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CONSERVATION AGRICULTURE IN KENTUCKY: INVESTIGATING NITROGEN LOSS AND DYNAMICS IN CORN SYSTEMS FOLLOWING WHEAT AND HAIRY VETCH COVER CROPS

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THESIS

A thesis submitted in partial fulfillment of the
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ABSTRACT OF THESIS

CONSERVATION AGRICULTURE IN KENTUCKY: INVESTIGATING NITROGEN DYNAMICS AND LOSS IN CORN SYSTEMS FOLLOWING WHEAT AND HAIRY VETCH COVER CROPS

Unintentional nitrogen (N) loss from agroecosystems produces greenhouse gases, induces eutrophication, and is costly for farmers; therefore, adoption of conservation agricultural management practices, such as no-till and cover cropping, has increased. This study assessed N loss via leaching, NH_3 volatilization, N_2O emissions, and N retention in plant and soil pools of corn conservation agroecosystems across a year. Three systems were evaluated: 1) an unfertilized organic system with cover crops *Vicia villosa*, *Triticum aestivum,* or a mix of the two; 2) an organic system with a *Vicia* cover crop employing three fertilization schemes (0 N, organic N, or a cover crop N-credit approach); 3) a conventional system with a *Triticum* cover crop and three fertilization techniques (0 N, urea N, or organic N). During cover crop growth, species affected N leaching but gaseous emissions were low across all treatments. During corn growth, cover crop and fertilizer approach affected N loss. Fertilized treatments had greater N loss than unfertilized treatments, and fertilizer type affected gaseous fluxes temporally and in magnitude. Overall, increased N availability did not always indicate greater N loss or yield, suggesting that N conserving management techniques can be employed in conservation agriculture systems without sacrificing yield.

KEYWORDS: Ammonia volatilization, Conservation agriculture, Cover crops, Nitrogen leaching, Nitrous oxide emissions

Rebecca Erin Shelton

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To my mother and father,

thank you for your unwavering support.

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1.1. **Human Alteration of the Nitrogen Cycle**

Nitrogen (N) is a critical nutrient, governing the dynamics of ecosystems, as the quantity of biologically available N is often a limiting factor for net primary productivity (Vitousek *et al.*, 2002; Galloway *et al.*, 2004). Globally, there are several large N pools that are relatively unavailable to plants and microbes. The largest pool of N is atmospheric N_2 which can be transformed into a small pool of biologically available N via the natural fixation processes of lightning (abiotic N fixation) and conversion by microorganisms (biological N fixation, often in symbiosis with leguminous plants) (Vitousek *et al.*, 1997; Galloway *et al.*, 2004). However, over the past 200 years, humans have drastically altered the global N cycle, and it is estimated that anthropogenic activities have more than doubled the amount of N in biologically available forms (Berendse *et al.*, 1993; Vitousek *et al.*, 1997). The anthropogenic activities primarily responsible for these changes are N fertilizer synthesis and application, fossil fuel combustion, utilization of N fixing crops (legumes), and N mobilization via biomass burning, land clearing and conversion, and the drainage of wetlands (Vitousek *et al.*, 1997; Galloway *et al.*, 2004).

Agricultural production and agroecosystem management are major contributors to the alteration of the global N cycle through such practices as N fertilization, fossil fuel combustion associated with tractor usage and other farm implements, incorporation of N fixing crops, land clearing, and the drainage of wetlands. It is estimated that agroecosystems receive approximately 75% of the bioavailable N created by human activities (Galloway *et al.*, 2004). Arguably, agriculture plays the largest role in human alteration of the N cycle (Mosier *et al.*, 1998).

In addition to adding large quantities of biologically available N into the global N cycle, humans have uncoupled the N cycle from the carbon cycle and increased turnover rates of both. Carbon (C) and N cycling are typically coupled in unmanaged ecosystems as biological N-fixation often occurs with C-fixation. However, application of synthetic N fertilizer adds N without adding C; thus agricultural practices have been identified as largely responsible for the uncoupling of these two cycles (Woodmansee, 1984). Tillage and regular disturbance of the soil also adds to the uncoupling of C and N cycling. Tillage influences soil structure and porosity, thereby affecting the interaction between soil, water, and gaseous exchange, which subsequently affects C and N transformations (Abdollahi *et al.*, 2014; Plaza-Bonilla *et al.*, 2014). Using tillage to incorporate organic residues, particularly those of legumes, can result in rapid decomposition of the residues, releasing N more quickly than occurs in undisturbed soils or soils with unincorporated organic matter (Drinkwater *et al.*, 2000). Disturbing soil by plowing at deeper depths results in less C accumulation in the soil profile (Zikeli *et al.*, 2013).

Lastly, not only do agricultural practices add N into the environment, uncouple the N cycle from the C cycle, and speed up the rate of nutrient cycling, but they further modify nutrient cycling by removing large quantities of N from the environment during crop harvest. It is largely due to repeated harvests that significant quantities of N and other nutrients must be added back into the system, often as synthetic fertilizer, prior to each cropping season. In fact, very little N accumulates within agroecosystems. According to Smil (1999), 50% of the N applied is removed via crop harvest and approximately only 2-5% of the applied N accumulates in agroecosystem soil. Similarly, Van Breemen *et al.* (2002) calculated that only 10% of added N is stored within the system. Though a large portion of the added N is taken up by the crop and lost from the system during harvest, the N use efficiency of most cropping systems is very low, with N also leaving agroecosystems via other loss pathways (Smil, 1999; Watson *et al.*, 2002). Models have predicted that approximately half of the N applied to agroecosystems in the form of mineral and organic fertilizers and via symbiotic N fixation is "lost" to the environment (Velthof *et al.*, 2009).

These N loss pathways include volatilization via the abiotic process of ammonium (NH_4^+) conversion to gaseous ammonia (NH_3), denitrification via an anaerobic microbial-driven biotic transformation of nitrate (NO_3^-), to nitrite (NO_2^-) to the gaseous N forms of nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2), nitrification via an aerobic microbial-driven biotic transformation of NH_4^+ to NO_2^- during which N_2O and NO are produced as intermediates, and loss via runoff and leaching. These loss pathways contribute to environmental issues such as the greenhouse effect, acid deposition, and eutrophication of coastal regions and other aquatic systems, impacting the productivity of both terrestrial and marine ecosystems (Galloway *et al.*, 2004). Approximately 25% of the N in agroecosystem soils is emitted annually to the atmosphere (Smil, 1999), and ~74% of U.S. nitrous oxide emissions, a potent greenhouse gas, are attributed to agricultural soil management (EPA, 2015). Nitrous oxide has a global warming potential approximately 298 times that of carbon dioxide (Forster *et al.*, 2007), and though the United States contains 11.5% of the world's arable land and 8.3% of the world's agricultural land use area, according to world development indicators produced by the (World, 2014), in 2010 the United states emitted 10.6% of global nitrous oxide (N_2O)

emissions (FAOSTAT, 2014).

Excess fertilization in agriculture is one of the two largest sources of nonpoint pollution to the surface waters of the United States (Carpenter *et al.*, 1998). Regression models have shown that 70% of the nitrate concentration in streamflow can be explained by the amount of fertilizer N used in that river basin (Boyer *et al.*, 2002). Approximately 20% of applied N accumulates in our water resources via nitrate leaching (Smil, 1999), and N loading of water sources is linked to algal blooms and disrupted aquatic ecosystem services (Suddick *et al.*, 2013).

These N losses are, in part, a consequence of maintaining N-saturated systems and management practices that do little to lessen or slow the cycling of N, such as N application timing that is not well synced with crop N uptake, frequent soil disturbance, and leaving soil bare during the non-growing season (Mohr *et al.*, 1999; Drinkwater and Snapp, 2007; Jan *et al.*, 2011). Altering farm management practices and minimizing N losses will not only impact the environment but also farm economics. Within the last decade, there has been growing public concern in the American Midwest about N loss from cropping systems (Crandall *et al.*, 2005). Because the cost of N fertilizer has substantially increased in the last decade (USDA-ERS, 2013b), unintentional off-farm losses negatively affect farmers' profit (they pay for something they are not able to capture and turn into profit/increased crop production). Therefore, for both environmental and economic reasons, it is critical to improve agroecosystem N use management so that N supply is well-coordinated with crop N demand (Fageria and Baligar, 2005).

1.2 Nitrogen Conservation and Loss in Agroecosystems

Research on agricultural systems to decrease N loss has shown that there is considerable variance in N loss pathways, spatially, temporally, and across different management practices and crops. Variability in nitrate (NO_3) leaching, N_2O emissions and NH_3 volatilization are widely acknowledged, and it is known that the driving factors- soil temperature, water filled pore space, soil aggregate structure, pH, and available C and N- depend on a combination of climatic conditions and the plant-soil environment (Six *et al.*, 2002). Some of these factors cannot be controlled (e.g. spatial variability in soil conditions, inter and intra-annual climate variability); however, by altering land management practices, such as the timing and form of nutrient inputs and optimizing soil structure by changing tillage practices, conditions can be modified to mitigate these losses. A particular combination of management practices, known as conservation agriculture, is one agricultural approach widely employed to decrease N loss (Scopel *et al.*, 2013; FAO, 2015).

