 (between day 60 and harvest on day 85), separately. The Kruskal-Wallis test was followed by Mann-Whitney pairwise test when a significant difference was detected. Unless otherwise noted, statistical significance in this study was determined at α=0.05. Statistical tests were conducted using PAST software (ver. 2.17c) downloaded from http://nhm2.uio.no/norlex/past/download.html (University of Oslo).

3.4. Results

3.4.1. Soil mineral nitrogen flux

Ammonium concentration in the PRS^{TM}-probes eluates were near zero in most cases. Therefore, no further description of this mineral N species is provided in this chapter. However, NO_3^- concentrations were detected and measured in the samples taken by the anion PRS^{TM}-probes during the experiment. Results of NO_3^- flux are shown in two different phases; phase 1: before second fertilizer application (Fig. 3-1) and phase 2: after second fertilizer application (Fig. 3-2). In phase 1, a similar pattern for NO_3^- flux was observed in both PT and NT soils (Fig. 3-1a and 3-1b), i.e. NO_3^- flux was the highest during the first two weeks and then gradually decreased in all treatments. This gradual decrease was more visible in PT soil (Fig. 3-1a). In both soils, cover crop amendment resulted in higher NO_3^- fluxes compared to controls. During the first two or three weeks of phase 1 of the experiment, there was no significant difference ($P > 0.05$) between controls and cover crop treatments with regard to NO_3^- fluxes (Fig. 3-1) (Table 3-2). However, from week three to the end of phase 1, cover crops amended treatments showed significantly higher NO_3^- flux than controls in both soils (Fig. 3-1). No significant difference ($P > 0.05$) was observed in NO_3^- flux between the two types of cover crops (Fig. 3-1) (Table 3-2).

After the second fertilizer application, in both PT and NT soils, there was an increase in NO_3^- flux which lasted about one week, and then gradually decreased over time (Fig. 3-2). During phase 2 (regardless of tillage and sampling time), NO_3^- flux was significantly higher ($P < 0.05$) in the cover crop treatments than in the controls. However,

during that phase, the cover crop treatments were not significantly different from each other ($P > 0.05$) in terms of NO_3^- flux (Table 3-3). Although NT soil showed higher NO_3^- flux than PT soil at some sampling times, comparison of PT and NT soils in terms of NO_3^- flux showed no significant difference ($P > 0.05$) (Table 3-3)., i.e. the studied soils under different tillage practices resulted in similar NO_3^- flux in both phase 1 and 2 of this experiment.

Measurements of NO_3^- concentration measured in different soil depths at the end of phase 1 showed that most of the NO_3^- pool was present in the first 5 cm of both PT and NT soil (Figs. 3-3 and 3-4). This depth distribution suggests limited leaching or NO_3^- accumulation at depth. Nitrate concentration in cover crop treatments was significantly higher ($P < 0.05$) than controls in the first 5 cm in both soils (Figs. 3-3 and 3-4) (Table 3-4).The rye treatments showed significantly ($P < 0.05$) higher NO_3^- concentration than vetch in the surface soil layers (0-5 and 5-10 cm) for both PT and NT soils (Figs. 3-3 and 3-4). Observations at the end of phase 1 also showed that the hairy vetch biomass was almost completely decomposed, with only a few stems left on the soil surface. However, rye residue was still observable and persisted to the end of the experiment.

Cumulative NO_3^- flux in both PT and NT soil also demonstrated that cover crop application resulted in higher NO_3^- fluxes than controls in both phases of the experiment (Figs. 3-5 and 3-6). Net cumulative NO_3^- flux from rye and vetch was almost similar to each other at the end of phase 1 and 2. At the end of phase 1, both cover crops resulted in an average net cumulative NO_3^- flux of 0.75 kg N ha^{-1} in PT soil (Fig. 3-5) and 1.52 kg N ha^{-1} in NT soil (Fig. 3-6). At the end of the experiment, the net cumulative NO_3^- flux (averaged over the cover crops) in PT (Fig. 3-5) and NT soil (Fig. 3-6) was 2.85 and 2.90

kg N ha^{-1}, respectively. At the end of phase 1, the net cumulative NO_3^- flux from cover crops was not significantly ($P > 0.05$) higher in NT (Fig. 3-6) than PT soil (Fig. 3-5) (Table 3-2), whereas, both soils resulted in almost the same net cumulative NO_3^- flux from cover crops at the end of the experiment.

3.4.2. Nitrous oxide flux

During phase 1, the highest fluxes of N_2O were measured in the first two (PT soil, Fig. 3-7a) and three (NT soil, Fig. 3-7b) weeks. Nitrous oxide emission then gradually decreased over time in all treatments. Cover crop treatments showed higher N_2O flux compared to controls in both soils. In general, in phase 1, the highest N_2O flux was observed in the vetch treatments in both PT and NT soils (Fig. 3-7), and the difference between cover crops was significant in the first two sampling weeks ($P < 0.05$) (Table 3-2). For the remainder of phase 1, in both NT and PT soils, the vetch treatments still had higher N_2O flux than rye, although this difference was not significant ($P > 0.05$) (Table 3-2). After the second fertilizer application (phase 2 of the experiment), N_2O fluxes increased again (Fig. 3-8), and this increase was at the highest in the PT rye treatments (Fig. 3-8a). After week 1 of phase 2, in both PT and NT soil, N_2O fluxes gradually decreased in all treatments (Fig. 3-8) and no significant difference was observed between the treatments (Table 3-3). Overall, N_2O flux was generally higher in NT (Figs. 3-7b and 3-8b) than PT soils (Figs. 3-7a and 3-8a). The difference between PT and NT soil was mainly noticeable in the vetch treatments.

The cumulative N_2O emission measured at the end of both phase 1 and 2, was significantly ($P < 0.05$) (Tables 3-2 and 3-3) higher in the vetch treatments than in the rye regardless of tillage (Figs. 3-9 and 3-10). Rye resulted in a small (≈ 0.64 kg N ha^{-1}) net cumulative N_2O flux in both soils. The cumulative N_2O emission was also higher in NT soil (Fig. 3-10) than PT soil (Fig. 3-9) at the end of both phase 1 and 2 and this difference was significant ($P < 0.05$) in the vetch treatment (Tables 3-2 and 3-3). In NT soil, the vetch treatment resulted in 2.18 and 2.24 kg N ha^{-1} of net cumulative N_2O flux at the end of phase 1 and 2, respectively (Fig. 3-10). However, these values for the vetch treatment in PT soil were 1.34 and 1.47 kg N ha^{-1} at the end of phase 1 and 2, respectively (Fig. 3-9). The amount of the N emitted as N_2O accounted for 0.72%, 1.5% and 2.1% of the mineral n fertilizer applied in the control, rye and vetch treatments, respectively in PT soil. In NT soil, N_2O emission represented 0.6%, 1.2% and 2.7 % after mineral N applied to these respective treatments. As mentioned earlier, the vetch treatment had higher N_2O flux than rye. While no clear trend between NO_3^- and N_2O fluxes was observed in NT soil, strong positive relationships between these variables were observed in PT soil (Fig. 3-11).

3.4.3. Corn shoot and root biomass

Corn growth observation in the greenhouse always indicated taller and bigger plants with rye application compared to control and vetch treatments regardless of tillage practice. This observation was also numerically supported by the measurements of corn height and biomass (Tables 3-5 and 3-6). Irrespective of tillage practices, control plants

were significantly shorter than cover crop-amended plants ($P < 0.05$) (Tables 3-7 and 3-8). The rye-amended plants were significantly taller than those in the vetch treatment ($P < 0.05$) (Tables 3-7 and 3-8). Corn height in PT and NT soils was not significantly different ($P > 0.05$), although in the rye-amended treatments better growth (height and biomass) was observed PT than in NT soil.

In both PT and NT soils, corn biomass was in the order rye > vetch > control (Tables 3-5 and 3-6). Corn plants grown in the rye-amended pots had significantly ($P < 0.05$) higher biomass than those in the vetch treatment (Tables 3-7 and 3-8). The shoot/root ratios (Table 3-6) were also much greater with the rye treatment compared to vetch and control. In general, no significant effect of soil tillage was detected with respect to corn biomass and shoot/root ratio (Table 3-8). The chemical composition (C, N, C/N) of corn shoot and root (Table 3-9) did not show any significant difference ($P > 0.05$) among treatments (Table 3-10). Total N uptake by corn plants in both PT and NT soils was in the order: rye > vetch > control (Table 3-11). This ranking is consistent with the observation of better corn growth with the rye than the vetch treatment. Results also showed that corn grown in NT soil had higher N uptake than corn grown in PT soil (Table 3-11).

Post-harvest analysis of corn tissue showed the lowest C/N ratio in the plants grown in the vetch-amended soils (Table 3-13). In other words, cover crop amendment resulted in lower soil C/N ratio compared to controls but this decrease was more noticeable in the vetch treatment, likely due to lower C/N ratio of the vetch than rye residue. Although PT and NT soils with cover crop added, had lower C/N compared to

control, no significant effect ($P > 0.05$) of the treatments on soil C and N content was detected (Table 3-14).

3.5. Discussion

3.5.1. Decomposition rate of cover crop biomass

Two months after the experiment began, the hairy vetch biomass was almost completely decomposed, whereas a good portion of the rye residue was still present on the soil surface in the experimental pots. This observation is similar to those reported in several previous studies (Zougmore et al., 2006; Nagumo and Nakamura 2013) in which only 5% of hairy vetch residue typically remaining at the end of a corn season. These results confirm the rapid mineralization and early release of mineral N from hairy vetch, and from the biomass of other leguminous cover crops that have low C/N ratios. This rapid decomposition of vetch may have led to the high NO_3^- fluxes measured at the beginning of the experiment. This enhancement in NO_3^- release was likely due to the sudden exposure of soil microbes to a large supply of C and N-rich substrates, including easily decomposable non-protein N and soluble protein N. Lee et al. (2002) noted that vetch lost 72-81% of its initial weight one month after application, leading to the conclusion that hairy vetch could be a substitute N starter fertilizer but not a sustainable N source due to its fast decomposition. Results of several past studies (Lee et al., 2002; Ruffo et al., 2003; Muhammad et al., 2011) suggested that, due to its higher N concentration, the leaf portion of crop residue decomposes faster than the stem and root portions. Although these factors may have played a role in the observed physical loss of

cover crop mass, this cannot be accounted for in the present study because the cover crop applied was a mixture of stems and leaves. Further, under field conditions, cover crop roots remain buried in the soils as they undergo decomposition; therefore, the N-supplying capacity of hairy vetch (and other legume cover crops) may last longer than the results of the present would suggest.

3.5.2. Mineral nitrogen release: Relative significance of tillage practice and cover crop composition

As noted previously, NH_4^+ flux was negligible in almost all treatments. That was an unexpected result that could be due to the rapid nitrification of the NH_4^+ evolved from ammonification of organic N. A study by Sainju et al. (2007), in which NH_4^+ fertilizer was applied, reported similar results; significant accumulation of NH_4^+ was only detected in treatments receiving N fertilizer in the 120-130 kg N ha^{-1} application rates, several fold greater than the amount applied in the present experiment.

The observed enhancement in NO_3^- release with cover crop addition is in accord with the findings of Frimpong et al. (2011) who reported increased NO_3^- release following soil incorporation of maize residue. Similar to the temporal pattern observed in the present study, pulses in soil NO_3^- were short-lived, generally lasting two weeks or less (Frimpong et al., 2011). In other words, following cover crop addition, N mineralization occurred rapidly due to the large amount of readily mineralizable C and N supplied by the fresh biomass, but the process decreased substantially over time as mineralized N was presumably consumed by growing corn plants and soil microorganisms.

Although difference was not always significant, NO_3^- flux was generally higher in the rye-treated soils than with the vetch (Fig. 3-1, Table 3-1). It should also be noted that the vetch treatment produced a few short-lived spikes in NO_3^- flux during the first 2 weeks of the experiment, especially in the NT soils (Fig. 3-1b).

Because of their chemical composition and physical structure, the biomass of legume plants should decompose faster than that of cereals. Observations consistent with that expectation were made in this experiment, as nearly all hairy vetch biomass was decomposed in about 7 weeks. In contrast, the rye biomass lasted much longer. These results suggest that N contained in hairy vetch biomass may have been released very rapidly, before the corn seedlings could fully utilize the available N. In contrast with rye, decomposition and mineral N release were more gradual and better synchronized with corn plants development. Similar patterns were reported in a study by Ruffo and Bollero (2003) in which hairy vetch and rye decomposition and NO_3^- release were compared. These authors (Ruffo and Bollero, 2003) concluded that rye was a more suitable material than vetch with regard to N supply to plants and soil conservation. In a decomposition study involving these same plant materials, Kuo and Sainju (1998) noted that rye, as a cover crop, could increase the crossover time of net N mineralization (i.e. the time when the amount of net N mineralized in the residue-amended soil is equal to that of control). Therefore, with rye addition the release of mineral N is more sustained, and its timing is better synchronized with crop N needs in the early growth phase. Vidal and Lopez (2005) noted that, due to the slow N release, rye could be a more appropriate cover crop to prevent NO_3^- leaching and water pollution. Therefore, from a soil fertility standpoint, rye can be proposed as a better cover crop option than vetch.

A central hypothesis of this study was that tillage management history could dictate the performance of different types of cover crops applied to agricultural soils. Specifically, it was hypothesized that legume cover crops (low C/N ratio) would be more suitable for NT soils, and that cereal cover crops (high C/N ratio) would be preferable for soils under conventional tillage (PT). The study results did not, however, support that hypothesis. In this study, tillage management (PT and NT) did not significantly affect NO_3^- flux (Fig. 3-1; Table 3-1) and had marginal effect on N_2O emission. These results are in accord with the findings of Dalal et al. (2011) who reported no significant effect of tillage practices (NT and PT) on mineral N release from agricultural soils in which crop residue was retained. However, in other studies (Sainju et al., 2007, 2012), involving vetch and rye as cover crops in NT and PT soils, higher NO_3^- concentration was measured in PT soil with vetch incorporation than in the other treatments. Further, these studies (Sainju et al., 2007, 2012) also reported higher rate of N leaching in the vetch compared to the rye treatment. Therefore, these authors (Sainju et al., 2007, 2012) proposed a mixture of rye and vetch for either NT or PT system. Results of the present study concur with that suggestion.