Conservation agriculture has fairly well-defined goals and practices. The primary goals are to reduce inputs and to conserve soil and water. These goals are achieved through reducing tillage, maintaining living or non-living organic residues on the soil surface, and practicing crop rotation and/or intercropping (Scopel *et al.*, 2013), and can be applied in either organic or conventional systems. Reducing inputs should directly reduce N loss through leaching, volatilization, and denitrification. Improved understanding of these loss pathways and their controlling environmental parameters should help inform management practices so that more N is retained within the soil or living biomass of the agroecosystem, thus decreasing the amount of N that must be added into the system each cropping season. This interest in improving N retention has resulted in a growing body of research that compares N loss between agroecosystems that use conservation agriculture practices and those that do not, but results are often contradictory and inconclusive.

Studies comparing N_2O and carbon dioxide (CO_2) emissions between systems with varying amounts of tillage have been particularly prominent in this field. Initially, it was hypothesized that no-till systems would reduce global warming potential compared to conventional tillage. This was predicted because no-till reduces soil C turnover which would increase C sequestered in the soil and reduce CO_2 emissions (Parkin and Kaspar, 2006). However, several studies have found that there is little-to-no difference in emissions across tillage systems (Kessavalou *et al.*, 1998; Robertson *et al.*, 2000; Grandy *et al.*, 2006; Parkin and Kaspar, 2006), and other studies have found evidence that soil C does increase under no-till, but that N_2O and/or CO_2 emissions also increase, negating a lower global warming potential (MacKenzie *et al.*, 1997; Six *et al.*, 2002; Baggs *et al.*, 2003; Venterea *et al.*, 2011). Interestingly, a long-term study by Six *et al.* (2004) found that in a conventional tillage vs. a no-tillage system, N_2O emissions were greater in the no-till system for the first five years, but emissions began to decrease after ten years, and then eventually became lower than the conventional tillage system twenty years after establishment. Clearly, the global warming potential of tilled versus no-till systems and the mechanisms controlling greenhouse gas production within these systems remains somewhat unknown. By continuing to study the effects of different tillage systems in other regions and climates of the world, we can continue to improve our understanding of how tillage impacts emissions and how those emissions are related to soil structure and soil C and N pools.

Organic residue management is also a primary management practice in conservation agriculture and likely to impact N loss pathways. Organic residues can be living or non-living, kept on the soil surface or incorporated, but the common goal is to leave more residues in the field so that less N is exported from the system. Non-living organic residues may be the residue from terminated crops or residue that is brought in to use as mulch, such as straw. Living organic residues are also known as cover crops.

Cover crops are grown after the cash crop, or in any off-season, to prevent N export via leaching and/or runoff. They stabilize the soil surface and take up N that remains in the soil system after harvest (McCracken *et al.*, 1994; Drinkwater and Snapp, 2005; Zhou *et al.*, 2012). When the cover crop is terminated prior to establishing the next cash crop, the residues are either left on the soil surface or incorporated so that they may decompose and release N to the cash crop. Incorporating the residue rather than leaving it on the soil surface is commonly practiced in tillage systems, and is known to stimulate rates of decomposition and nutrient cycling (Beare *et al.*, 1993; Varco *et al.*, 1993). However, no-till conservation agriculture systems promote keeping the residues on the soil surface, which is achieved by mowing, rolling down, or killing cover crops with herbicide. To increase complexity, even if the residue is left on the surface, its mineralization rate is influenced by the type of termination employed: studies have found that rolling down the cover crop results in slower mineralization than if it were flail mowed (Dabney *et al.*, 1991). N dynamics and loss from cover crops will clearly vary depending upon the specific combination of management practices employed.

Though conservation agriculture may, or may not, increase N retention in the agroecosystem, some N must be added as there will always be N exported from the system in the form of the harvested crop. Thus, not only does research continue to explore the best combination of practices for N retention in the form of tillage and residue management, but also with the type of fertilizer, how it is applied, and how it interacts with tillage and residues. Synthetic fertilizer is commonly applied as urea, ammonium nitrate, solutions of urea and ammonium nitrate, or injected as anhydrous ammonia, with urea being the most readily volatilized as ammonia (Battye *et al.*, 1994). Organic fertilizers are applied in many different forms with varying concentrations and ratios of C and N. Common forms are fresh manure, composted manure, green plant material, composted plant material, and blood/feather/meat meal (USDA-AMS, 2015). Biotically mediated N transformation pathways are strongly influenced by type of fertilizer, as fertilizers that provide a C source often stimulate heterotrophic microbial activity (Fairchild *et al.*, 1999; Mitchell *et al.*, 2013). Along with environmental factors, the type of fertilizer plays a role in dictating the timeline of when N is available for plant uptake, but so does the timing of the application and the method of application (Venterea *et al.*, 2011). Fertilizer can be applied pre-planting, at-planting, split between planting and another time in a crop's life cycle when its N demand is at its peak, or applied only at peak N demand. It can be applied by broadcasting, with or without incorporation, banding, with or without incorporation, or injecting it directly into the soil. All of these fertilizer management strategies play a role in determining N loss via biotic and abiotic gaseous loss and leaching, and they also interact with the presence or absence of organic residues and tillage regime, making it difficult to isolate which combination of practices is best for reducing N loss.

Many studies suggest that, in addition to C and N substrate availability, microbial respiration and activity are influenced by both soil temperature and soil moisture (Feng *et* *al.*, 2003). The previously discussed conservation management practices - tillage and cover crop termination - have important effects on N loss because they directly influence environmental factors that control N transformations in the soil environment, such as soil moisture, soil temperature, and soil structure (aggregate structure and porosity), which then indirectly affect the microbial community and conditions for N loss pathways.

Tilled systems leave greater soil surface area exposed, allowing for increased sun exposure that heats the soil and evaporates soil moisture (Licht and Al-Kaisi, 2005; Salem *et al.*, 2015). This could affect microbial activity because increased temperature has a pronounced positive effect on microbial activity and denitrification (DeKlein and VanLogtestijn, 1996). In contrast, no-till systems have been promoted for their ability to retain soil moisture, particularly when the soil is covered with an organic residue that prevents evaporative loss (Das *et al.*, 2015), and for eliminating the physical soil disturbance that tillage introduces. However, these conditions also create a cooler soil environment longer into the growing season which can impact soil respiration rates (lesser CO_2 released) and N transformation rates (DeKlein and VanLogtestijn, 1996; Soane *et al.*, 2012; Hu *et al.*, 2013). Commonly, environmental conditions in no-till systems result in an overall positive correlation between nitrification, denitrification, and soil moisture (Hu *et al.*, 2013).

In a Kentucky Maury silt loam, Rice and Smith (1982) found that there was greater denitrifying activity (N_2O is a byproduct of denitrification) in a no-till compared to a conventional till system and attributed the difference to soil moisture conditions. Doran (1980) also found that denitrification potential was higher in no-till systems, but that mineralization and nitrification potential were higher in conventional till systems.

Alternatively, some studies have found that the nitrification of ammonium was more rapid in no-till soils because of more favorable moisture conditions (Rice and Smith, 1983) and that decreases in soil water potential decreased rates of ammonification and nitrification (Chen *et al.*, 2011).

The physical structure and organic matter content of the soil are also affected by tillage, and these factors influence soil moisture and substrate availability, thus impacting gaseous diffusion from the soil to the atmosphere. Soil aggregate stability and size distribution typically influences water holding capacity, diffusion of gases and water, C occlusion, and habitats for soil organisms (Mangalassery *et al.*, 2013; Al-Kaisi *et al.*, 2014; Bandyopadhyay and Lal, 2014; Du *et al.*, 2015; Guo *et al.*, 2015; Salem *et al.*, 2015). Compared to conventional tillage practices, reducing tillage has been found to improve soil structure by increasing soil organic matter, reducing soil bulk density and increasing the proportion of larger aggregates (Daraghmeh *et al.*, 2009; Al-Kaisi *et al.*, 2014). Research has found that no-till systems have more macro-aggregates and that these macro-aggregates contain an increased number of micro-aggregates holding soil organic C, especially when mulch is part of the no-till system (Liang *et al.*, 2011; Al-Kaisi *et al.*, 2014; Andruschkewitsch *et al.*, 2014). However, systems in which more C is contained within macro-aggregates rather than micro-aggregates, may have increased C and N gaseous emissions, as substrates within macro-aggregates are more labile and subject to mineralization (Elliott, 1986; Drury *et al.*, 2004; Manna *et al.*, 2006; Bandyopadhyay and Lal, 2014). It is clear that there are differences in soil moisture, soil temperature, and soil structure across different tillage and residue management regimes, and further research is needed to characterize the relationships between these variables and N transformation and loss within different systems.