Both rye and vetch treatments resulted in NO_3^- fluxes and soil NO_3^- pool 1.5-2-fold higher than controls. These results demonstrated that cover crops can be a source of N for growing crops, and therefore inclusion of cover crops in farming systems could result in a reduction of N fertilizer applied to agricultural soils. That should prove economically and environmentally beneficial given the deleterious ecological impacts of increased reactive N in natural systems. Although cover crop can be advocated as a viable N management strategy (Lee et al., 2002; Sainju et al., 2007, 2012), it is important

to recognize that the practice can, for most crops, only provide a portion of the needed N. As stated previously, N fertilizer had to be applied around day 60 in this experiment when corn growth was visibly impaired by a lack of mineral N.

3.5.3. Nitrous oxide emission: Relative significance of tillage practice and cover crop composition

In accord with several past studies (He et al., 2007; Muhammad et al., 2011; Mitchell et al., 2013), N_2O fluxes were highest in the 2-3weeks following fertilizer application, and higher in the cover crop treatment than controls (Fig. 3-3).Largely a product of denitrification in soils, the intensity of N_2O production is often controlled by the availability of NO_3^- and organic C to support the activity of heterotrophic denitrifiers. Mitchell et al. (2013) and Pimentel et al. (2015) suggested that increased N_2O emission in cover crop-amended soils can be linked to increased availability of labile C to denitrifiers as a result of cover crop application.

Although the cover crops did not significantly differ with regard to N_2O emission, vetch (low C/N ratio) addition resulted in higher N_2O flux compared to rye (high C/N ratio). A similar negative trend between cover crop C/N ratio and N_2O flux was reported by Huang et al. (2004). This result is also in accord with the finding of Zschornack et al. (2011) who, in an investigation of N_2O emission from soils treated with the legume species serradella and ryegrass, measured higher emission with the legume cover crop. These results are also in agreement with those of others (Constantinides and Fownes, 1994; Millar and Baggs, 2004), and the general negative relationship between N_2O

emission and C/N ratio of crop residue (Huang et al., 2004). Additionally, results of the present study showed strong positive relationships between N_2O emission and NO_3^- flux, as a measure of mineral N availability. The slope of the relationship was much steeper (3 times) in vetch-amended soil compared to rye. This is an important and novel observation, and it underscores the propensity of NO_3^- evolved in the vetch treatment to undergo denitrification. That could be due to greater biodegradability of vetch-derived organic C, as well as poor synchronization between crop needs and the timing of NO_3^- release from decomposing vetch biomass. When plant N uptake is negligible, the conversion of NO_3^- to N_2O becomes the most favored fate. Finally, it is conceivable that the composition of the soil denitrifying population was differently affected by each type of cover crop, and that ultimately may have resulted in variable enhancement in N_2O production. To assess the merit of that contention, laboratory-scale investigations must be conducted to examine shifts in the size and diversity of the denitrifying community in soils treated with cover crops of varying composition.

Cumulative N_2O emission was not significantly affected by tillage practices, but was strongly related to the type of cover crop. Several studies have reported statistically significant interactions between cover crop and tillage practice, with higher N_2O emission generally observed with reduced tillage than with PT under similar cover crop treatment (Peterson et al., 2011; Abdalla et al., 2012). During phase 2 of the present study, a borderline interaction between these factors was detected (Table 3-2). However, the most striking observation is the consistently lower (2 times on average) N_2O emission with rye compared to vetch. The lower N_2O emission with rye (and other biomass with higher C/N ratios) could be linked to enhanced N immobilization (Zschornack et al., 2011) as well as

more efficient use of N by plants. As long as this N immobilization does not result in a complete exhaustion of the mineral N pool (thus impeding plant growth), this should be viewed as beneficial because it leads to the retention of N which otherwise could be lost from the soil system as N gases. These results have significant management implications and support the view that, rye adoption as a cover crop, could help mitigate N_2O emission from agricultural soils.

3.5.4. Effects of cover crop addition on corn growth

Cover crop addition resulted in significant improvement in corn growth and N uptake. Nearly all parameters related to corn growth were 4-5 times greater in the cover crop treatments compared to control. Similar results have previously been reported (Lee et al., 2002; Nagumo and Nakamura, 2013). The enhancement in corn growth with cover crops application can be ascribed to greater N availability to growing plants. Providing strong support for that interpretation is the consistently greater biomass production and the higher N uptake (2-5 times) measured in cover crop-treated corn than in controls. These results confirm the positive effects of cover crops on corn growth, and these beneficial effects were more prominent in the rye than in the vetch treatments. Improved corn growth with rye amendment could be due to the greater resistance of rye to decomposition (compared to vetch) allowing the release of N to occur over a period of time that is longer and better matches with plant N needs. As noted above, vetch addition also resulted in increased N uptake but much less so than with rye. As the data presented

in Fig.4 suggest, instead of being incorporated into corn biomass, a non-negligible portion of vetch-derived N is denitrified resulting in higher cumulative N_2O loss.

During the experiment, the amount of N added as cover crop was < 20 kg N ha^{-1}, and that amendment was the only difference between the control and the treatments. Inspection of the data presented in Table 3-4 showed that the difference in N uptake between treatments and controls is 6-8 times greater than the amount N added in the cover crop. This observation suggests that the impact of cover crop on plant N nutrition could far outweigh the amount of N contained in the cover crop biomass. Through its effect on soil microbes and enzyme activity, cover crop addition could stimulate the conversion of native soil N into available N for plant uptake. This view would be consistent with the concept of added nitrogen interaction (ANI) that was suggested by Jenkinson et al. (1985). Future studies, using ^{15}N-labelled cover crop biomass, should be conducted to examine this mechanism and underlying processes.

3.5.5. Limitations of the study

Since the current study was conducted in a greenhouse, environmental variables such as temperature and moisture were under control. This is not typical of field conditions. Studies by Dietzel et al. (2011), and Peterson et al. (2011) showed that application of crop residues did not affect N_2O emission, but the environmental variables (e.g. freezing events, rainfall) played more important role in affecting the N_2O emission from soils. Therefore, in order to investigate the N_2O emission mitigation potential of cover crops in agroecosystems, one needs to consider both the quality of cover crops and

the environmental variables controlling nutrient cycling in soils. This greenhouse experiment was conducted over period of 12 weeks which is shorter than the normal corn growing season. Therefore, there was no opportunity to investigate the effect of cover crops on late season mineral N dynamics. In this experiment, two types of soil (PT and mid-term NT) and two types of cover crop (hairy vetch and rye) were studied, which was a limited number of soils and cover crops. Therefore, the transferability of the results to other soil conditions remains uncertain. Moreover, in this study, N in the cover crop materials was not labeled, and therefore it was not possible to quantify how much of the N uptake was from the added cover crop or from mineralization of native soil organic N pool. All these limitations need to be considered and resolved in future studies.

3.6. Conclusion

The present study was undertaken with the overall objective of identifying the type of cover crop suitable to agricultural soils under different tillage practices. In comparison, to controls (without cover crop), application of cover crop biomass resulted in enhanced NO_3^- flux, corn N uptake and N_2O emission, but the extent of these enhancements was largely dependent on the cover crop composition and not on tillage. While no significant effect of tillage practices was detected, the increase in NO_3^- and N_2O fluxes was significantly higher in soil treated with vetch than with rye. On the other hand, corn growth was better and N incorporation into corn biomass was significantly greater with rye amendment comparted to vetch. Overall, the results indicate that, regardless of

tillage practices, rye is a better cover crop option than vetch for enhancing soil fertility and N nutrition during early growth of corn.

Acknowledgements

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Table 3-1. Chemical properties of soils (0-10 cm) used in the study.

Soil tillage	Texture	pH	Total C %	Total N %	C/N ratio (before cover crop addition and planting)	NO_3^- (mg kg^{-1} dry soil)
PT	Silt loam	6.41	1.33±0.23	0.19±0.06	7.00	1.06±0.08
NT	Silt loam	7.42	1.85±0.16	0.17±0.02	10.88	0.59±0.14

Table 3-2. Summary statistical analysis of the effect of soil tillage and cover crop amendment on NO_3^- and N_2O fluxes during phase 1 (corresponding to the first 60 days) of the experiment.

Source of variation		NO_3^- flux	Cumulative NO_3^- flux	N_2O flux	Cumulative N_2O flux
Soil tillage (PT, NT)	Week 1	ns	ns	*	*
	" 2	ns	ns	*	*
	" 3	ns	ns	*	*
	" 4	ns	ns	ns	*
	" 5	ns	ns	*	*
	" 6	ns	ns	*	*
	" 7	ns	ns	*	*
Cover crop (control vs. cover crop)	" 1	ns	ns	*	*
	" 2	ns	ns	ns	*
	" 3	ns	ns	ns	*
	" 4	*	*	ns	*
	" 5	*	*	ns	*
	" 6	*	*	ns	*
	" 7	*	*	ns	*
Cover crop (rye vs. vetch)	" 1	ns	ns	*	*
	" 2	ns	ns	*	*
	" 3	ns	ns	ns	*
	" 4	ns	ns	ns	*
	" 5	ns	ns	ns	*
	" 6	ns	ns	ns	*
	" 7	ns	ns	ns	*
Tillage × cover crop	" 1	ns	ns	*	*
	" 2	ns	ns	*	ns
	" 3	ns	ns	ns	ns
	" 4	ns	ns	ns	ns
	" 5	ns	ns	ns	ns
	" 6	ns	ns	ns	*
	" 7	ns	ns	ns	*

ns, and * represent no significance, and significance at $P < 0.05$, respectively.

Table 3-3. Summary statistical analysis of the effect of soil tillage and cover crop amendment on NO_3^- and N_2O fluxes during phase 2 (between day 60 and harvest on day 85) of the experiment.

Source of variation		NO_3^- flux	Cumulative NO_3^- flux	N_2O flux	Cumulative N_2O flux
Soil tillage (PT, NT)	Week 1	ns	ns	*	*
	〃 2	ns	ns	ns	*
	〃 3	ns	ns	ns	*
Cover crop (control vs. cover crop)	〃 1	*	*	*	*
	〃 2	*	*	ns	*
	〃 3	*	*	ns	*
Cover crop (rye vs. vetch)	〃 1	ns	ns	*	*
	〃 2	ns	ns	ns	*
	〃 3	ns	ns	ns	*
Soil tillage × cover crop	〃 1	ns	ns	*	*
	〃 2	ns	ns	ns	*
	〃 3	ns	ns	ns	*

ns, and * represent no significance, and significance at $P < 0.05$, respectively.

Table 3-4. Summary statistical analysis of the effect of soil tillage and cover crop amendment on soil NO_3^- concentration at the end of phase 1 (corresponding to the first 60 days) of the experiment.

Source of variation	Soil depth	
	0-5 cm	5-10 cm
Soil tillage (PT, NT)	ns	ns
Cover crop (control vs. cover crop)	*	*
Cover crop (rye vs. vetch)	*	*
Soil tillage × cover crop	ns	ns

ns, and * represent no significance, and significance at $P < 0.05$, respectively.

Table 3-5. Corn height and total biomass at the end of phase 1 (corresponding to the first 60 days). Corn (1 plant per pot) was growing in pots filled with PT or NT soils amended with rye (C/N: 82) or vetch (C/N: 11). No cover crop was added to the control pots. Values are mean ± standard deviation of three replicates.

Soil tillage	Cover crop	Corn height (cm)	Corn total biomass (g)
PT	Control	43±12.00	11.06±2.71
	Rye	120±8.33	50.18±3.49
	Vetch	91±6.83	41.93±1.83
NT	Control	23±4.01	9.64±1.68
	Rye	101±8.74	42.21±3.66
	Vetch	73±7.23	30.47±3.0

Table 3-6. Corn height and total biomass and shoot/ root biomass ratio at the end of phase 2 (after harvest). Corn (1 plant per pot) was grown in pots filled with PT or NT soils amended with rye (C/N: 82) or vetch (C/N: 11). No cover crop was added to the control pots. Mineral N fertilizer was applied on day 61 due to observed signs of N deficiency. Values are mean ± standard deviation of three replicates.

Soil type	Cover crop	Corn height (cm)	Corn total biomass (g)	Shoot/Root biomass ratio
PT	Control	47±4.61	19.57±7.80	5.90
	Rye	142±19.35	69.39±5.42	16.69
	Vetch	100±4.36	42.67±3.11	7.09
NT	Control	36±8.50	11.52±1.88	4.51
	Rye	129±10.81	50.83±2.43	14.69
	Vetch	92±10.15	49.42±7.51	7.92

Table 3-7. Summary statistical analysis of the effect of soil tillage and cover crop amendment on corn height and biomass at the end of phase 1 (corresponding to the first 60 days) of the experiment.

Source of variation	Corn height	Corn biomass
Soil tillage (PT, NT)	ns	ns
Cover crop (control vs. cover crop)	*	*
Cover crop (rye vs. vetch)	*	*
Soil tillage × cover crop	ns	ns

ns, and * represent no significance, and significance at $P < 0.05$, respectively.

Table 3-8. Summary statistical analysis of the effect of soil tillage and cover crop amendment on corn height and biomass at the end of phase 2 (after harvest) of the experiment.

Source of variation	Corn height	Corn biomass
Soil tillage (PT, NT)	ns	ns
Cover crop (control vs. cover crop)	*	*
Cover crop (rye vs. vetch)	*	*
Soil tillage × cover crop	ns	ns

ns, and * represent no significance, and significance at $P < 0.05$, respectively.

Table 3-9. Total carbon (TC), total nitrogen (TN), carbon/nitrogen (C/N) ratio in corn biomass harvested from PT and NT soils; control and amended with rye or vetch.Values are mean of three replicates.

Soil type	Cover crop	Shoot			Root		
		TC%	TN%	C/N	TC%	TN%	C/N
PT	Control	44.26±1.79	10.22±2.10	4.33	42.51±1.23	7.64±0.54	5.56
	Rye	40.25±7.83	8.16±2.98	4.93	41.46±2.08	7.80±0.94	5.31
	Vetch	44.01±7.83	7.75±2.64	5.68	42.65±5.03	7.40±1.65	5.76
NT	Control	44.50±1.53	7.79±1.47	5.71	41.58±1.59	8.17±0.63	5.09
	Rye	47.61±3.32	13.43±0.50	3.54	39.23±4.42	6.92±0.43	5.66
	Vetch	46.20±1.69	10.77±2.05	4.29	39.65±5.11	8.53±1.75	4.65

Table 3-10. Summary statistical analysis of the effect of soil tillage and cover crop amendment on C/N ratio of corn tissues.

Source of variation	Corn C/N ratio
Soil tillage (PT, NT)	ns
Cover crop (control vs. cover crop)	ns
Cover crop (rye vs. vetch)	ns
Soil tillage × cover crop	ns

ns represents no significance at $P < 0.05$, respectively.

Table 3-11. Total nitrogen (TN) (g) and biomass (g), TN uptake in produced biomass (g) of shoot and root, and TN uptake (kg N ha^{-1}) in corn harvested from PT and NT soils amended with rye or vetch. No cover crop was added to controls.

Soil type	Cover crop	Shoot			Root			TN uptake (g N in total produced biomass)[#]	Corn TN uptake (kg N ha^{-1})[Ψ]
		TN (g)	Biomass (g)	TN in shoot biomass (g)	TN (g)	Biomass (g)	TN in root biomass (g)		
PT	Control	0.10	16.73	1.71	0.08	2.84	0.22	1.93	99.48
	Rye	0.08	65.47	5.34	0.08	3.92	0.31	5.65	291.24
	Vetch	0.08	37.88	2.94	0.07	4.79	0.35	3.29	169.59
NT	Control	0.08	9.43	0.73	0.08	2.09	0.17	0.9	46.39
	Rye	0.13	47.59	6.40	0.07	3.24	0.22	6.62	341.24
	Vetch	0.11	43.88	4.73	0.09	5.54	0.47	5.2	268.06

[#] Corn TN uptake was calculated using the following formula:

$$Corn\ TN\ uptake\ (kg\ N\ ha^{-1}) = \frac{TN\ uptake\ in\ shoot\ and\ root\ (g)}{Area\ of\ each\ pot\ (cm^2)} \times \frac{1\ (kg)}{10^3\ (g)} \times \frac{10^8 (cm^2)}{1\ (ha)}$$

Ψ area of each pot= 1940 cm^{-2}

Table 3-12. Summary statistical analysis of the effect of soil tillage and cover crop amendment on corn total N uptake.

Source of variation	Corn total N uptake (kg N ha^{-1})
Soil tillage (PT, NT)	*
Cover crop (control vs. cover crop)	*
Cover crop (rye vs. vetch)	*
Soil tillage × cover crop	*

* represents significance at $P < 0.05$.

Table 3-13. Total carbon (TC), total nitrogen (TN) and carbon/nitrogen ratio (C/N) in PT and NT soils (0-10 cm) amended with rye or vetch. No cover crop was added to controls. Values are the mean ± standard deviation of three replicates.

Soil tillage	Cover crop	TC%	TN%	C/N
PT	Control	14.3±0.6	1.63±0.4	8.7
	Rye	15.9±1.2	1.84±1.1	8.3
	Vetch	15.6±0.3	2.05±1.3	7.6
NT	Control	17.6±1.5	1.61±0.7	10.9
	Rye	19.9±2.3	1.92±1.5	10.4
	Vetch	18.4±1.5	1.96±1.1	9.4

Table 3-14. Summary statistical analysis of the effect of soil tillage and cover crop amendment on soil C/N ratio.

Source of variation	Soil C/N ratio
Soil tillage (PT, NT)	ns
Cover crop (control vs. cover crop)	ns
Cover crop (rye vs. vetch)	ns
Soil tillage × cover crop	ns

ns represents no significance at $P < 0.05$.

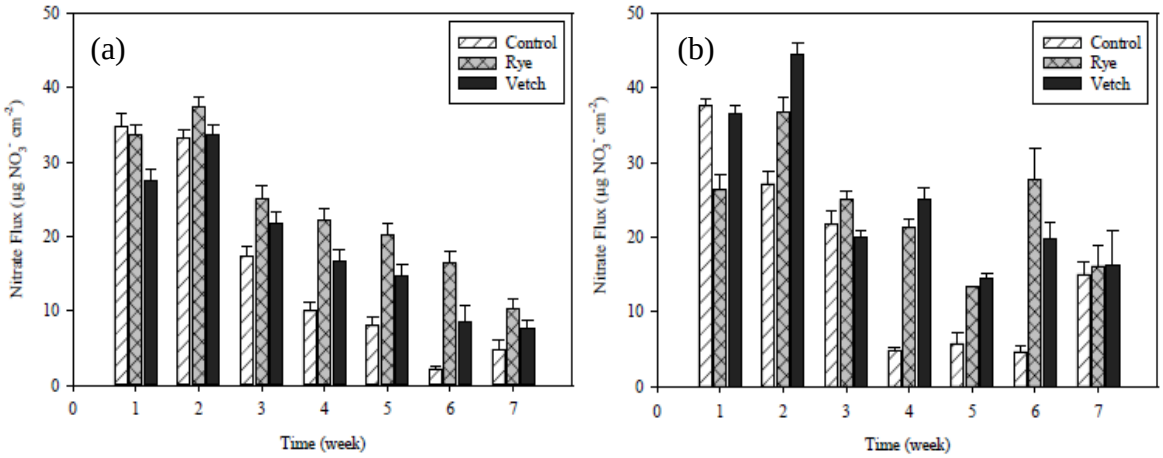

Fig. 3-1. Nitrate flux in (a) plow-till (PT) and (b) no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 1 (corresponding to the first 60 days) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

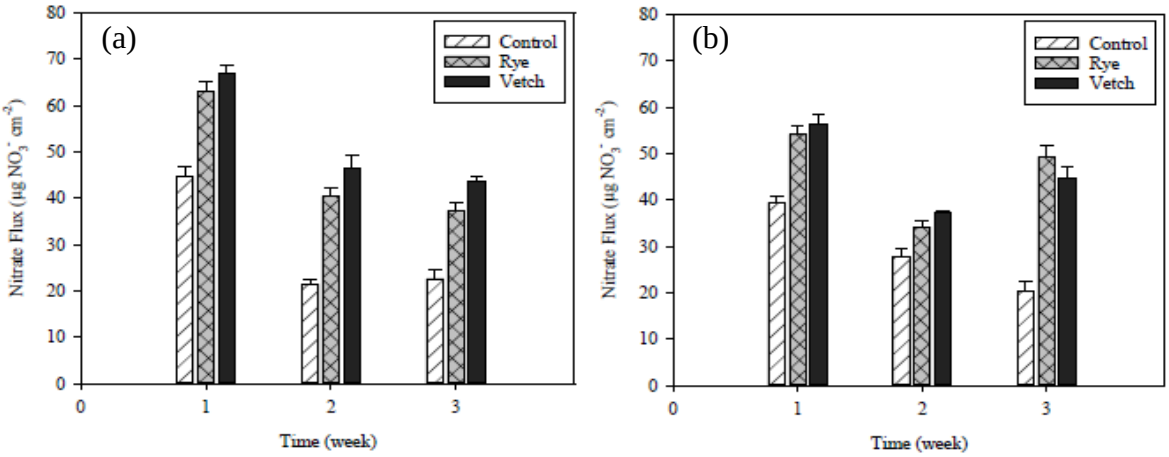

Fig. 3-2. Nitrate flux in (a) plow-till (PT) and (b) no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 2 (between day 60 and harvest on day 85) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

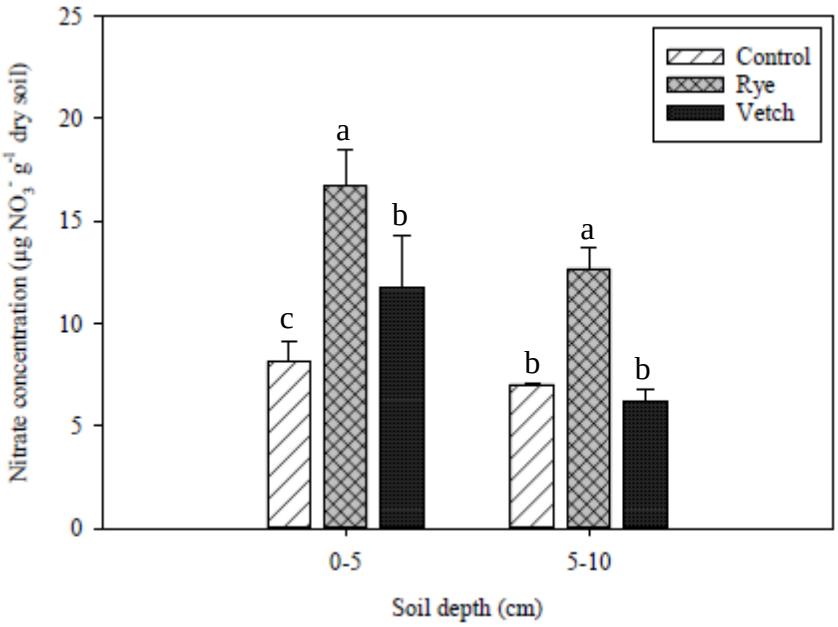

Fig. 3-3. Nitrate concentration in plow-till (PT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively). No cover crop was added to controls. Soil samples were taken from different depth (0-5 cm and 5-10 cm) on day 60 and before the second fertilizer application event. Error bars represent standard deviation from a mean of three replicates.

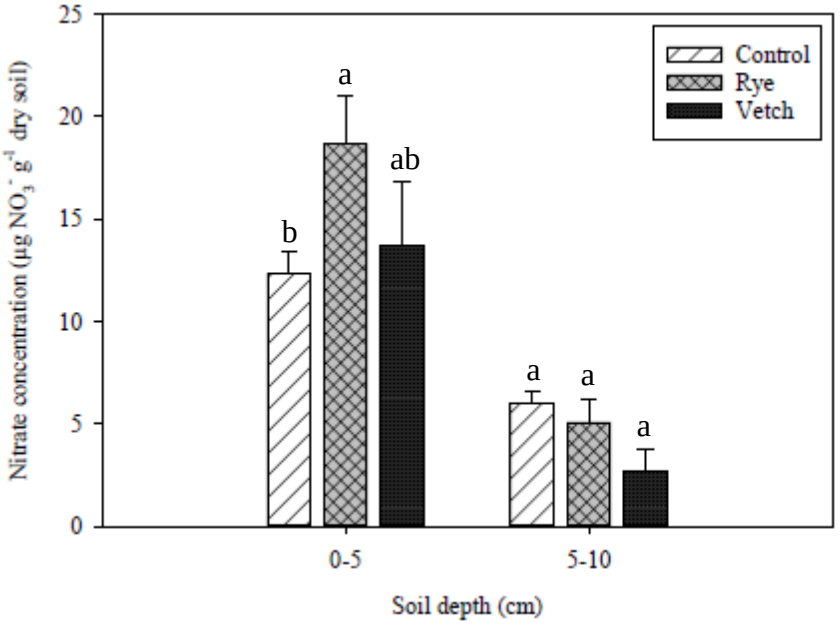

Fig. 3-4. Nitrate concentration in no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively). No cover crop was added to controls. Soil samples were taken from different depth (0-5 cm and 5-10 cm) on day 60 and before the second fertilizer application event. Error bars represent standard deviation from a mean of three replicates.

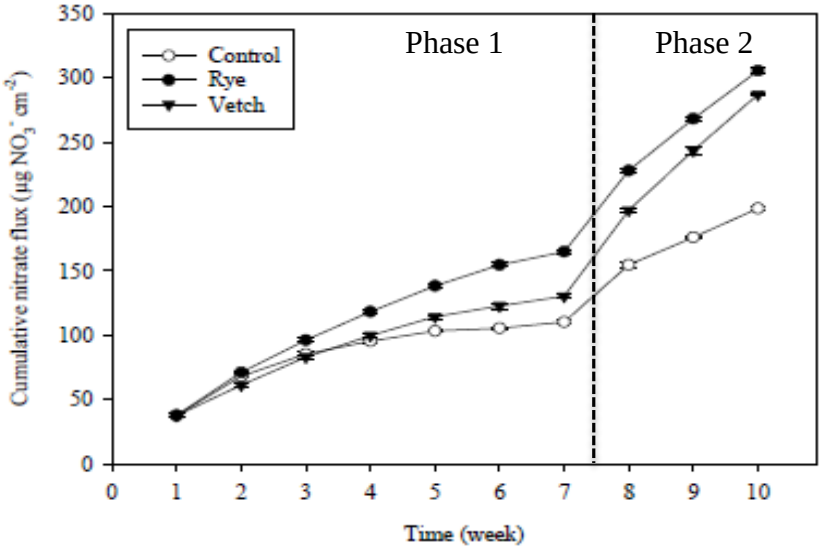

Fig. 3-5. Cumulative nitrate flux in plow-till (PT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 1 (corresponding to the first 60 days) and phase 2 (between day 60 and harvest on day 85) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