It is important to determine the influence of tillage and organic residues on microbial processes so that the biotically mediated N pathways are better understood. However, the abiotic pathways affected by soil temperature, soil moisture, and other environmental conditions must also be taken into account as ammonia volatilization is one of the primary N loss pathways of N applied as fertilizer (Bouwman *et al*., 2002; Vitousek *et al*., 2009). A study by Fan *et al.* (2011) found that an increase in temperature from 20 to 30 degrees C increased cumulative NH_3 volatilization loss and that a loam soil had less volatilization than a sandy soil. A supporting study also found that ammonia volatilization from urea was decreased in clay soils compared to sandy soils and, additionally, was greater in systems with organic residues present (Francisco *et al.*, 2011). Volatilization in a sandy soil may be attributed to decreased ability to fix ammonium (due to lesser cation exchange capacity) (Harrison and Webb, 2001) and, despite increasing moisture retention and decreasing soil temperature (Baggs *et al.*, 2003; Das *et al.*, 2015), conditions typically associated with reduced volatilization, organic residues may increase volatilization as they can act as a barrier between the applied urea and the soil surface, preventing assimilation of the urea into the soil profile (Rochette *et al.*, 2009). Additionally, Mohr *et al.* (1998) found that systems in which organic residues are left on the soil surface without any applied fertilizer are subject to increased levels of ammonia volatilization compared to systems in which the residue is incorporated, possibly because residues are a C source thus stimulating the growth of urease producing bacteria and fungi (Deng and Tabatabai, 1996; Hamido and Kpomblekou-A, 2009).

Despite strong correlations between nitrification, ammonification, and denitrification with soil moisture and temperature, some studies of CO_2 and N_2O emissions have shown very weak or no correlation with soil moisture or temperature (Liu *et al.*, 2002; Dyer *et al.*, 2012), indicating that there may be other regional or land-use factors influencing environmental interactions and the production and emission of these gases. Stange and Neue (2009) suggested that temperature sensitivity of nitrification differs between sites and with site history. Additionally, C and N substrate availability is a critical factor in determining the fate of N in agroecosystems. Conclusively, this body of prior work illustrates the complexity of developing and issuing recommendations that are best for all regions, climates, and soil types; thus, to predict soil N dynamics and develop management strategies to improve agroecosystem N retention, site specific research is needed.

In Kentucky, there is some existing research quantifying N loss pathways in no-till, conservation agriculture systems. Parsons *et al.* (1991) studied the denitrification rates of two central Kentucky soils - a Lanton silt loam and a Maury silt loam. They found that water filled pore space and soil respiration were correlated with N gas loss and indicative of denitrifier activity, but that this relationship was stronger for the Lanton soil than the Maury soil, as the Maury soil N_2O emission rates were very low. These low rates were attributed to the superior drainage qualities of the Maury soil. Rice and Smith studied denitrification in a Kentucky Maury silt loam in tilled and no-till systems. In 1982, they found higher denitrifying activity in no-till corn systems with a rye cover crop and attributed it to higher soil moisture, but in 1984, they found no consistent trend for the effect of tillage on N lost (Rice and Smith, 1982, 1984). Though they did not study specific N loss pathways, they concluded that leaching and denitrification may be of less importance than N immobilization in determining tillage effects on fertilizer N availability. The findings of Kitur *et al.* (1984) supported this theory and also determined that immobilization in rye residue seemed to be the most important sink for fertilizer N in no-till systems with a rye cover crop.

McCracken *et al.* (1994) studied no-till corn systems with different cover crops and found that rye cover crops had less nitrate leachate than vetch cover crops. Stoddard *et al.* (2005) found that no-till systems with manure and fertilizer applied tended to have higher NO_3 concentrations in leachate (collected at 90 cm) than tilled systems with the same fertilizer treatments. However, very few studies have looked at leaching and gaseous loss simultaneously in no-till corn systems. One study by Fairchild *et al.* (1999) studied a Kentucky Zanesville silt loam containing a fragipan. The study found that denitrifiers may indirectly help reduce NO_3 leaching via denitrifcation and the release of N_2O and, in preliminary studies, found that NO_3 removal at the fragipan layer was stimulated by C input and suppressed by a winter wheat cover crop, but this study was specific to soils with a fragipan layer. No Kentucky study to date, that I am aware of, has compared no-till corn systems and simultaneously assessed N dynamics in biomass and the soil, while quantifying a variety of N loss pathways, including gaseous, aqueous, and solid phases.

1.3 Organic vs. Conventional Management Effects

Though some work has been done to produce site-specific recommendations for Kentucky (Bitzer *et al.*, 2000; Murdock and Ritchey, 2014), recommendations for both organically managed and conventionally managed conservation agriculture systems are needed. Specifically, it is critical to address corn (*Zea mays L.*) systems as corn is the dominant row crop in Kentucky (Bitzer *et al.*, 2000) and the dominant cash crop

(KyCGA, 2014) covering 1,520,000 acres of Kentucky in 2014 (USDA-NASS, 2015). No-till operations are increasing (Horowitz *et al.*, 2010); in 2001, ~37% of corn acres in Kentucky were managed as no-till operations that kept more than 30% of the soil surface covered in residue, and by 2010, this percentage had increased to approximately 47% of Kentucky corn acres (USDA-ERS, 2014). In Kentucky, corn systems are primarily conventionally managed. However, organic acreage has been increasing over the last decade. In 2005, the USDA reported that only 159 acres in Kentucky were utilized for organic corn production; however, by 2011, production had increased to cover 1,123 acres (USDA-ERS, 2013a). This trend will likely continue in Kentucky, as it has nationwide. Conventional and organic systems have different primary management concerns and to achieve systems that are economically realistic, goals and strategies for reducing N loss may differ. These management differences are often manifested in the type of N fertilization, the species of cover crop planted, and the timing of cover crop planting and termination.

1.3.1 Type of Fertilizer

First, the type of fertilizer used in these systems typically differs. In conventional no-till systems, common fertilizers include anhydrous ammonia, urea applied with urease and/or nitrification inhibitors, polymer coated urea, manure, or a urea ammonium nitrate (UAN) solution applied alone or with urease and/or nitrification inhibitors. Inhibitors and polymer coatings often enhance the efficiency of fertilizer N, i.e. improve crop N retention, as N is released more slowly throughout the season rather than solely at application. In terms of N_2O emissions, these fertilizers release less N at initial application but then have higher fluxes throughout the season after rainfall events (Hatfield and Venterea, 2014; Parkin and Hatfield, 2014). In a no-till system with a clay loam soil, Halvorson *et al.* (2014) found that a polymer coated urea fertilizer, ESN, reduced N_2O emissions by 42% compared to urea and 14% compared to UAN, while applying UAN with AgrotainPlus, a urease and nitrification inhibitor, reduced emissions by 61% compared to urea and 41% compared to UAN alone. On a Crider silt loam in Bowling Green, Kentucky, Sistani *et al.* (2011) found that applying poultry litter with AgrotainPlus to a no-till corn system produced greater N_2O emissions than urea, UAN, and SuperU (urea nitrogen granule with nitrification and urease inhibitors) fertilizers. Though several studies have examined N_2O emissions released from enhanced efficiency fertilizers, results have yet to identify one that consistently emits the least N_2O. In central Kentucky, the most commonly used fertilizers are urea and UAN with and without inhibitors (Edwin Ritchey, personal communication, 28 January 2014), but additional research is needed in this region to determine N losses using these products in conventional no-till corn systems.

In organic systems, N is typically added to the system via N fixation by legumes, as Chilean nitrate, in animal manure, in compost, or in the form of animal/plant byproducts (Teasdale, 2012). Unlike conventional sources that have readily available N, these fertilizers are often naturally slow in releasing N and do not require additional inhibitors or coatings. Also, similar to manure in conventional systems, adding N in one of these forms (excluding Chilean nitrate) also adds C to the system. Despite the slow release nature of these sources, this added C may be largely responsible for increasing N loss from systems using these organic N sources. Mitchell *et al.* (2013) conducted a series of soil incubations with soil collected from fertilizer bands in a field planted with a corn crop following a rye winter cover crop. The results showed that mineralizable C limited N_2O emissions rather than NO_3 availability. Additionally, (Cavigelli *et al.*, 2009) found that an organic system amended with poultry litter produced significantly more N_2O than a conventional no-till system, and the organic system had greater soil C content. Overall, there is limited research on N loss from organic, no-till systems and much is unknown about the interaction of soil C and applied N in these settings. However, it is understood that in organic C rich systems heterotrophic nitrification and denitrification is stimulated as heterotrophic microbial populations are reliant on obtaining C from organic C sources, whereas, in contrast, autotrophic bacteria are reliant on atmospheric CO_2 for meeting carbon requirements rather than organic C and thus autotrophic nitrification and denitrification does not require organic C if inorganic N sources are available (Subbarao *et al.,* 2007).

1.3.2 Cover Crop Species

Second, the cover crop species used in these corn-cropping systems is often affected by whether the system is organically or conventionally managed. Three primary considerations when choosing a species are: 1) the potential N contribution, 2) the ability to re-capture and retain N in the soil, and 3) biomass production capability. Leguminous species are valued for potential N fixation and subsequent contribution and, thus, may reduce input costs for the following summer crop, increasing profit potential compared to grass cover crop species (Roberts *et al.*, 1998). Because organic farmers depend more on alternative N sources compared to those that are chemically synthesized, this increases incentives to plant a leguminous cover crop. However, the legume is typically not used as the only source of N in organic systems, as legumes are only profitable when they increase the yield of the following crop, which, in most cases, requires additional N (Allison and Ott, 1987).