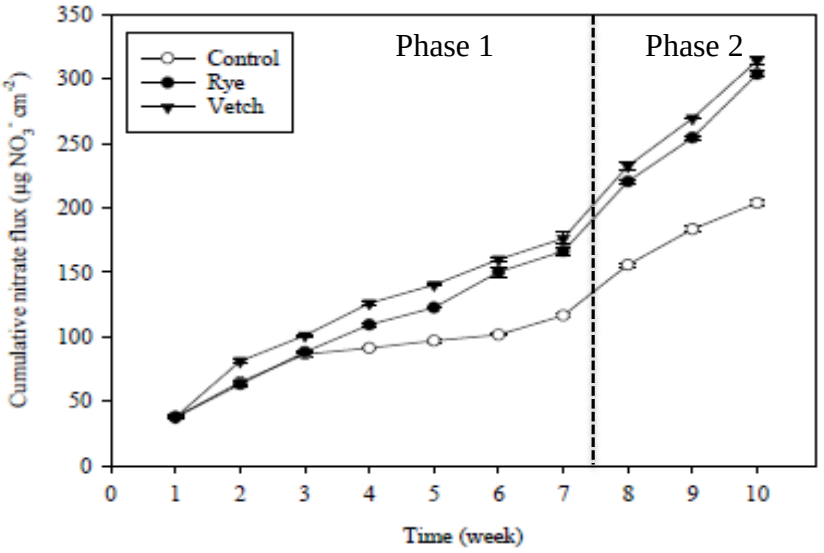

Fig. 3-6. Cumulative nitrate flux in no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 1 (corresponding to the first 60 days) and phase 2 (between day 60 and harvest on day 85) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

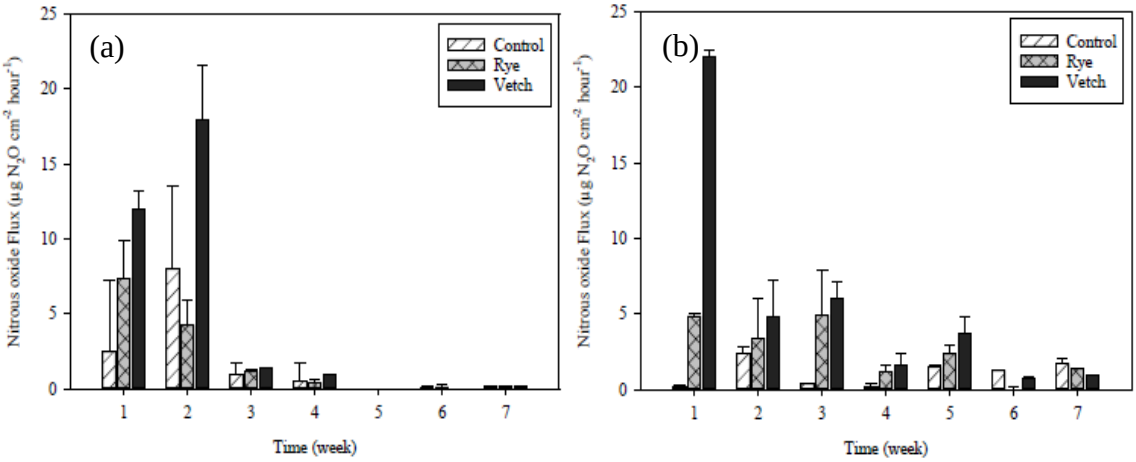

Fig. 3-7. Nitrous oxide flux in (a) plow-till (PT) and (b) no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 1 (corresponding to the first 60 days) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

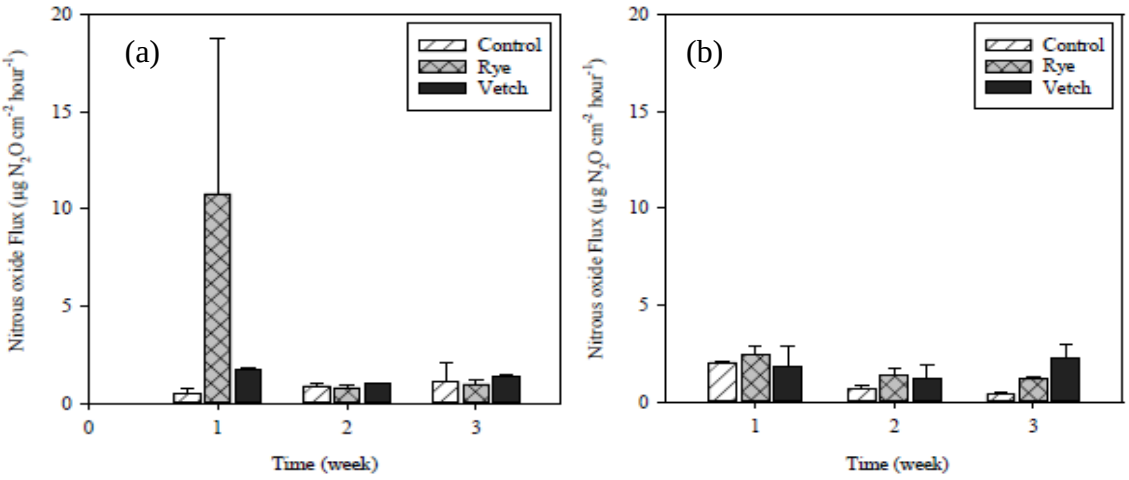

Fig. 3-8. Nitrous oxide flux in (a) plow-till (PT) and (b) no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 2 (between day 60 and harvest on day 85) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

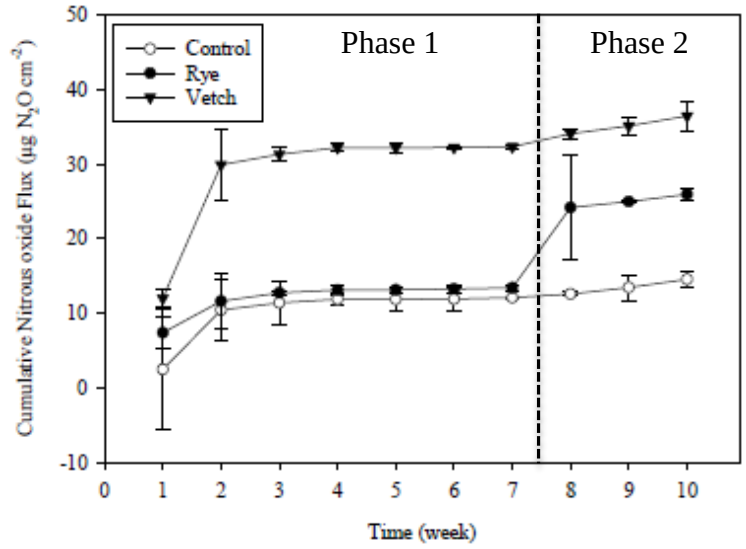

Fig. 3-9. Cumulative nitrous oxide flux in plow-till (PT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 1 (corresponding to the first 60 days) and phase 2 (between day 60 and harvest on day 85) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

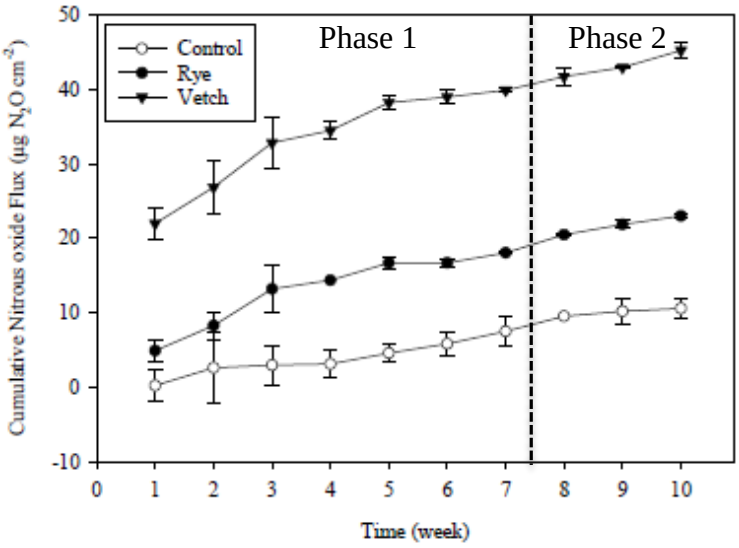

Fig. 3-10. Cumulative nitrous oxide flux in no-till (NT) soil amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) during phase 1 (corresponding to the first 60 days) and phase 2 (between day 60 and harvest on day 85) of the experiment. No cover crop was added to controls. Error bars represent standard deviation from a mean of three replicates.

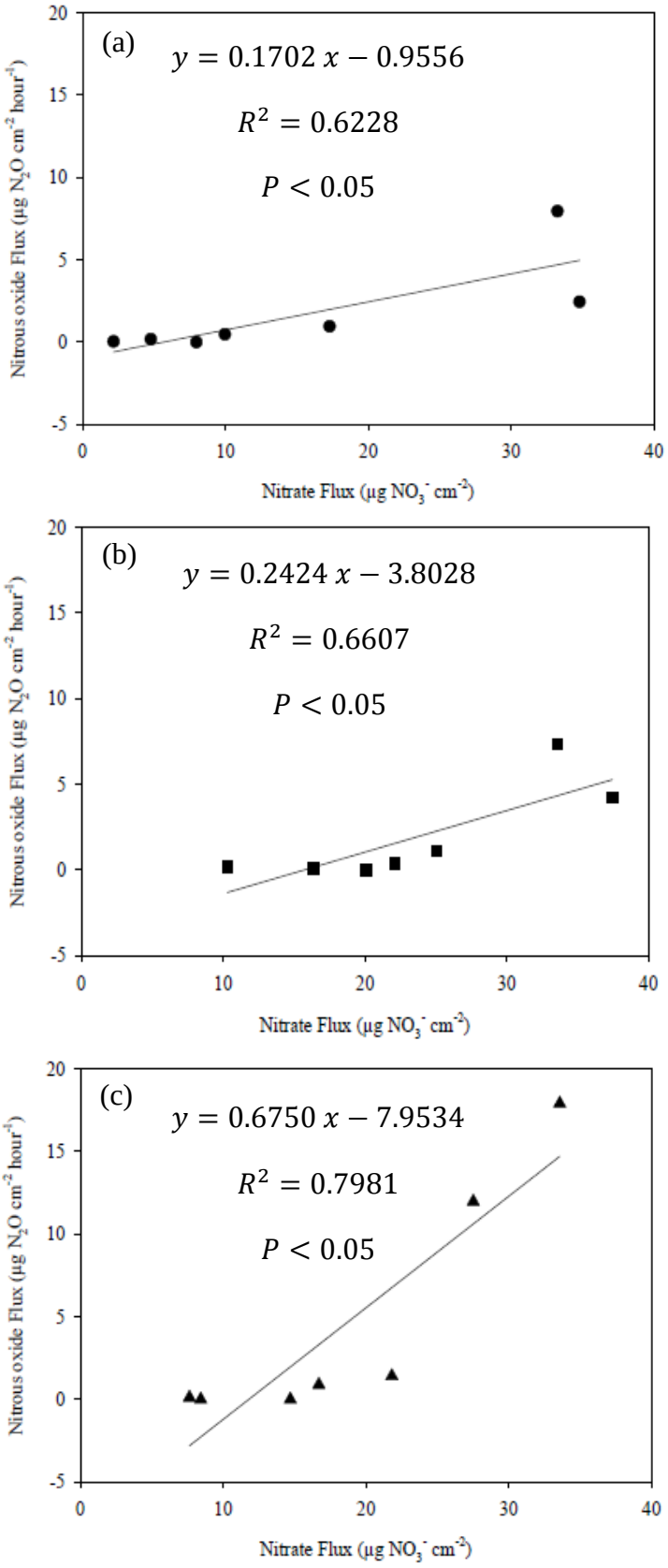

Fig. 3-11. Relationship between nitrate flux and nitrous oxide flux in plow-till (PT) soil. (a) non-amended, (b) rye-amended, and (c) vetch-amended, during the first 60 days of the experiment.

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Chapter 4

Soil microbial biomass and diversity as affected by different tillage practices and cover crops

4.1. Abstract

Agricultural practices affect the physical and chemical characteristics of soils, which can influence the soil microbial community. No-tillage practice and cover crop incorporation are two important components of a sustainable cropland management system. Cover crops could provide the soil microflora with a source of carbon and nitrogen, but it remains unclear how the chemical composition of cover crop biomass affects soil microbial biomass and diversity in cropland under different tillage management. A laboratory experiment was conducted to investigate the effects of two commonly-used cover crops in US Midwest agricultural fields, hairy vetch (*Vicia villosa*; C/N:11) and rye (*Secale cereal*; C/N:82), on microbial biomass and diversity in soils under no-till (NT) (11 years) and plow-till (PT). Results showed that both types of cover crop increased soil microbial biomass carbon; however, significant increase ($P < 0.05$) in microbial biomass nitrogen and nitrate (NO_3^-) production was only observed with the vetch treatment. Shannon's diversity index showed a similar degree of bacterial diversity in the NT and PT soils, whereas fungal diversity was higher in NT compared to PT soil. Overall these results suggest that vetch, and perhaps other cover crops with low C/N ratios, can induce an increase in microbial biomass and diversity in both NT and PT soils.

Results of this study could shed light on some unknown effects of NT management and cover crop application on soil microbial communities in agroecosystems.

4.2. Introduction

Soil ecosystems harbor an immense and diverse population of organisms that affect various biogeochemical processes including formation of organic matter, recycling of nutrients, modification of soil physical and chemical properties and suppression of pests and diseases (Wall and Virginia, 1999; Cairney, 2000; Nair and Ngouajio, 2012). Soil microbes primarily consist of bacteria and fungi, which account for more than 98% to the total soil microbial biomass (Gattinger et al., 2002). Soil microorganisms influence soil quality through enhancement of soil structure, improved soil fertility and increased availability of nutrients to growing plants (Dodd et al., 2000; Kirk et al., 2004). A well-functioning soil microbial community is essential for a healthy soil ecosystem, and is the cornerstone of a sustainable agricultural production system (Sharma et al., 2010). A healthy soil is defined as a soil ecosystem with high microbial diversity given the essential contribution of subset of the soil microflora to many biological processes and nutrient cycling (van Bruggen et al., 2006). In addition, a diverse soil microbial community is an important measure of sustainable land use (Huaiying et al., 2006).

Over the past decade, there has been numerous attempts to link soil health and soil microbial diversity. Some studies have shown that climate, plant biomass composition, and soil physical-chemical properties (pH, texture, water availability) are important factors controlling that linkage (van Bruggen and Semenov, 2000; Jackson et

al., 2003), but it is still not clear how these factors can be impacted by land management. It is of interest to understand how the intensity of tillage practices can affect the diversity of soil microbial communities.

Changes in soil management, such as tillage practices, can affect soil bacterial and fungal populations (Coleman, 2008; Holtkamp et al., 2008). Annual plowing has been the predominant method of land preparation around the world (Feng et al., 2003). The primary reasons for tillage are to: 1) provide a smooth and suitable seedbed, 2) reduce soil compaction, although temporarily, to enhance crop rooting, 3) incorporate fertilizers and herbicide, 4) control weeds, 5) bury crop residues to control plant disease, 6) create a warmer soil environment, especially in humid and cold regions, and 7) follow tradition (Gebhardt et al., 1985).

The primary reason of conventional plow tillage (PT) is to break up soil clods and loosen the soil, thereby creating a soil physical environment suitable for plant roots development. However, this practice generally leads to loss of organic matter, and eventually more soil compaction (Doran, 1980). As farmers become more aware of the negative impacts of PT on soil quality, there has been a shift in land management strategy with a greater adoption of no-till (NT) farming, both in the US and around the world (Huggins and Reganold, 2008). It is well-known that NT practice increases soil organic matter content as well as soil aggregate stability (Nyamadzawo et al., 2009). Increase in soil organic matter can improve soil fertility, and ultimately result in better plants growth and the deposition of larger amounts of plants residue on land surface (Wright et al., 2008). No-till practice has been shown to affect soil microbial community by creating a

better habitat for soil microorganisms through alteration in soil physical and chemical properties (Doran, 1980).

Several studies have shown that microbial biomass can be significantly affected by tillage practices. In general, soil microbial biomass is higher in soils under NT than PT (Feng et al., 2003; Spedding et al., 2004; Nyamadzawo et al., 2009; Gonzalez Chavez et al., 2010). In fact, through its effects on soil moisture, soil temperature and organic carbon availability (Venterea 2011), NT practice can induce changes in both the size (biomass) and composition of the soil microbial community (Frey et al., 1999). In a study by Feng et al. (2003), the microbial community was examined in soils supporting continuous cotton (*Gossypium hirsutum* L.) cultivation, under PT and NT practice for 24 years. Results of that study showed that the effect of tillage practices on soil microbial communities was seasonal; the effect of tillage practices was significant in February and May, but not in October. This seasonal pattern was attributed to tillage-induced difference in soil moisture and temperature conditions during the growing season. The study by Lagomarsino et al. (2009) showed that NT practice resulted in increased soil microbial diversity, and enhancement of soil enzymes activity. Helgason et al. (2010) studied microbial biomass and community dynamics in PT and long-term NT soils using phospholipid fatty acid analysis, and found that bacteria, fungal and total biomass was greater in NT than PT. Jiang et al. (2011) studied soil total microbial biomass C, microbial biomass N, and fungal and bacterial biomass in a 20-year old NT soil. Results of that study showed that NT adoption resulted in a proportional increase in both bacterial and fungal biomass, although no significant dominance of either bacteria or fungi was observed.

Although NT has generally been shown to result in increased soil microbial population and diversity, crop residue composition is an additional factor that could also affect soil biological attributes. Cover crop is an emerging nutrient management strategy in which a crop (a grass or a legume) is grown during the dormant season to scavenge residual soil nutrients. Cover crop not only protects cropland from erosion and physical damages but also provides microbes with a source of readily available C and N (Lu et al., 2000; Ndaw et al., 2009). In agricultural systems, both tillage and cover cropping can influence soil microbial community structure through complex interactions between these factors (Buckley and Schmidt, 2001; Carrera et al., 2007). In recent decades, cover crop-based farming systems have received considerable attention. In the United States, the amount of lands dedicated to cover crop farming has steadily increased (Liang et al., 2014). Cover crops have traditionally been used to reduce soil erosion and build soil quality; however, they are also considered as green manures, thus providing N to growing crops. Cover crop adoption can help to reduce the application of mineral N fertilizers, and mitigate nitrous oxide (N_2O) emission from agricultural soils (Martens, 2001; Bavin et al., 2009; Gomes et al., 2009). Cover crops have also been adopted as a weed managing strategy (Batten et al., 2006; Wortman et al., 2013). Cover crops can benefit agricultural soil sustainability through improved soil microbial properties including increase in total microbial biomass, bacterial and fungal densities (Lienhard et al., 2013), but much remains to be learned about the underlying drivers of these impacts.

Locke et al. (2012) investigated microbial biomass in soils treated with different types of cover crops including rye (*Secale cereale* L.) or Balansa clover (*Trifolium michelianum* Savi var. *balansae Azn*), and showed that soils amended with rye had higher

microbial biomass than those treated with clover. An investigation about the effect of cover crops on different groups of soil microorganisms (bacteria and fungi) was conducted by Grunwald et al. (2000). In that study, the short-term effects of a mixture of oat (*Avena sativa* L.) and lana wooly pod vetch (*Vicia dasycarpa* cv. lana) application on soil microbial biomass was investigated. Results of that study showed that cover crops incorporation led to immediate increase in bacterial biomass; however, soil fungal biomass response was delayed and only increased 3 and 5 weeks after the cover crops application.

The chemical property of cover crops can have an important role in the reported changes in soil microbial communities (Buyer et al., 2010). Soil microbial communities can be strongly influenced by the carbon:nitrogen (C/N) ratios of the cover crops. Cover crop biomass with low C/N ratios generally decomposes and release C and N faster than cover crop biomass with high C/N ratios (Huang et al., 2004; Ndaw et al., 2009). Buyer et al. (2010) studied the effect of cover crops application (such as hairy vetch and rye) on soil microbial community structure in a tomato cropping system. Results of that study indicated generally higher populations of bacteria, fungi and arbuscular mycorrhiza in soil amended with cover crops compared to controls. Liang et al. (2014) investigated soil microbial responses to amendment with winter legume cover crops [Austrian winter pea (*P. sativum*), hairy vetch (*V. villosa*), crimson clover (*T. incarnatum*) and balansa clover (*T. balansae*)] with different C/N ratios. Their results showed that legume species, even with small differences in C/N ratio, and lignin and cellulose content, had variable effects on soil microbial properties. For instance, the Austrian winter pea treatment showed the greatest positive effects on nitrification, β-glucosidase, and β-glucosaminidase activity.

The ratio of C mineralization to microbial biomass C was also found to differ among cover crops, being the lowest with Austrian winter pea addition.

Soil microbial communities respond to a broad range of management factors, and are often considered as early indicators of the changes induced by management practices in the soil environment (Wortman et al., 2013). Therefore, knowledge of soil microbial composition and diversity and their effects on soil C and N dynamics can provide valuable insight into the changes induced by different management practices, such as tillage methods and cover crop incorporation. Increase in soil microbial biomass as a result of NT adoption or cover crop application has been reported by several studies, but it is not clear how cover crops with largely different chemical compositions can affect soil microbial biomass and diversity in soils under different tillage practices. In other words, there is an urgent need to investigate the interacting effects of cover crops composition and tillage practices on soil microbial diversity, and biomass and N dynamics (mineralization and immobilization).

In order to address these questions, a laboratory experiment was conducted using soils under plow-till (PT) and no-till (NT) practices amended with different cover crops (varying C/N ratios) to investigate their effects on soil microbial biomass C and N, soil microbial diversity and initial N release from applied cover crops. It is hypothesized that, regardless of tillage practice, cover crops with low C/N ratio (more readily available C and N) would result in higher soil microbial biomass and diversity than cover crops with high C/N ratios. It is also hypothesized that low C/N cover crops would result in higher microbial biomass and diversity and mineral N release in NT than in PT soils.

4.3. Materials and methods

4.3.1. Soil samples collection

This study was conducted with soil samples (0-25 cm) collected in November 2014 from agricultural fields in Indiana (39°51′ 49″N, 86°21′31″W) (USA) under corn-soybean rotation. Management practices at the sampling sites included conventional tillage (plow-till, PT) and medium-term no-till (NT, 11 years). Soybean was the last crop grown on fields prior to soil sampling. The dominant soil series at the site included Crosby (fine-silt loamy mesic aeric Epiaqualfs) and Brookston (fine-silt loamy mesic typic Epiaqualfs). Composite soil samples (taken from multiple points) were transported to the laboratory in plastic bags, sieved (2 mm) and kept in a refrigerator (4 ^{o}C) until used in the experiment described below. Field-moist soil was extracted with K_2SO_4 to determine initial inorganic N concentration. A portion of each soil sample was air dried and used for determination of chemical properties (Table 4-1).

4.3.2. Cover crop collection

Cover crops selected for this study are among the most commonly-used cover crops in US Midwest agricultural fields, and included hairy vetch (*Vicia villosa*) and rye (*Secale cereale*). These cover crops have contrasting chemical composition, i.e. hairy vetch has a C/N ratio of 11:1 and rye has a CN ratio of 82:1. Rye was collected from Starkey farm in Brownsburg, Indiana. Vetch was collected from a field maintained by INDOT near Lebanon, Indiana. Collected cover crops were transported to the laboratory, air and oven-dried and cut.

4.3.3. Laboratory experiment design

This experiment was conducted during a 5-week period in the laboratory. Field moist (≈0.16±0.07 g water g^{-1} soil) soil was weighed (200 g) into mason jars (500 mL). Then, 10 g of each cover crop was weighed, added to each jar and thoroughly mixed. Duplicate jars for each soil type (PT or NT) not amended with cover crop were also included as controls. Unlidded mason jars containing soil and cover crops were placed on a laboratory bench at room temperature (22 ^{o}C), and were watered as needed to maintain constant soil moisture (≈0.16 g water g^{-1} soil) during the experiment. After 1, 2, and 5 weeks, jars were sampled ad the soil samples immediately used in the biochemical assays described below.

4.3.4. Soil microbial biomass C and N measurement

Soil microbial biomass C and N was measured using the fumigation and extraction method (Brookes et al., 1985). After week 1, 2 and 5 of the experiment, approximately 16 g moist soil samples were taken from each mason jar and split into two parts (8 g each). One portion (non-fumigated) was immediately extracted with 0.5 M potassium sulfate (K_2SO_4) (soil dry weight/extractant ratio of 1:4 w/v).The suspension was shaken for 15 minutes, and the supernatant was filtered. The other portion of moist soil (8 g) was poured into 25 mL glass beakers and subjected to chloroform fumigation. The fumigant consisted of ethanol-free chloroform (CH_3Cl, 20 mL) placed in a glass beaker along with a few boiling chips. All beakers were stacked in a desiccator lined with a wet tissue paper. The desiccator was evacuated until the CH_3Cl boiled for 2 minutes.

The desiccator was then closed allowing CH_3Cl vapor to accumulate. Soil was incubated in the dark at 25 ºC for 24 hours. After fumigation, soil samples were ventilated and transferred into 50 mL centrifuge tubes, and extracted with 0.5 M K_2SO_4 as described for the non-fumigated samples. All extracts were stored frozen prior to analysis. Soil extracts were diluted with DI water (samples/ DI water ratio of 1:20 v/v) and analyzed for dissolved organic carbon (DOC) using a Vario-Cube analyzer (Elementar Americas). Microbial biomass C and N was computed using the equations below:

$$Biomass\ C = \frac{F_C}{k_C}$$

where F_C is the difference between total organic carbon in fumigated and non-fumigated extracts, and k_C=0.45 is the fraction of killed biomass C (Jenkinson 1988).

$$Biomass\ N = \frac{F_N}{k_N}$$

where F_N is the difference between total organic nitrogen of fumigated and non-fumigated extracts measured by CN analyzer and k_N=0.57 is the fraction of killed biomass N (Jenkinson 1988).

4.3.5. Community-level physiological profile (CLPP)

Over the past decade, the diversity of microbial communities has been increasingly characterized using the utilization pattern of sole C substrates generated with commercially available 96-well Biolog microtiter plates (Insam, 1997). These CLPPs provide a rapid approach of assessing differences in soil microflora. In this study,

substrate utilization pattern of culturable soil microbial population was determined using Biolog GEN III (Catalog No. 1030) and FF plates (Catalof No. 1006) (Biolog, Inc, Hayward, CA, USA). GEN III and FF plates contain a blank (control well with water) and 95 unique C substrates that are developed for identifying bacteria and fungi, respectively (Classen et al., 2003). GEN III microplates contain tetrazolium redox dyes, which are used to colorimetrically indicate utilization of the carbon source or resistance to inhibitory chemicals. Bacterial respiration causes the reduction of the tetrazolium redox dye and formation of a purple color. Tetrazolium has inhibitory effect on fungal activity and that is how GEN III plates are designed to identify bacteria. Fungi microplates (FF plates), designated a broad range of fungi including both filamentous and yeast forms, contain iodonitrotetrazolium violet as a redox dye which makes reddish orange wells to indicate utilization of carbon sources.

The procedure used to determine C substrate utilization by soil microbial communities in this study was adapted from Buyer et al. (2001). All solutions and bottles used in these assays were autoclaved. For each soil sample, duplicate 1g sub-samples were suspended in 100 mL deionized (DI) water (pH 7.2) and the suspension vortexed for a few minutes. Then, 1 mL of that suspension was diluted in another 100 mL of DI water and vortexed. Finally, 150 µL aliquots of that final dilution (10^{-4}) were added to each of 96 microplate wells. For FF plates, 1 mL of the bactericide bronopol solution (1 g L^{-1}) was added to the final suspension to eliminate bacterial growth. Then, all plates were incubated at 22 ^{o}C and absorbance in the GENIII (λ:595 nm) and FF (λ:650 nm) microplates wells was read on a daily basis for 10 days using a VersaMax microplate

reader (Molecular Devices, Sunnyvale, CA). Shannon's diversity index (H) was computed:

$$H = -\sum p_i \ln p_i$$

Where, p_i is the ratio of absorbance for a given substrate to the sum of absorbance of all wells in a microplate (Jacinthe et al.,2010; Nair and Ngouajio, 2012).