Commonly used winter legumes include *Vicia villosa* (hairy vetch), *Trifolium incarnatum* (crimson clover), and *Pisum sativum* (austrian winter pea), with hairy vetch being the most commonly used cover crop in the United States (Baldwin and Creamer, 2009). Vetch is a winter hardy legume, produces approximately 297 kg ha^{-1} of biomass for every 100 growing degree days, and can provide up to 179 kg $ha^{-1}$of N (Teasdale, 2012). Additionally, research at the University of Kentucky found the benefits of hairy vetch to be greater than that of crimson clover or big flower vetch in terms of yield advantage beyond that of legume N contribution (Bitzer *et al.*, 2000). Hairy vetch improves soil structure, soil water holding capacity, and increases the effectiveness of additional applied N for the subsequent crop (Hanson *et al.*, 1993; Lichtenberg *et al.*, 1994).

If the primary reason for planting a cover crop is to re-capture N left in the soil profile from the preceding growing season and prevent nitrate leaching over the winter months, cereal grass cover crops such as *Triticum aestivum* (winter wheat), *Secale cereale* (winter rye), and *Avena sativa* (oat) are recommended. These species are preferred for N retention as they establish more quickly than legume monocultures, and their root growth remains active in cooler temperatures (Ranells and Wagger, 1997). As a result, cereal grasses often produce as much as 6,720 kg ha^{-1} of biomass but the N concentration is low, between 1-2% N (Baldwin and Creamer, 2009). In Kentucky, winter wheat is the most common winter cover crop grown in conventional corn systems (Lee and Knott, 2014).

Biomass production by the cover crop is of concern for both organic and conventional farmers. Both systems benefit from the organic matter building potential provided by more biomass, but conventional farmers may prefer to plant high biomass grass cover crops as they tend to grow more vigorously than legumes during the winter, resulting in greater soil N uptake and reduced soil erosion. In contrast, organic farmers value cover crop biomass production for its contribution to weed control during the summer growing season, as well as additional N input. With a legume cover crop, an organic farmer may benefit in terms of N contribution, but it may not provide the biomass production needed for weed control. Typically, biomass production greater than 8,960 kg ha^{-1} is needed for weed suppression, and the maximum production of hairy vetch is only approximately 5,600 kg ha^{-1} (Teasdale, 2012).

In addition to insufficient biomass production, legume biomass decomposes more quickly than cereal grasses, as it has a lower C:N ratio. If the C:N ratio of biomass is less than 20 to 25 to 1, the N contained within the material is typically released rather than immobilized and breakdown occurs quickly (Wagger, 1989). The C:N ratio of hairy vetch is generally between 10 and 20 to 1, whereas that of a cereal grass terminated in mid to late May can be as high as 50:1 (Baldwin and Creamer, 2009). In a no-till system, Wagger (1989) monitored the release of N from cover crop residue and found that 87% of hairy vetch N and 86% of crimson clover N was released within 16 weeks following termination, but that only 47% of that contained in rye residue was released. Additionally, the quantity of N initially found in the rye residue was much lower. The increased biomass production, high C content, and slow decomposition rate of cereal grasses is beneficial for weed suppression, but does not provide the N contribution like that of a leguminous cover crop. One benefit is often sacrificed for the other; however, a compromise may be achieved by growing a cover crop mixture.

Growing legumes and cereal grasses together in a bi-culture cover crop can be more beneficial than growing either in monoculture. Growing legumes in bi-culture with cereal grasses may increase legume winter hardiness and result in a quantity of biomass greater than if either of the species were grown in monoculture; more biomass results in increased weed suppression and greater N and organic matter contribution (Teasdale and Abdul-Baki, 1998; Snapp *et al.*, 2005). In addition, mixing a cereal grass species with a legume decreases the C:N ratio of the biomass, resulting in increased residue decomposition and N release (Snapp *et al.*, 2005). The C:N ratio is decreased not only because the legume species has an innately lower C:N ratio, but some research has found that the C:N ratio of the rye component of a rye-vetch mixture was lower than when rye is grown alone (Salon, 2012). However, (Clark *et al.*, 1997) and Rosecrance *et al.* (2000) both found that vetch alone still released more N than a rye-vetch bi-culture and that N immobilization still occurred in the rye-vetch bi-culture. Finally, it is theorized that because non-legumes more efficiently scavenge N from the soil than legumes, in bi-cultures the non-legume may deplete soil N promoting increased N fixation and nodulation in the legume species (Snapp *et al.*, 2005; Salon, 2012). Overall, growing bi-cultures over the winter may be beneficial for weed suppression and N contribution in organic systems.

1.3.3 Cover Crop Management and Timing

Third, the timing of cover crop planting and termination may differ between organic and conventional systems. In Kentucky corn systems, the cover crop is typically planted in the fall between late September and early November and terminated in spring between April and May. In conventional systems, the cover crop is typically terminated at least two to three weeks prior to the ideal corn planting date (early May) via herbicide burn down using glyphosate or paraquat (Lee and Knott, 2014). However, organic farmers terminate via rolling or mowing the cover crop, and the species they plant may be influenced by the kill effectiveness of these technologies. If rolling the cover crop in the spring, they must wait to terminate the cover crop until a certain stage of physiological maturity is reached. For effective kill and reduced re-growth, vetch is terminated when the crop is at 50% flower and wheat is terminated at soft dough stage (Bowman *et al.*, 2012). The weather conditions are also critical in determining when termination can occur; if the soil is too moist rolling the crop will result in unwanted soil compaction. Additionally, an organic farmer may choose to terminate later than a conventional farmer in order to achieve maximum biomass production and N-fixation prior to termination.

It is difficult to provide a single set of recommendations to reduce N loss that are suitable for all systems due to management constraints, like those required for organic certification, and naturally varying edaphic and climatic conditions. On top of the previously mentioned factors controlling N loss, even the type of corn variety planted can affect N use (Caviglia *et al.*, 2014). However, it is possible to determine practices that reduce N loss within organic and conventional conservation agriculture systems in Kentucky with research that examines the N dynamics specific to each of these agroecosystems. Significant economic and environmental concerns justify the need for this type of research.

Corn production covers more area in the United States than any other crop, including ~6% of Kentucky's total land area (KyCGA, 2014; USDA-NASS, 2015). In Kentucky, total corn acres harvested increased by 17% and the number of Kentucky farmers selling corn for grain increased by 36% from 2007 to 2012 (KyCGA, 2014).

Economically it is important to mitigate N loss as the average price received for corn grain is declining while the cost of fertilizer N continues to increase (USDA-ERS, 2013b). In 2012, the price received for corn grain was $6.67 per bushel with conventional fertilizer N cost averaging $588 per short ton (USDA-ERS, 2013b; NASS, 2014). However, in 2013 fertilizer N costs increased to an average of $606 per short ton, and USDA projected corn grain prices for 2014-2015 were expected to fall within the range of $3.65 to $4.35 per bushel (Thiesse, 2014).

Environmentally, agricultural systems are a significant human alteration of the global N cycle, contributing to increases in atmospheric N_2O and NH_3 and eutrophication of water bodies. However, because agricultural systems are managed systems, they present great opportunity for manipulation that can reduce N loss and increase economic and environmental sustainability.

1.4 Objectives and Hypotheses

This project addresses several research gaps in the current literature. It will quantify N loss for conservation agriculture systems in Kentucky that leave cover crop residue on the soil surface, and assess how soil N dynamics are affected by cover crop species across the entirety of the year as opposed to solely post-termination of the cover crop. It will also contribute to data on organic, no-till systems as organic corn production is increasing, but to date, there is little research to provide cover crop recommendations for no-till systems using organic production practices. The primary objective of this research is to generate data aimed at improving recommendations for best systemic on-farm management practices that reduce N loss in Kentucky conservation agricultural systems, both conventional and organic. This will be achieved by measuring N loss via leaching, NH_3 volatilization, and N_2O emissions and N retention in plant and soil pools in both types of system throughout a full year. To fulfill the primary objective, three secondary objectives were identified: 1) quantify the effects of cover crop type (winter wheat vs. hairy vetch vs. a bi-culture of the two species) on N loss and dynamics in an organic corn system; 2) quantify the effects of fertilizer approach (full application of organic fertilizer vs. a fertilizer N-credit approach that reduces the applied fertilizer by taking into account the N contribution of the cover crop) on N loss and dynamics in an organic corn system planted with a hairy vetch cover crop; and 3) quantify N loss and dynamics between two fertilizer types (urea with a urease inhibitor vs. an organic slow-release pellet) in a conventional corn system planted with a winter wheat cover crop.