4.3.6. Analytical methods

Soil samples were collected in October 2014 at the study sites to determine soil properties. Soil samples were air-dried, and sieved (2-mm sieve) for determination of chemical properties. Soil pH was measured with an Accumet model 25 pH/ion meter (soil/water ratio of 1:2 w/v), calibrated using pH 4 and 7 buffers. Particle size was determined by the hydrometer method after dispersion of soil with sodium hexametaphosphate ($Na_6P_6O_{18}$) (50 g L^{-1}) (Table 4-1). Soil extracts (before the experiment, and non-fumigated soil samples) were analyzed for nitrate (NO_3^-) using US Environmental Protection Agency (EPA) method 353.1 on a Konelab Aquakem analyzer. Soil extracts were also measured for C and N using a Vario-Cube analyzer (Elementar Americas) with 960 ^oC oven temperature (Elementar Americas, New Jersey, USA).

4.3.7. Statistical Analysis

Data were first tested for normality using the normality test available in the Sigma Plot software (Systat, San Jose, CA). Since most of the data were not normally distributed, Kruskal-Wallis test was used (factors: soil tillage and cover crop) to examine the differences in mineral nitrogen (NO_3^-) concentration and microbial biomass carbon and nitrogen during the experiment. The Kruskal-Wallis test was followed by Mann-Whitney pairwise test when a significant difference was detected. Unless otherwise noted, statistical significance in this study was determined at $\alpha=0.05$. Statistical tests were conducted using PAST software (ver. 2.17c) downloaded from http://nhm2.uio.no/norlex/past/download.html (University of Oslo).

4.4. Results

4.4.1. Microbial biomass carbon and nitrogen

In all treatments, microbial biomass C increased over time during the incubation (Fig. 4-1). In general, the increase in microbial biomass carbon was greater with vetch than with rye amendments. As expected, the lowest values for microbial biomass C were observed in the controls (Fig. 4-1). In both soils, vetch addition resulted in significantly higher microbial biomass carbon than control ($P < 0.05$) (Fig. 4-1), and although the rye treatment resulted in higher microbial biomass carbon compared to control, this difference was only significant in week 5 with the NT soil ($P < 0.05$) (Table 4-2). Although a significant difference was only observed in week 5 ($P < 0.05$) (Table 4-2), the

effect of vetch on microbial biomass C varied with soil, being generally greater in NT than PT soil.

A similar pattern for microbial biomass N was observed in both PT and NT soils (Fig. 4-2). Just as with microbial biomass C, there was an increase in microbial biomass N over time in all treatments (Fig. 4-2). Microbial biomass N in PT and NT soils showed similar values in controls and rye treatments after week 1, 2 and 5 and. In both PT and NT soils, the vetch treatment resulted in an increase in microbial biomass N over time; however, this increase was only significant ($P < 0.05$) after week 5 in the NT soil (Fig. 4-2b). In the vetch treatments, microbial biomass N (Fig. 4-2b) was significantly higher ($P < 0.05$) in the NT than in the PT soil (Fig. 4-2a) after week 5 (Table 4-2).

4.4.2. Soil nitrate concentration

During the experiment, NO_3^- concentrations in the controls and rye treatments were very low, in both PT and NT soils (Fig. 4-3). However, in the vetch treatment significantly higher NO_3^- concentration ($P < 0.05$) was detected in both PT and NT soils (Table 4-2). In the vetch treatment, NO_3^- level also exhibited an increasing trend over time in both PT and NT soil (Fig. 4-3). In the vetch treatment, although NO_3^- concentration was generally higher in the PT than in the NT soil, the difference was only significant ($P < 0.05$) after week 5.

4.4.3. Soil microbial diversity

Cover crop incorporation led to increased bacterial diversity compared to controls (Fig. 4-4). This increase was more noticeable in the PT (Fig. 4-4a, b and c) than in the NT soil (Fig. 4-4 d, e, f). In the controls, bacterial diversity was higher in NT than in PT soil (Fig. 4-5). Rye and vetch resulted in similar increase in bacterial diversity, as illustrated by almost similar values for diversity index (e.g. 168 hours) in all the cover crop treatments in both soils (Fig. 4-4). In both PT and NT soils, the GEN III microplate wells started to develop purple color after 24 hours after inoculation and that purple color was noticeably darker in the microplates inoculated with the NT soil compared to the PT.

Soil fungal diversity measurements using FF microplates showed that cover crop incorporation resulted in increased fungal diversity (Fig. 4-6). In general, there was an increase in soil fungal diversity over time in the cover crop-amended treatments, and this increase was higher and more visible in the NT soil (Fig. 4-6d, e and f). In the PT soil, both cover crops resulted in similar increase in fungal diversity at irrespective of sampling time; however, in the NT soil, the vetch treatment resulted in higher fungal diversity compared to the rye treatment.

In the non-amended controls, higher values of Shannon's index were measured in NT soil compared to PT soil, indicating higher diversity of fungal communities in NT than in PT soil (Fig. 4-7). In both PT and NT controls, bacterial diversity was greater than fungal diversity (Figs. 4-5 and 4-7), but both bacterial and fungal diversity was higher in NT than in PT soil (Figs. 4-5 and 4-7). The FF microplates generally started to develop orange to red color after 72 hours, and similar to bacteria microplates, the FF plates

inoculated with the NT soil had darker and more visible red color thean those inoculated with the PT soil.

4.5. Discussion

4.5.1. Soil microbial biomass carbon and nitrogen, and soil mineral nitrogen as affected by different tillage practices and cover crop incorporation

In this study, results of microbial biomass C analysis showed higher values for NT soil compared to PT, although this difference was only significant ($P < 0.05$) after week 5 (Fig. 4-1). However, in both PT and NT soils, microbial biomass N was no different between cover crop-amended treatments and controls at all sampling events (Fig. 4-2). It has been widely reported that microbial biomass can be altered by tillage practices and is generally higher in soil under NT than PT management (Mullen et al., 1998; Kandeler et al., 1999; Spedding et al., 2004; Gonzalez-Chavez et al., 2010). The lack of significant differences in microbial biomass C and N between NT and PT soils observed in this study suggests (Figs. 4-1 and 4-2) that 11 years of NT management was probably too short to make noticeable changes in microbial biomass of the studied NT soil. Microbial biomass C in both PT and NT soils responded positively to rye or vetch amendments. Microbial biomass C in the treatments was not only higher than controls but also an increase over time culminating in a significant difference ($P < 0.05$) between treatments in week 5 (Fig. 4-1). This temporal pattern in microbial biomass C is in accord with the results reported by Buyer et al. (2010), involving vetch and rye as cover crops in a tomato cropping system.

The greater enhancement microbial biomass C with vetch than with rye addition (Fig. 4-2) suggests the induction of a larger population of soil microbes by the decomposing vetch tissues. This could be due to the lower C/N ratio of vetch, an attribute that has been related to rapid decomposition of residues and nutrients mineralization (Liang et al., 2014). As far as microbial decomposition is concerned, cover crops with low C/N ratios are generally superior to those with high values (Melillo et al., 1982; Cotrufo et al., 1995; Carreiro et al., 2000). The low C/N ratio of vetch not only resulted in higher microbial biomass C, but may have also contributed to the trend in microbial biomass N observed during the experiment in both PT and NT soils (Fig. 4-2). While the rye treatment only produced a modest increase in soil microbial N, soils amended with vetch exhibited a consistent increase in soil microbial biomass N over time, and more markedly so the NT soil (Fig. 4-2b).

Results of NO_3^- measurements (Fig. 4-3) also indicated higher NO_3^- concentrations in the vetch-treated soil compared to rye and controls at all sampling times. These results could also be ascribed to the lower C/N ratio of vetch residues, leading to higher and faster N mineralization as was discussed earlier. This interpretation is in agreement with the findings of Liang et al. (2014), from an investigation of N mineralization in soils treated with biomass from different legume species. These authors reported that legumes with lower C/N ratios resulted in more rapid mineral N release from cover crops to the amended soils. Regardless of the type of cover crop incorporated, the results of the experiment showed lower microbial biomass N and higher NO_3^- concentration in the PT soil, and in contrast, higher microbial biomass N along with lower NO_3^- concentration in the NT soil. Although other processes could play a role,

these results suggest a greater degree of NO_3^- immobilization by resident microbes in the NT than the PT soils. Experiments using ^{15}N-labeled cover crops are needed to evaluate the merit of that suggestion.

4.5.2. Soil microbial diversity as affected by different tillage practices and cover crop incorporation

At all sampling times, higher bacterial and fungal diversities were measured in the NT soil compared to the PT soil(Figs. 4-4 and 4-6).This observation adds to past results regarding enhancement in soil enzyme activity, and functional diversity of soil microbial communities with NT management (Lagomarsino et al., 2009). This result is also in agreement with the study hypothesis and the results reported by others (Helgason et al., 2010, Jiang et al., 2011 and Wortman et al., 2013) that NT management generally result in larger soil microbial diversities in comparison to PT management. However, Feng et al. (2003) suggested that the effects of tillage practice on soil microbial communities are primarily determined by the soil conditions during a growing season. Specifically, these authors (Feng et al., 2003) argued that soil moisture and temperature are the key parameters that determine changes in soil microbial communities under different tillage practices. Since tillage is often a strong driver of soil microbial community structure (Drijber et al., 2000), and due to less soil disturbance under NT than PT management, NT adoption can improve soil physiochemical characteristics (aggregate stability, organic C, moisture, and cation exchange capacity), and indirectly creates an environment in which soil fungi and bacteria can grow and expand (Lienhard et al., 2013).

The present study documents positive impacts of cover crop addition on both bacterial and fungal diversities (Figs. 4-4 and 4-6). Similar responses have reported in several past studies (Calbrix et al., 2007; Steenwerth and Belina 2008; Nair and Ngouajio 2012; Liang et al., 2014). Cover crop incorporation into soils provides soil microbes with a new source of C and N, allowing increase in both the size and diversity of the microbial population (Ndaw et al., 2009). While the vetch and rye treatments had almost similar impacts on bacterial diversity (Fig. 4-4), the vetch treatment had a much higher impact on fungal diversity than does rye addition. Overall, results of this study further demonstrate the positive effects of cover crop application and NT management on soil microbial biomass and diversity. This current study was conducted in the laboratory under controlled environmental conditions. This study was also conducted for only 5 weeks, which is not long enough to assess the temporal dynamics of mineral N and soil microbial community composition. Field-scale investigations are needed at sites under NT management with varying length of time to validate the results of this bench-scale experiment.

4.6. Conclusion

Based on the results of this study both cover crops led to an increase in soil microbial biomass C; however, only the vetch application enhanced microbial biomass N and nitrate production in both NT and PT soils. Soil microbial diversity increased with either cover crop compared to controls. In addition, our results indicated that although bacterial diversity was not different in NT and PT soils, fungal diversity was higher in the

NT than the PT soil. Generally, cover crop incorporating into soils disregarding of their tillage practice could increase microbial biomass and diversity and benefit soil with not only a C and N supply but also enhance soil structure and quality. Although 11 years of NT management was probably not long enough to generate drastic changes in the diversity of soil bacterial communities, fungal diversity was consistently higher in soil from the mid-term NT management compared to the PT, whether with or without cover crop amendment. Further investigation is needed to study the changes in microbial diversity in soils under long-term NT management.

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Table 4-1. Chemical properties of soils (0-10 cm) used in the study

Soil tillage	Texture	pH	Total C (%)	Total N (%)	NO_3^- (mg kg^{-1} dry soil)
PT	Silt loam	6.41	1.33±0.23	0.19±0.06	1.06±0.08
NT	Silt loam	7.42	1.85±0.16	0.17±0.02	0.59±0.14

Table 4-2. Summary analysis of variance for the effect of tillage and cover crop amendment on soil microbial biomass and nitrate.

Source of variation		Microbial biomass carbon	Microbial biomass nitrogen	Nitrate concentration
Soil tillage (PT, NT)	Week1	ns	ns	ns
	Week 2	ns	ns	ns
	Week 5	*	*	*
Control vs. rye (PT)	Week1	ns	ns	ns
	Week 2	ns	ns	ns
	Week 5	ns	ns	ns
Control vs. rye (NT)	Week1	ns	ns	ns
	Week 2	ns	ns	ns
	Week 5	*	ns	ns
Control vs. vetch (PT)	Week1	*	ns	*
	Week 2	*	ns	*
	Week 5	*	ns	*
Control vs. vetch (NT)	Week1	*	ns	ns
	Week 2	*	ns	*
	Week 5	*	*	*
Rye vs. vetch (PT)	Week1	ns	ns	*
	Week 2	ns	ns	*
	Week 5	ns	ns	*
Rye vs. vetch (NT)	Week1	*	ns	ns
	Week 2	*	ns	*
	Week 5	*	*	*

ns, and * represent no significance, and significance at $P < 0.05$, respectively.

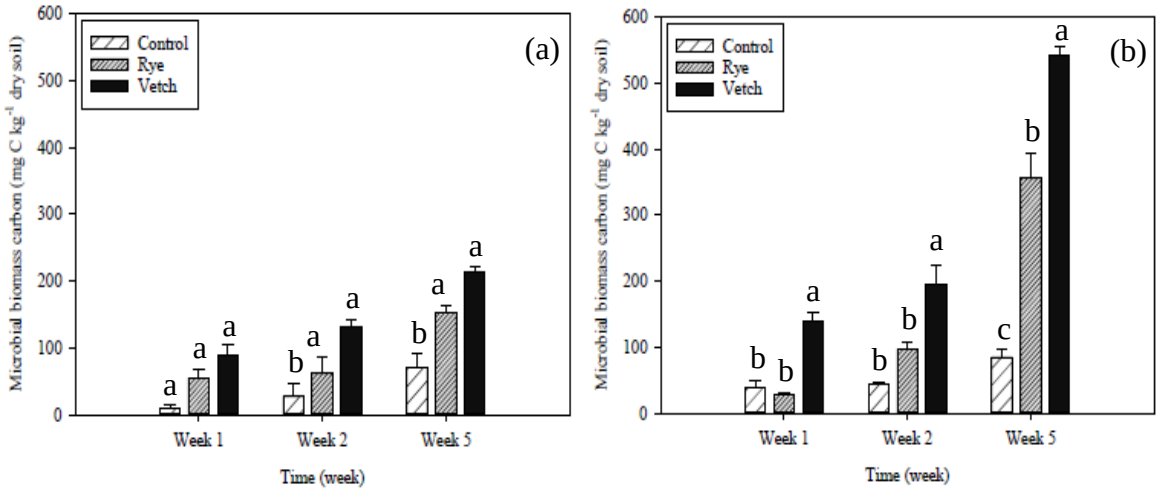

Fig. 4-1. Microbial biomass carbon in (a) plow-till (PT) and (b) no-till (NT) soils, amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) at the end of week 1, 2 and 5 of the experiment. No cover crop was added to controls. Error bars represent standard deviation from the mean.