For the organic management systems, I hypothesized that: 1) N loss would be greater in systems planted with a legume only (hairy vetch) cover crop in comparison to the grass only (wheat) or the bi-culture (hairy vetch-wheat) treatment, because legumes grow less vigorously during the winter, and thereby take up less residual soil N, making it more available for loss, and at maturity, legumes have a lower C:N ratio, providing an organic residue that is N rich and decomposes rapidly; 2) in treatments with a hairy vetch cover crop, I hypothesized that as the quantity of organic N fertilizer applied increased, N loss would also increase. For the conventional management systems with wheat cover crops, I hypothesized that 3) N loss would be greater in treatments receiving fertilizer vs. those without and that the dominant gaseous N loss pathways (NH_3 volatilization, N_2O emissions) would differ between treatments with different types of fertilizer N sources due to dissimilar rates of N release.

Chapter 2 : Materials and Methods

2.1 Research Site

This experiment was established in October 2013, and was conducted at the University of Kentucky's Horticulture Research Farm in Fayette County, Kentucky in an organic field (37°58'25"N, 84°32'9 "W) and a conventional field (37°58'28"N, 84°32'10"W), located within 100 m of one another (Fig. 2.1). The organic field was certified organic according to USDA National Organic Program guidelines by the Kentucky Department of Agriculture in 2009. It was kept fallow with a weed cover and tilled once per year for the three years prior to plot establishment. The conventional field was similiarly managed, but was planted with a fall strawberry crop one year prior to plot establishment. Both field sites had been in production for the past 35 years and had not been under no-till management. The soil series at the research site is a Maury silt loam (well drained, fine, mixed, active, mesic Typic Paleudalfs).

The climate of the site is warm, moist, and temperate with a mean annual temperature of 13.1 °C and precipitation of 114.7 cm. The mean daily maximum temperature in the summer is 29.4 °C with a low of 18.1 °C, while in the winter the mean daily maximum is 6.3 °C with a low of -2.8 °C (NOAA). Precipitation is typically distributed equally throughout the year. The year of the study, October 2013 to November 2014, was cooler (mean temperature of 11.5°C) and wetter (143.7 cm precipitation) than the historic average. Specifically, temperatures were cooler than average in January through March and in July, and precipitation was notably higher in December, April, and August (Fig. 2.2).

2.2 Experiment Design

In October 2013, fifteen and nine, 25 m^2 plots (5 m by 5 m) were established in the organic and conventional fields, respectively. In the organic field, the plots were assigned to one of five treatments, each with three replicates, and in the conventional field, the plots were assigned to one of three treatments, also with three replicates (Figs. 2.3, 2.4). The treatments in both fields were arranged in a completely randomized design. Treatments were designed to measure N loss within organic and conventional conservation agriculture corn systems and incorporated cover crop species and fertilizer types commonly found within the Kentucky landscape. Both fields were spaded with an Imants Rotary Spader (Imants BV, Reusel, Netherlands) in September 2013, and winter cover crop treatments were broadcast planted the first week of October and terminated 20 May 2014. On 28 May 2014 a summer corn (*Zea mays indenata*) crop was planted by hand to simulate a no-till planter (91.44 cm between row spacing, 15.24 cm within row spacing). Each plot was 5 m wide and 5 m long, and contained five rows of corn. Fertilizer N was broadcast applied to selected treatments the same day as corn planting. The corn crop was hand harvested on 6 October 2014 (Table 2.1).

2.2.1 Organic Field Treatments

In the organic field, five treatments were designed to compare: 1) the effect of cover crop species on N loss and 2) the effect of fertilizer approach on N loss within systems using a hairy vetch cover crop. To compare the effect of cover crops, three different cover crop species were planted: hairy vetch (*Vicia villosa*) seeded at 33.6 kg ha^{-1}, winter wheat (*Triticum aestivum*) seeded at 134.5 kg ha^{-1}, and a mix of hairy vetch and winter wheat seeded at 22.4 and 67.3 kg ha^{-1}, respectively (Fig. 2.3). To capture the effect of cover crop species alone, each of the three cover crop treatments were compared under no fertilizer conditions.

To quantify the effect of fertilizer approach on N loss, only a hairy vetch cover crop treatment was examined because it is a leguminous species and is capable of N-fixation, and I was interested in comparing N loss from systems receiving the recommended application of organic fertilizer in addition to the leguminous N versus those that account for the N contribution of the cover crop residue as a N source. Two different organic N fertilizer application treatments were compared to the hairy vetch no fertilizer added treatment. One fertilizer treatment received 168 kg ha^{-1} N of pelleted Nature Safe 13-0-0 organic N (Griffin Industries LLC, Cold Spring, Ky), and a cover crop N credit approach was taken with the other fertilizer treatment. The organic N fertilizer was 40% C and 13% N (0.19% ammoniacal N, 12.04% water insoluble N, and 0.77% water soluble N) with a pH of 5.5 (Kirk Carls, Nature Safe Natural and Organic Fertilizers, personal communication, 14 March 2015).

For the cover crop N credit approach, the quantity of N contained in the cover crop is measured and the quantity of fertilizer N is reduced to account for the N contribution of the cover crop. Five days prior to cover crop termination, a cover crop biomass sample was taken to determine biomass yield per hectare and multiplied by 3.5% to estimate the quantity of N per hectare (Sarrantonio, 2012). Due to the atypically cold winter, the shoot biomass yield of the hairy vetch was low, (1741 kg ha^{-1} dry wt), and the N content averaged 61 kg ha^{-1}. To mimic a more typical year, the hairy vetch cover crop was supplemented with hairy vetch cuttings from an adjacent field to bring the N content of the cover crop up to 112 kg ha^{-1} (Smith *et al.*, 1987; Cline and Silvernail, 2002). Then, an additional 56 kg ha^{-1} of organic fertilizer was applied, so that the N added to the system was equal to 168 kg ha^{-1}, the recommended rate of N for corn (Murdock and Ritchey, 2014). However, after lab analysis of the cover crop biomass samples, it was determined that the actual N content of the cover crop was less than 3.5%. The cover crop in the treatments was 2.98% (±0.13%) N, and the added cover crop was 3.44% N. Additionally, because N-credit calculations were conducted four days prior to cover crop termination, the biomass N content of the hairy vetch had increased by the time of termination; thus, in total, the combined N content of the hairy vetch and the fertilizer was 232 kg N ha^{-1} in the N-credit treatment, 242 kg N ha^{-1} in the organic fertilizer treatment, and 78 kg N ha^{-1} in the unfertilized treatment.

The cover crops were terminated via flail mowing on 20 May 2014 (when hairy vetch was at 50% flowering) and were weed whacked prior to corn planting on 28 May 2014. The corn variety used was 71T77cnv from BlueRiver Organics (114 day, untreated conventional, non-GMO). Between row weed pressure was managed as needed with four mowing events using a BCS flail-mower (13 & 25 June, 10 July, 22 August 2014) and within-row weed pressure was managed with one hand cultivation event on 2 July 2014. No weed biomass was removed from the plots. Pest pressure was managed with three applications of Bt (Javelin®) and Spinosad (Entrust®) using a spreader sticker (Nu-film 17) (Table 2.1).

2.2.2 Conventional Field Treatments

In the conventional field, three treatments were designed to compare the effect of two different fertilizers on N loss in conventional systems seeded with a winter wheat (*Triticum aestivum*) cover crop at 134.5 kg ha^{-1}. Winter wheat was chosen for the conventional systems as it is a cereal grass and is the most commonly planted cover crop in Kentucky conventional corn systems (Lee and Knott, 2014). The N treatments were 0 N, 168 kg ha^{-1} N applied as urea 40-0-0 with a urease inhibitor (AgrotainUltra®), and 168 kg ha^{-1} N applied as the same fertilizer used in the organic treatments (Nature Safe 13-0-0, Griffin Industries LLC, Cold Spring, Ky) (Fig. 2.4). Urea applied with or without inhibitors is commonly used as a fertilizer in the central region of Kentucky (Edwin Ritchey, personal communication, 28 January 2014) and a urease inhibitor is recommended for surface application of urea after 1 May (Bitzer *et al.*, 2000). The organic fertilizer was used to represent systems that add N in a biological form, a N source that releases more slowly than inorganic fertilizer. On farms, slow release N may be in the form of manure or chicken litter, but the packaged source of organic fertilizer was used in this study in order to eliminate adding additional phosphorus and/or potassium to the treatment.

The cover crop was terminated via flail mowing followed by glyphosate application (Roundup Pro; Monsanto, St Louis, MO, U.S.A.) on 20 May 2014. Corn was planted and fertilizer applied on 28 May 2014. The corn variety used in the conventional field was REV24BHR93 from Terral Seed, Inc. (114 day, corn borer resistant, rootworm resistant, round-up ready, gluphosinate tolerant), a variety that had performed well in the University of Kentucky's 2013 variety trials (Kenimer *et al.*, 2013). Weeds were managed with one additional application of glyphosate on 10 July 2014 and no pest management was deemed necessary (Table 2.1).

2.3 Measured Parameters

2.3.1 Gaseous Emissions

The static chamber method was employed to measure gaseous emissions (Parkin and Venterea, 2010). In each of the 24 plots (8 treatments, 3 replicates), a rectangular stainless steel chamber (16.35 x 52.70 x 15.24 cm) was inserted into the soil so that the top was nearly flush with the soil surface. Chambers were inserted at random locations in each plot ten days after cover crop seed was broadcast at initiation of the experiment in the fall. Chambers were removed prior to cover crop termination in order to avoid damage from heavy machinery. The day of corn planting, the pans were re-inserted into the plots and were placed perpendicular to the corn rows so that they were between two corn plants and spanning soil surface area both within and between rows (Fig. 2.5).

To measure nitrous oxide (N_2O) and ammonia (NH_3), a 'cap' made from an identical stainless steel chamber, equipped with a vent tube and lined with Teflon© tape (Bytac©, Saint Gobain Performance Plastics), was clipped to the pan to create a sealed chamber. The chamber was connected to a photoacoustic spectroscopy gas analyzer (Innova Air Tech Instruments Model 1412, Ballerup, Denmark) via Teflon© tubing (Fig. 2.6). Measurements were taken continuously for ten minutes on the days of sampling and NH_3, N_2O, and CO_2 concentrations (ppm) were recorded simultaneously. Gaseous flux was calculated using the equations described by Iqbal *et al.* (2013). Annual fluxes were estimated by interpolating between sampling dates and calculating the area under the curve using the trapezoidal rule. During sampling periods, additional environmental parameters were also recorded, including soil moisture at 5 cm depth (DELTA-T HH2 moisture meter using a ML2x 6 cm theta probe, Delta-T Devices, Cambridge, England), soil temperature at 5 cm depth, and ambient air temperature (Taylor Digital Pocket Thermometer, Model 9878E, Taylor Precision Products, Oak Brook, IL).

Measurements commenced on 28 October 2013 and continued until 29 October 2014. Sampling intensity varied throughout the experiment, with increased intensity during periods when fluxes were expected to be high or affected by management practices. During the cover crop growing season and prior to corn planting (28 October 2013 – 13 May 2014) measurements were taken twice a month. Following corn planting and fertilization (28 May 2014), measurements were taken daily for a week and then every other day until 12 June 2014, on which date fluxes from fertilized treatments appeared similar to those from unfertilized treatments. During the rest of the corn growing season, measurements were taken every seven to ten days, returning to the twice a month sampling scheme in October. Measurements were taken on all plots between 10 am and 3 pm, and care was taken to change the starting position and the order in which the plots were sampled from one sampling date to the next.

2.3.2 Nitrogen Leachate

Ion exchange resin lysimeters were used to measure N leachate, after Susfalk and Johnson (2002). Cation and anion exchange resins (25 g) (LANXESS NM-60, Klenzoid Equipment Company, Wayne, PA) were placed between Nitex® nylon cloth and sand layers that were enclosed in polyvinyl chloride tubes 5 cm in diameter. Leachate was monitored for two measurement periods. The first round of lysimeters were deployed during cover crop growth from 10 October 2013 until 16 May 2014 and the second round occurred during the summer corn growing season from 16 May 2014 until 20 October 2014. Two lysimeters were installed at 40 cm depth in each plot under an undisturbed soil profile. The first round was placed randomly in each plot, but during the corn growing season, one lysimeter was placed within the cornrow and one was placed between rows.

When lysimeters were harvested, inorganic ammonium and nitrate was extracted by shaking the resin in 100 ml of 2.0 *N* KCL for one hour. The extract was then filtered (Slow flow, fine porosity, 12.5 cm diameter, Fisher Scientific, Pittsburgh, PA) and a 1 ml aliquot was taken for analysis. Ammonium concentration was determined using a modification of the Berthelot reaction (Chaney and Marbach, 1962) and nitrate quantified via reduction to nitrite using a copperized cadmium reduction microplate device (ParaTechs Co., Lexington, KY) as described by Crutchfield and Grove (2011). Colorimetric analysis was conducted using a microplate reader (Molecular Devices, VERSAmax, Sunnyvale, CA).

2.3.3 Soil Nutrients and Bulk Density

Prior to cover crop establishment, at the beginning of the study, soils were sampled to determine differences between and within fields. Three cores (0-15 cm) were taken per plot, bulked for a single analysis, homogenized, air-dried, and analyzed by the University of Kentucky Regulatory Services Soil Testing Laboratory in Lexington, Ky. Soil P, K, Ca, Mg, and Zn were extracted with Mehlich III and analyzed by inductively coupled plasma spectroscopy (Varian, Vista Pro CCD, Palo Alto). Total C and N were analyzed by combustion (LECO Corporation, St. Joseph), soil pH was measured with a glass electrode in 1:1 soil:water solution and calculated using an equation determined from an analysis of 240 soil samples in March of 2009 (soil-water pH = 0.91 x 1 *N* KCL soil pH + 1.34), and buffer pH was measured with a glass electrode using a Sikora buffer. Percent organic matter was calculated from percent total carbon (Nelson and Sommers, 1982) (Appendix 1).

To monitor soil N dynamics, three types of measurements were taken during the course of the year. First, cation and anion exchange resin bags (LANXESS NM-60, Klenzoid Equipment Company, Wayne, PA) were made with 10 g of resin tied in a porous material (Gibson *et al.*, 1985). Each month from November 2013 to November 2014, three resin bags per plot were inserted at 15 cm depth, at the same time those from the prior month were removed for analysis of inorganic ammonium and nitrate. There were two instances when the resin bags were deployed for six weeks as opposed to one month, as the ground was frozen and it was not possible to remove the resin bags. These instances occurred January to mid-February and from mid-February to the first of April. After removal from the field, resin bags were rinsed with deionized water until free of soil and other debris and extracted with 40 mL 2.0 *N* KCL. They were shaken in KCL for one hour and then filtered (Slow flow, fine porosity, 12.5 cm diameter, Fisher Scientific, Pittsburgh, PA). Extract was stored overnight at 4 °C and analyzed colorimetrically as described above.

Soil samples were also taken five times during the course of the year and extracted for inorganic and potentially mineralizable N content. Three 0-15 cm soil cores per plot were taken prior to cover crop termination (13 May 2014), at corn V6 growth stage (1 July 2014), at corn R1 growth stage (8 August 2014), at corn harvest (6 October 2014), and post-harvest (3 November 2014). For inorganic N extraction, soils were passed through a 2 mm sieve and extracted at field moist conditions within 24 hours of collection. For the extraction, 5 g of soil was extracted with 20 mL of 1.0 *N* KCL, shaken for 1 hour, filtered, stored overnight and colorimetrically analyzed (as previously described). Gravimetric moisture content was determined by drying a subsample of the soil at 55 °C until dry.

A chemical method for assessing potentially mineralizable nitrogen (PMN), as described by Gianello and Bremner (1986), was used to determine PMN on the five soil samples in addition to the pre-planting soil sample collected on 1 November 2013. This procedure is insensitive to air-drying (Gianello and Bremner, 1986) and, thus, was performed on samples after air-drying and storage. 20 mL of 2.0 *N* KCL was added to 3 g of soil in glass centrifuge tubes and placed in a block digester (Benchmark, Digital dry Bath II, BSH1004) to incubate for 4 hours at 100 °C. Samples were then filtered and analyzed colorimetrically as described above. Gravimetric moisture content was determined by drying a subsample of the soil at 55 °C until dry.

To calculate soil N content on a per hectare basis, a soil bulk density sample was taken from each plot in October 2014 using a slide hammer. A non-compacted, 4.8 cm in diameter, soil core was taken from 0-15 cm and dried at 110 °C for 48 hours. Bulk density was calculated as the weight of the dry soil divided by the volume.

2.4 Plant Biomass Sampling

2.4.1 Cover Crop Biomass

Beginning in May 2014, cover crop biomass was sampled monthly through September. The first sampling was conducted the day of cover crop termination after flail mowing. During the months of June through September, the decomposing cover crop residue on the soil surface was collected. In the organic plots, weed residue from mowing was also collected. Two samples were collected randomly from each plot using a 25x25 cm quadrat, were dried at 55 °C for 48 hours, and then weighed. The same location was never sampled twice. Samples were processed on a grinding mill to pass through a 1 mm sieve (Cyclotec 1093, FOSS™ , Eden Prairie, MN), and sub-samples were then ground on a ball grinder (Cianflone Scientific Instrument Corporation, Pittsburgh, PA) or a jar-mill (U.S.Stoneware, East Palestine, OH). Cover crop biomass was analyzed for C and N content via flame combustion (Flash EA 1112 elemental analyzer, CE Elantech Inc., Lakewood, CA).

Additionally, cation and anion exchange resin bags were placed beneath the cover crop residue, but above the soil surface, to qualitatively capture the ammonium and nitrate coming from the decomposing residue to the soil surface. Resin bags contained 10 g of resin beads (LANXESS NM-60, Klenzoid Equipment Company, Wayne, PA), and plastic mesh was used to slightly elevate resin bags off the soil surface. These resin bags were collected monthly from late May through September and extracted and analyzed colorimetrically as previously described.