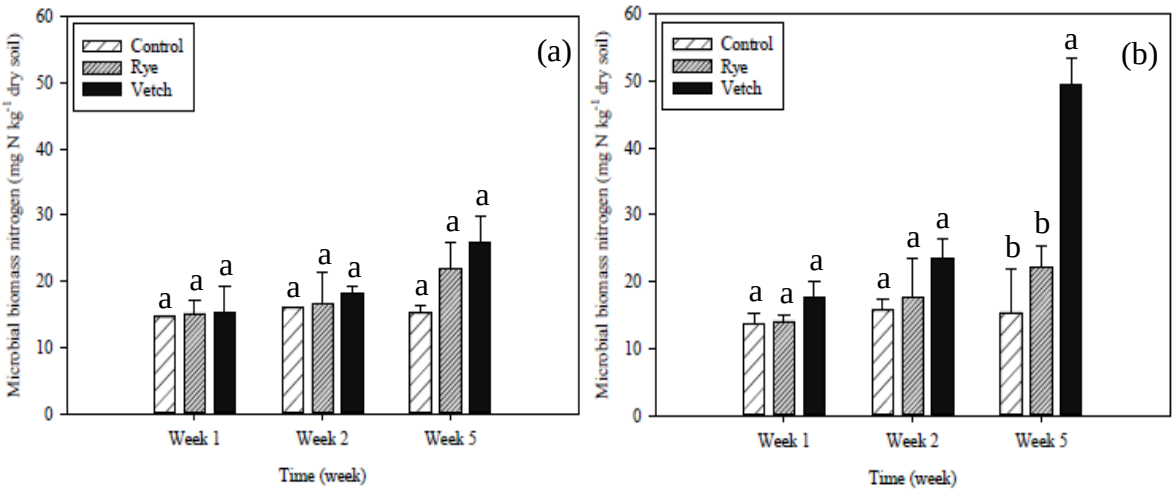

Fig. 4-2. Microbial biomass nitrogen in (a) plow-till (PT) and (b) no-till (NT) soils, amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) at the end of week 1, 2 and 5 of the experiment. No cover crop was added to controls. Error bars represent standard deviation from the mean.

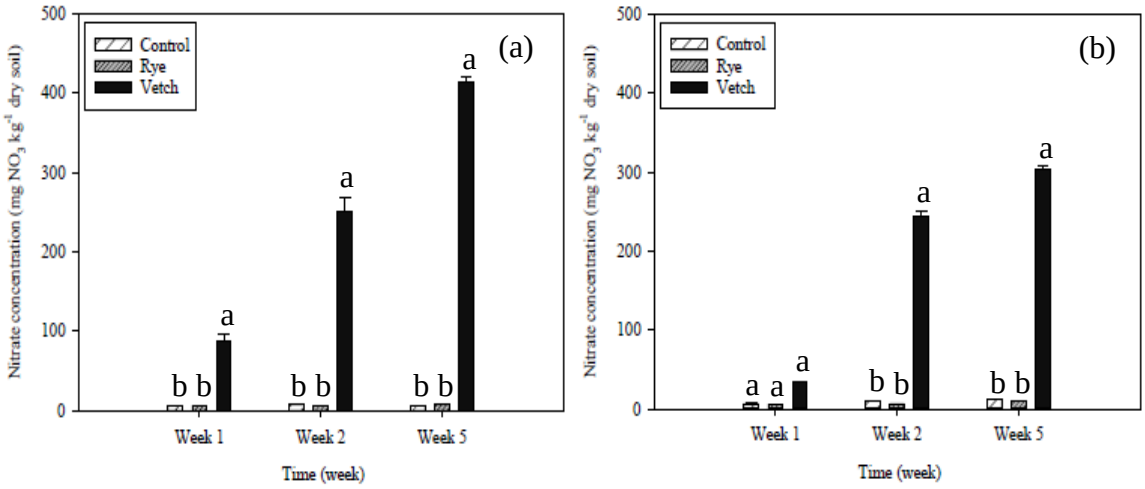

Fig. 4-3. Nitrate (NO_3^-) concentration in (a) plow-till (PT) and (b) no-till (NT) soils, amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) at the end of week 1, 2 and 5 of the experiment. No cover crop was added to controls. Error bars represent standard deviation from the mean.

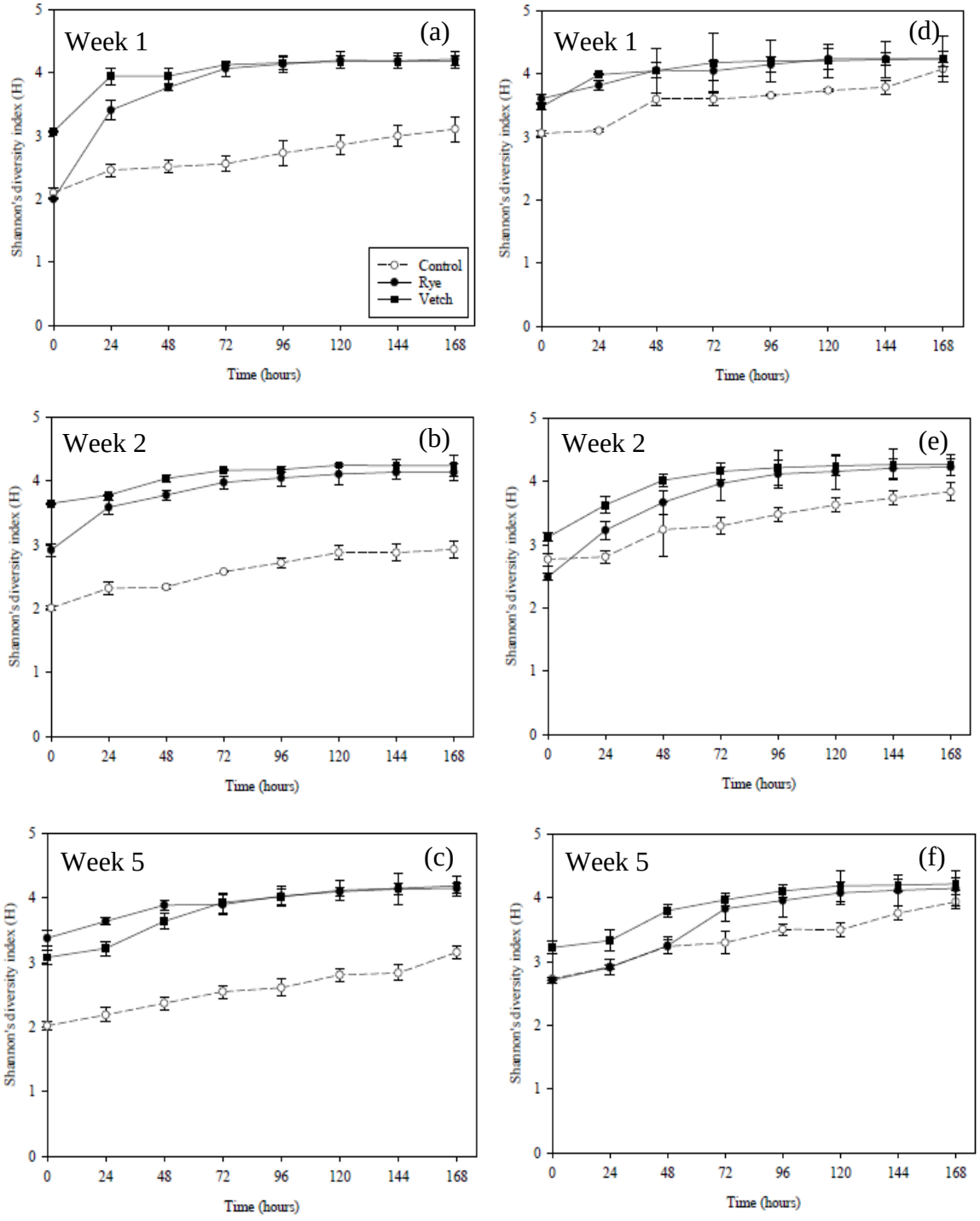

Fig. 4-4. Bacterial diversity in plow-till (PT) (a, b, c) and no-till (NT) (d, e, f) soils, amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) at the end of week 1, 2 and 5 of the experiment. No cover crop was added to controls. Error bars represent standard deviation from the mean.

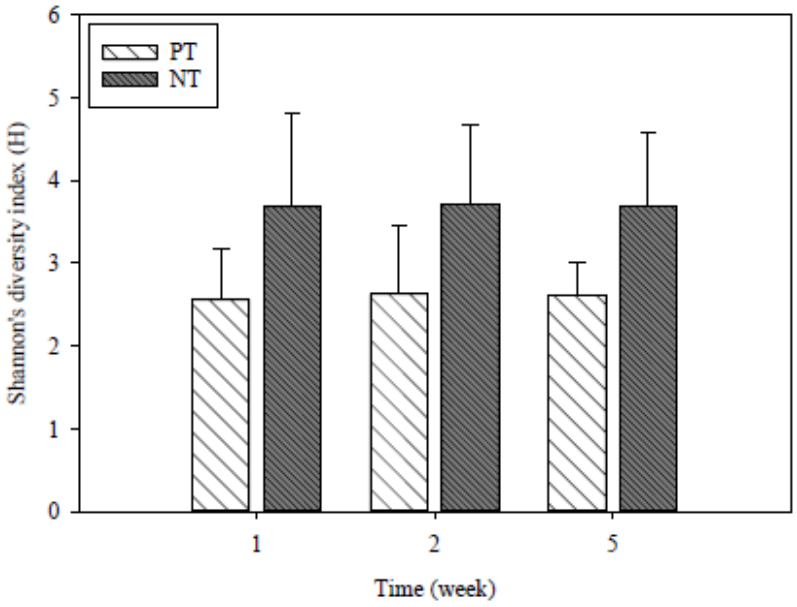

Fig. 4-5. Average bacterial diversity in control plow-till (PT) and no-till (NT) soil (without cover crop addition) at the end of week 1, 2, and 5 of the experiment. Error bars represent standard deviation from the mean.

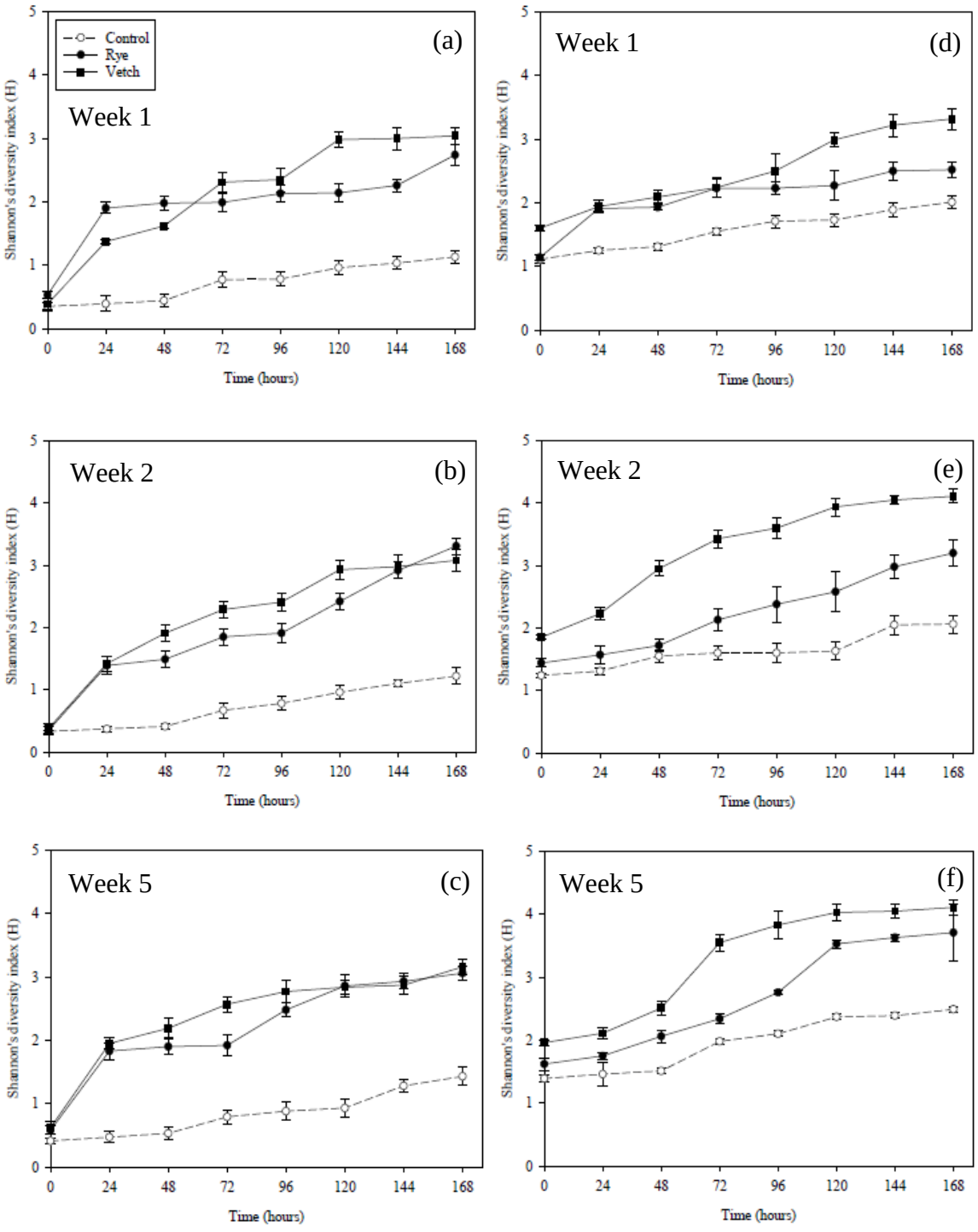

Fig. 4-6. Fungal diversity in plow-till (PT) (a, b, c) and no-till (NT) (d, e, f) soils, amended with different cover crops (rye and vetch with C/N ratio of 82 and 11, respectively) at the end of week 1, 2 and 5 of the experiment. No cover crop was added to controls. Error bars represent standard deviation from the mean.