2.4.2 Corn Biomass

Corn biomass samples were collected three times over the course of the experiment. Corn leaves were collected at corn R1 growth stage, entire corn plant samples and weed biomass were collected at corn maturity, and grain samples were collected for yield analysis at harvest. At R1 growth stage, three corn plants from each plot were randomly selected, and from these plants, a mature, healthy leaf was collected. Samples were dried at 55 °C for 48 hours (Model SA-350, The Grieve Corporation, Round, Lake Illinois), weighed, ground, and analyzed via flame combustion for carbon and N content. At corn maturity, a 0.25 m^2 quadrat was randomly placed within one of the inner three cornrows in each plot and entire corn plants were cut at the base, flush with the soil surface. Roots were not collected. Additionally, any weed biomass growing within the quadrat was also collected. Samples were dried at 55 °C for one week, and the corn plants were sub-divided into stalks, leaves, husks, tassels, cob and shank, and grain and weighed. Stalks and cobs were ground with a Thomas Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ) until they could pass through a 2 mm sieve. Leaves, husks, tassels, and weeds were ground through a 1 mm sieve on a grinding mill (Cyclotec 1093, FOSS™, Eden Prairie, MN), and grain was ground using an electric coffee grinder. Sub-samples of corn and weed biomass were analyzed for C and N content via flame combustion (Flash EA 1112 elemental analyzer, CE Elantech Inc., Lakewood, CA).

Corn was harvested by hand on 6 October 2014. Yield was calculated from the grain produced by the inner three rows of each plot. Additionally, to minimize edge effects, the two outermost corn plants of each of the three rows were not included in yield calculations. Corn was dried at 55 °C for 48 hours and then allowed to air dry for five weeks. Corn was shelled and weighed and a sub-sample was collected and dried at 55 °C for 24 hours to obtain moisture content so that yield data could be corrected for bushel weight at 15% moisture content.

2.5 Nitrogen Balance Calculation

Using some of the measured parameters, a N balance calculation was computed for the post-fertilization time period (28 May 2014 – 1 November 2014) in each of the treatments to quantify whether the system experienced a net positive N gain or a net negative N loss. The post-fertilization time period was analyzed rather than an annual budget because the N loss that occurred during the cover crop growing season (October 2013 – 28 May 2014) was not representative of the fertilizer treatments, as fertilizer was not applied until the following May. A N mass balance approach was used subtracting average N export from average N input:

N Balance = (cover crop N + fertilizer N) – (gaseous N loss + corn grain N export)

Leaching data was not included in this equation as it was measured in ppm and was not scaled up to kg N ha^{-1} as that would require many assumptions about the drainage patterns of the field site.

2.6 Statistical Analyses

This experiment was designed to investigate the effects of cover crop species and fertilizer on N loss in organically and conventionally managed corn systems. From eight total treatments, three separate contrasts were developed that addressed the three specific objectives of the study. Within the organic field, unfertilized plots differing in cover crop type were compared (hairy vetch vs. wheat vs. bi-culture), and treatments planted with hairy vetch, but receiving different fertilizer approaches (0 N vs. N-credit vs 168 kg ha^{-1} organic N), were compared. In the conventional field, the effect of fertilizer type was compared across treatments (0 N vs. 168 kg ha^{-1} organic N vs. 168 kg ha^{-1} urea).

General and mixed linear models (proc GLM, proc mixed) (9.3 SAS Institute Inc., Cary, NC) were utilized for the analyses. Fixed effects were either cover crop species or fertilizer approach/type. Replicate was also a fixed effect in the general models and a random effect in the mixed models. For parameters that were measured at multiple times over the study year, time was included as a repeated effect (in GLM) or as a fixed effect (in mixed), with the repeated effect of the treatments over time specified using a subject option.

For parameters that were only measured once during the study, a general linear model (proc GLM) (9.3 SAS Institute Inc., Cary, NC), employing type 1 sums of squares and a least squares means statement to produce pairwise comparisons, was used to test for differences across treatments. These parameters included: soil bulk density, corn yield, corn grain N export, N balance, leaching data for each of three different time periods (pre-fertilization, post-fertilization, and the summed annual total), and N_2O-N and NH_3-N flux estimates that were calculated over three distinct time periods (pre-fertilization, post-fertilization, and annually). All response variables and residuals were assessed for normality and transformed when necessary.

Parameters measured more than once during the study varied in their sampling frequency. For soil resin NO_3-N and NH_4-N concentrations, monthly data were analyzed for two time periods (pre-termination/fertilization; October – May and post-termination/fertilization; May – November) using a repeated statement in GLM, as we anticipated treatment associated differences in this parameter would be strongly influenced by the fertilization event. For parameters that included missing values or were better modeled using a more general co-variance structure than GLM provides, a mixed linear model (proc mixed) (9.3 SAS Institute Inc., Cary, NC) was used. These parameters included: cover crop N content, %N, and C:N ratio analyzed monthly across five months (May-September); biomass resin N concentrations analyzed monthly across four months (June-September); soil inorganic N across four collection periods (corn V6 growth stage, corn R1 growth stage, corn harvest, and one month post-harvest), hot KCL extracted N across five collection periods (November 2013, corn V6 growth stage, corn R1 growth stage, corn harvest, and one month post-harvest); and N_2O-N emissions, NH_3-N emissions, soil moisture, and soil temperature measured at various frequencies over the course of the study (n=36 dates in total). Similar to the soil resin N data, repeated measures tests for N_2O-N emissions, NH_3-N emissions, soil moisture, and soil temperature were performed separately for three distinct time periods that corresponded with major management activities: the pre-fertilization/termination period (28 Oct 2013 –

28 May 2014), the post-fertilization intensive measurement period (29 May – 12 June 2014), and the post-fertilization period (19 June – 1 November 2014).

For each analyzed parameter, the covariance-structure was modeled using either a first order auto-regressive (AR(1)) structure or a heterogeneous auto-regressive structure (ARH(1)), depending upon which best fit the error variability expressed in the dataset. All response variables and residuals were assessed for normality and, where possible, transformed to achieve normality. Though normality of the data and/or the residuals is preferred for statistical accuracy, transformation to achieve normality for N_2O-N and NH_3-N datasets was not possible for all three of the analyzed contrasts. However, assuming correct linearity of the model and independent and homoscedastic errors (achieved by using an appropriate covariance structure – ARH(1)), normality may be bypassed, as the central limit theorem applies due to a large sample size $n > 30$, and the smallest sample size we used was $n=30$ during the post-fertilization intensive measurement period. Thus, the model estimates for each effect follow a normal distribution and the accuracy of the statistic is not compromised (Norman, 2010). A least squares means statement was used to produce pairwise comparisons across treatments on each measurement date for all parameters when significant main effects were identified.

Table 2.1: Timeline of field management and sampling events.

2013

Month	Tasks
September	Constructed stainless steel pan bases for trace gas measurements Spaded field site and laid measurements for plot installation
October 8-9	Collected soil samples, 0-15 cm Broadcast planted cover crops Installed lysimeters Began taking bi-monthly trace gas measurements and installed first round of monthly resin bags at 15 cm depth (resin bags continued monthly for entirety of project and trace gas measurements continued bi-monthly until corn planting and fertilization)

2014

May 15	Collected hairy vetch biomass samples for N-credit calculations from organic field
16	Removed resin lysimeters and installed second round
19	Cut and applied additional hairy vetch from adjacent field to N-credit treatments
20	Terminated cover crops in organic field via flail mowing and in the conventional field via flail mowing and glyphosate application Collected cover crop biomass samples and install biomass resin bags (continue monthly through September) Collected soil samples (0-15 cm) for inorganic N and Hot KCL extraction
28	Weed-whacked organic field No-till hand planted corn and broadcast applied fertilizer in organic and conventional fields
29-31	Daily Trace Gas Measurements
June 1-4, 6, 9, 12	Trace gas measurements
19	Began weekly trace gas measurements until September when they were reduced to bi-monthly
13	Mowed organic field
25	Mowed organic field
July 1	Collected soil samples (0-15 cm) at corn V6 growth stage
2	Within row hand weeding of organic field
10	Mowed organic field and applied glyphosate to conventional field
August 8	Collected corn leaf samples and soil samples at corn R1 growth stage
22	Mowed organic field and applied Bt & spinosad
25	Applied Bt & spinosad to organic field
29	Applied Bt & spinosad to organic field
September 3	Collected soil bulk density samples
29	Collected final cover crop biomass samples and biomass resin bags
October 6	Hand harvested corn biomass and grain in organic and conventional fields, collected soil samples (0-15 cm)
20	Removed resin lysimeters
26	Mowed corn stover in organic and conventional fields
29	Final trace gas measurement
November 3	Final soil samples collected and trace gas rings removed

Figure 2.1: Site map of organic and conventional fields at the University of Kentucky's Horticulture Research Farm located in Fayette County, Kentucky.