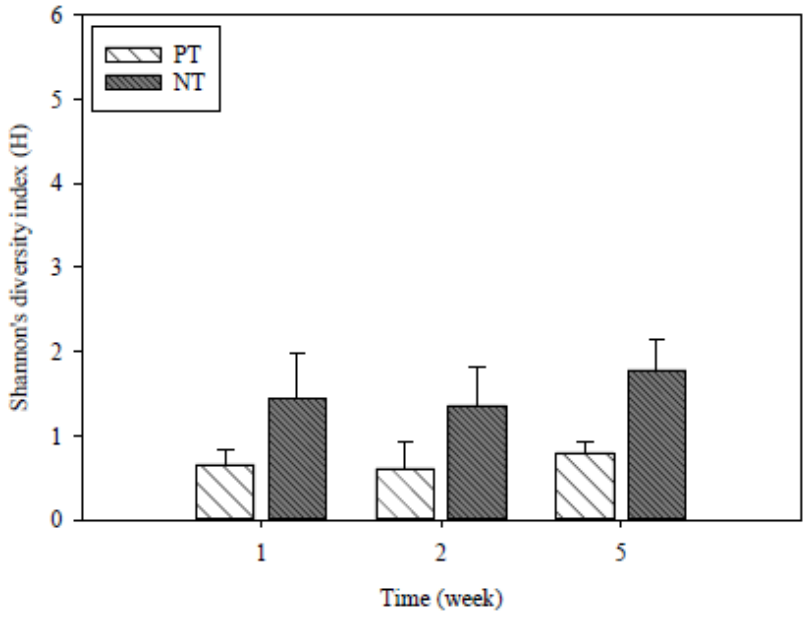

Fig. 4-7. Average fungal diversity in control plow-till (PT) and no-till (NT) soil (without cover crop addition) at the end of week 1, 2, and 5 of the experiment. Error bars represent standard deviation from the mean.

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Chapter 5

Summary

In the last century, the rise in the world population and the increased demand for food has resulted in the intensification of agricultural production. This increase has had important ramifications on our environment and the sustainability of agroecosystems. For instance, in order to increase crop production, intensive tillage practices have been adopted and heavy application of nitrogen (N) fertilizers has often been made by farmers, which both have been shown to substantially contribute to nitrous oxide (N_2O) emission from agroecosystems (Forster et al., 2007). Nitrous oxide, mainly a by-product of the biologically-mediated denitrification process in soils, has been linked to depletion of the stratospheric ozone layer. This long-lived (lifetime of 166±16 years) atmospheric constituent also participates in the greenhouse effect, thus contributing to the warming of the atmosphere. Because of concerns regarding the impact of N_2O on the environment, researchers have come up with N management approaches to mitigate N_2O emission from agriculture. No-till (NT) farming and cover crop are two practices that have been evaluated, among other important efforts, for their potential to minimize N_2O emission in agroecosystems. Past studies have yielded variable results with regard to the impact of NT adoption and cover crop application on N_2O emission. Therefore, the purpose of this dissertation research was to provide a detailed examination of the impact of different tillage practices (plowed-till and no-till) and cover crops (with different chemical composition) on N_2O emission through investigation of the soil microbial community and N cycling processes in response to these land management options.

In chapter 2 of this dissertation research, experiments were conducted to identify the main group of microbes contributing to N_2O production in soil ecosystems. Different types and levels of biocides were applied to cropland (PT and NT) and forest soils in order to identify the product leading to optimum inhibition of N_2O production. The two most commonly-used biocides, streptomycin and cycloheximide, not only did not result in any significant inhibition of N_2O production, but streptomycin even stimulated N_2O production. Streptomycin and cycloheximide have long been used as antibiotics and due to their widespread dispersion in the environment, soil microorganisms might have developed resistance against these two old biocides. This could be a factor for the non-efficiency of these biocides. However other biocides, captan and bronopol, inhibited N_2O and CO_2 production with an optimum concentration of 16 mg g^{-1} for both biocides. The dominance of bacteria in N_2O production was demonstrated by the greater inhibition obtained with bronopol (bactericide) compared to captan (fungicide). In addition, fungi:bacteria ratios, which were smaller than 1 in all the soils tested, further confirmed that bacteria are the main contributors to N_2O production in the soil investigated. The highest fungi:bacteria ratio was observed in a soil under long-term NT, indicating that a larger population of fungi has evolved in that soil compared to the others, and this could be due to less soil disturbance and higher moisture content in soils under NT management. Through these alterations in soil moisture regime, NT provides soil microorganisms with a more favorable environment to grow and expand (MacKenzie et al., 1997; Guggenberger et al., 1999; Bateman and Baggs., 2005). Although the results of this study improve our understanding of the biotic control of N_2O production in agroecosystems, one needs to recognize the limitations of the results. The experiments

were conducted in water-saturated soils (anaerobic condition), and these are generally not the prevailing conditions in agricultural soils. Therefore, it is unclear if similar results would have been obtained if the soils were tested under aerobic condition. Further studies using molecular biology (e.g. PLFA) and genomics should also be conducted to gain greater insights into the microbial groups contributing to N_2O production in soils under NT or PT practices.

Chapter 3 of this dissertation research described a greenhouse experiment dedicated to the identification of cover crops that are well-suited to agricultural soils under different tillage practices. Specifically, the experiment investigated the effect of soil amendment with cover crop of varying chemical composition on mineral N flux, crop N uptake and N_2O emission. Experimental pots, filled with PT and NT soils, were amended with rye or vetch biomass (C/N ratio of 82 and 11, respectively) and seeded with corn. Results of this experiment showed that the application of cover crop residue generally enhanced nitrate (NO_3^-) and N_2O fluxes, especially during the first two or three weeks after cover crop addition. Results indicated that the increase in NO_3^- and N_2O fluxes was dependent on C/N ratio of the cover crops applied. In other words, rye with greater C/N ratio resulted in higher NO_3^- fluxes and lower N_2O emission than vetch. Corn growth (height and biomass) was also better in rye-amended than vetch-amended soils. Overall, regardless of tillage practice, rye emerged as a better cover crop for enhancing soil fertility and N nutrition during early growth of corn.

Since several past studies have reported major shifts in soil microbial communities in response to agricultural land management (Feng et al., 2003; Spedding et al., 2004; Nyamadzawo et al., 2009; Gonzalez Chavez et al., 2010), a question that arose

from the findings reported in chapter 3 is whether alterations in the soil microbial community can be detected when exposed to cover crops of different chemical composition. A laboratory experiment was conducted to obtain a better microbiological understanding of the effects of the rye and vetch addition to PT and NT soils. Results of this laboratory experiment, reported in chapter 4, showed that regardless of tillage practice cover crop incorporation into soils increased soil microbial biomass and diversity. These soil biochemical characteristics have been proposed as sensitive indicators of soil health, the cornerstone of agricultural sustainability. Therefore, as a farming practice, cover crop addition represents more than just a source of N, but could also be considered as a practice that enhances soil fertility. Overall, results of this investigation demonstrated that soil biological functions benefit from cover crop application regardless of the soil tillage management.

Although the greenhouse (Chapter 3) and laboratory study (Chapter 4) provided useful information with regard to cover crop selection for soils under different tillage practices, the limitations of these experiments cannot be overlooked. For example, the greenhouse experiment was conducted with all environmental variables (e.g. soil moisture and temperature) kept under control, which is not a condition not normally found in agricultural fields. As it was shown by Dietzel et al. (2011), and Peterson et al. (2011), environmental variables such as freezing events, rainfall, etc could play important roles in affecting N transformation processes (e.g. nitrification, denitrification). Further, two types of soil (PT and mid-term NT) and two types of cover crop (hairy vetch and rye) were studied in this experiment, which was a limited number of soil types and cover crops. Thus, further studies should include soils with different duration of NT (short-,

mid-, and long-term) and cover crops with a broader range of C/N ratios. These investigations would provide a stronger basis for the selection of cover crops suitable to the most common types of agricultural soils of the US Midwest. In agricultural fields, when the cover crop is terminated, the whole plant system (shoot and roots) undergoes decomposition. However, in both the greenhouse and the laboratory experiments conducted in this dissertation, cover crops were collected off-site and used to amend soils in the laboratory. With this approach, the contribution of cover crops rhizosphere to the processes investigated remains unknown. Elimination of cover crops root system is therefore another limitation of this study. In addition, non-labeled crop residues were used in this study. This can be considered as a limitation because it was not possible to track the transformations of N released from the cover crops applied. In other words, it cannot be stated for certain that the increase in mineral N flux, N_2O emission and N uptake in corn grown on cover crop-amended soils was due to N released from the cover crops. For instance, cover crops addition might result in some changes in soil microbial activity leading to mineralization of some immobilized N that already existed in the soil and not provided by the cover crops. Therefore, it is recommended that further studies should apply ^{15}N-labeled crop residues in order to be able track N transformations more accurately.

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Curriculum vitae

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Education

Doctor of Philosophy, Indiana University-Purdue University Indianapolis, Indiana, USA
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- Concentration in Biogeochemistry with minor in spatial analysis in health and natural science.

Master of Science, Tarbiat Modares University, Tehran, Iran February 2010

- Major in Soil Sciences Engineering with focus on soil pollution and remediation.

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- Major in Soil Sciences Engineering with focus on soil classification.

Relevant experience

Research Assistant, (IUPUI), Department of Earth Sciences, Indianapolis, Indiana
August 2011-May 2016

Teaching Assistant, (IUPUI), Department of Earth Sciences, Indianapolis, Indiana
August 2012-May 2014

Researcher, Bio Bank, Ministry of Agriculture, Tehran, Iran June 2008-October 2010

Intern, Soil and Water Research Centre, Tehran, Iran May 2006-August 2006

Conference presentations

Bacterial and fungal contribution to N_2O production in midwest soils under different tillage practices and land uses, *Urban Health Conference*, Indianapolis, Indiana.
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Assessing microbial contribution to N_2O production in soils under no-till management, *Soil Science Society of America*, Tampa, Florida.
November 2013

Bacterial and fungi contribution to N_2O production in agroecosystems, *Midwest Geobiology Conference*, Indianapolis, Indiana.
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Linking N_2O production in no-till soils to microbial community diversity and activity, *Soil Science Society of America*, Cincinnati, Ohio.
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Efficiency assessment of bacterium assisted root stabilization of arsenic by kale (*Brassica oleraceae* var. *viridis*), *EcoSummit*, Columbus, Ohio.
October 2012

Publications

Ladan, S., Jacinthe, P.A. (2016). Nitrogen availability and early corn growth on plowed and no-till soils amended with different types of cover crop. Plant and Soil Journal (*under review*).

Ladan, S., Jacinthe, P.A. (2015). Evaluation of potential inhibitors of bacterial and fungal denitrification in soils under different tillage practices. Applied Soil Ecology Journal (*under review*).

Malakouti, M.J., & **Ladan, S**. (2010). Hazardous Effects of Arsenic on Plants and Human Beings in Iran (A review). 4th International Symposium on Trace Elements and Minerals in Medicine and Biology. St. Petersburg, Russia.

Ebrahimi, S., **Ladan, S**., & Malakouti, M.J. (2009). Investigatin of Remediation Possibility of Different Types of Oil Contaminants in Soil and Algorithm Presentation According to Contaminant Type. 11th Congress of Soil Science, Gorgan, Iran.

Malakouti, M.J., **Ladan, S**., & Tabatabaei, S.J. (2009). Nitrate in the Edible Parts of Vegetables in Iran: Origin, Safety and Toxicity Limits. In: Shahid Umar et al (Eds) Nitrate in Leafy Vegetables: Toxicity and Safety Measures (UC).

Ebrahimi, S., **Ladan, S**., & Malakouti, M.J. (2008). Stubble Burning, A Serious Threat to Crop Production and Soil Fertility in Iran. University of Natural Resources and Applied Life Sciences (BOKU), Eurosoil, 350, Vienna, Austria.

Relevant course work

Biology, Math, Physics, Soil Physics, Soil Chemistry, Soil Biology, Soil Pollution, Soil Biogeochemistry, Geochemisrty, Organic Chemistry, Inorganic Chesmisty, Isotopes Geochemistry, Statiscis, Remote Sensing, GIS

Skills

- Microsoft Office, ArcGIS, ILWIS, SigmaPlot, SAS, PAST, Data Analysis, Teaching, Research, Greenhouse and Labrotary Activities, GC, Mass Spectrometry, HPLC
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Organizational involvement

- Member of Indiana Association of Environmental Professionals (INAEP) 2015-Present

- Member of Indiana Geologists Association 2015-Present
- Member of Earth Sciences Women's Network (ESWN) 2015-Present
- Member of Soil Science Society of America (SSSA) 2011-Present
- Member of School of Science Graduate Students Counsil, IUPUI 2011-2014
- Member of Graduate Professional Students Government, IUPUI 2011-2015

Honors

- Received Dr. Arthur Mirsky Geology Scholarship, 2011
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