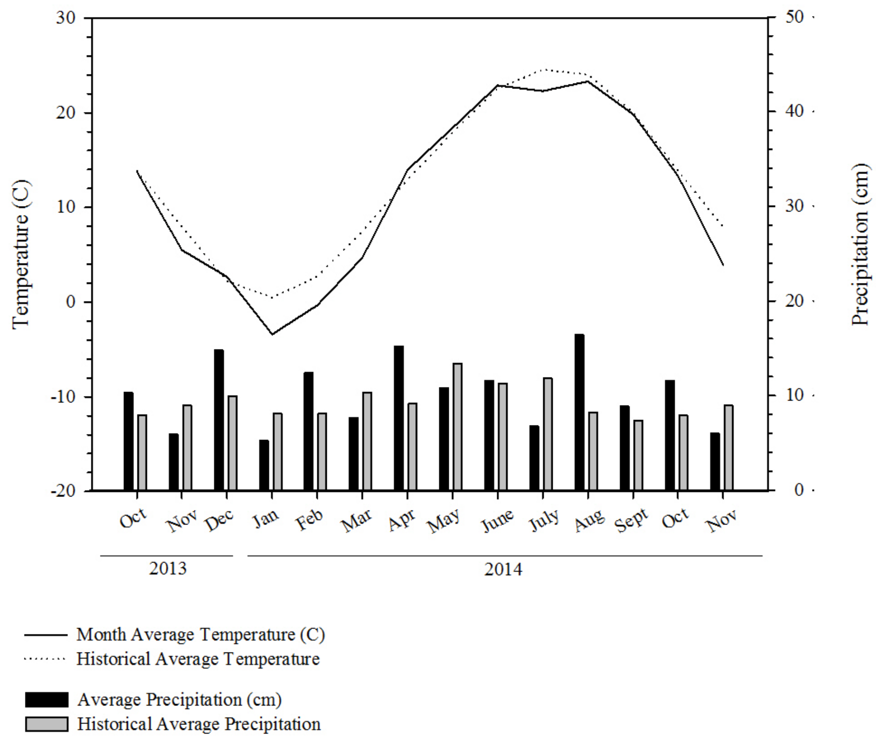


Figure 2.2: Temperature and precipitation the year of study at the experimental site at the University of Kentucky's Horticulture Research Farm in Fayette County, Kentucky and historic temperature and precipitation data as recorded at the Lexington Bluegrass Airport.

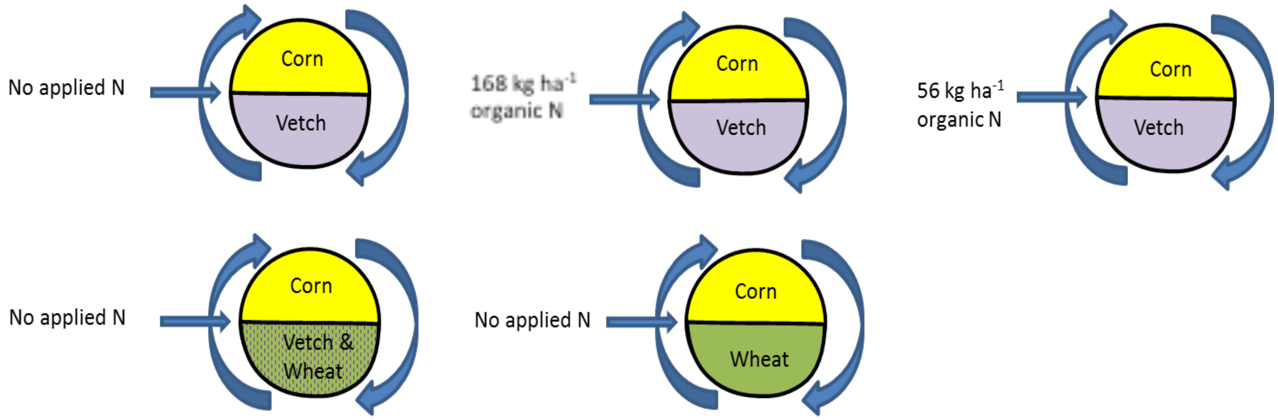


Figure 2.3: Schematic of organic treatments. A cover crop (vetch, wheat, or a mixture of the two) was planted in October 2013 and was terminated 20 May 2014. A corn crop was planted with or without fertilizer on 28 May 2014 and harvested 6 October 2014. The quantities of applied fertilizer were added in addition to the pre-existing N content of the cover crop.

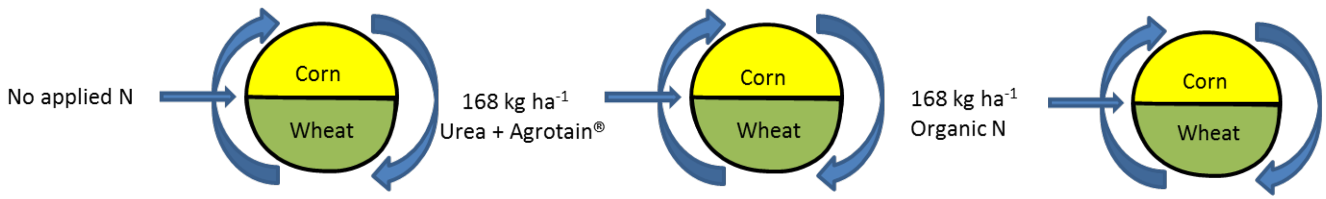


Figure 2.4: Schematic of conventional treatments. A cover crop (wheat) was planted in October 2013 and was terminated 20 May 2014. A corn crop was planted with or without fertilizer on 28 May 2014 and harvested 6 October 2014.

Figure 2.5: Trace gas pan placed between corn plants and spanning soil surface area both within and between rows.

Figure 2.6: Field measurements of gaseous emissions taken using the static chamber method and a photoacoustic spectroscopy gas analyzer.

Chapter 3 : Results

3.1 Organic Field: Cover Crop Comparisons, 0 N Treatments

3.1.1 Cover Crop Growing Season

Biomass of the cover crops at termination was significantly greater in the vetch-wheat bi-culture and wheat than the hairy vetch alone ($p<0.0096$, $p<0.0118$, respectively) with dry weights of 2791 (hairy vetch), 3590 (wheat), and 4968 kg ha^{-1} (bi-culture). N content of biomass was 100 kg N ha^{-1} in the vetch-wheat bi-culture, 78 kg N ha^{-1} in hairy vetch alone, and 27 kg N ha^{-1} in the wheat (Fig 3.1A, Table 3.1). During cover crop growth (Oct.-May), soil moisture varied from a low of 13% on 28 October 2013 to a high of 35% on 18 February 2014, but was comparable across treatments, differing slightly (<6%) at only three time points (Fig. 3.2A, Table 3.2). Similarly, overall soil temperature did not differ between treatments, though there were slight differences (<5^{o}C) at four time points, primarily in March and April (Fig. 3.3A, Table 3.3).

Soil resin NO_3-N concentrations were highest in the vetch treatments prior to cover crop termination in Jan./Feb. (Fig. 3.4A and Table 3.4). Early in the growing season (e.g. Dec.), soil resin NO_3-N concentration in the bi-culture treatment was more similar to vetch than wheat, but the bi-culture became more similar to wheat as the growing season progressed (e.g., Feb/Mar; Fig. 3.4A and Table 3.4). N loss measured as NO_3-N leachate differed between cover crop species, with hairy vetch > vetch-wheat > wheat (Table 3.5). Though N_2O-N fluxes were slightly higher in the wheat treatment on 3 March and 17 & 29 April 2014, linear contrasts indicated there were no significant differences in either N_2O-N or NH_3-N loss across treatments when analyzed throughout the entire cover crop growing season (Table 3.5). NH_3 and N_2O-N fluxes were quite low at this time of year (Figs. 3.5A, 3.6A, Tables 3.7, 3.8).

3.1.2 Corn Growing Season

Post-termination of cover crops (May-November), soil temperature did not differ between treatments, but there were significant differences in soil moisture. Soil moisture in the vetch-wheat and wheat alone treatments was greater than that in the vetch treatment, particularly in June (Fig. 3.2B, C, Table 3.2), though this general trend held throughout the post-fertilization period. Nitrogen dynamics in the decomposing cover crop residue also differed across treatments. After cover crop termination, the differences in C:N ratio of the cover crops (wheat > vetch-wheat > hairy vetch alone) largely reflected differences in %N as that with the highest %N had the lowest C:N (Fig. 3.1B, C, Table 3.1). Overall, N content and %N of residue in hairy vetch and vetch-wheat declined during decomposition, whereas the N content of the wheat treatment stayed fairly constant at a low level, and %N of the residue slightly increased (Figs. 3.1B, C), indicating N mineralization was occurring in the hairy vetch and vetch-wheat and immobilization in the wheat. These differences in cover crop residue N release were also apparent in the NO_3-N and NH_4-N concentrations extracted from the resins placed under the biomass. NO_3-N release was greatest from hairy vetch alone (hairy vetch > vetch-wheat > wheat) and NH_4-N release from hairy vetch and vetch-wheat was greater than from the wheat alone (Fig. 3.7A, B, Table 3.1).

Soil N dynamics also differed between treatments post-termination and reflected the differences in N released by the decomposing cover crop residue (hairy vetch, vetch-wheat > wheat) (Table 3.4). In August, soil resin bags indicated that there was a significantly higher concentration of NO_3-N in the hairy vetch and vetch-wheat soils compared to wheat alone (Fig. 3.4A). Though not statistically significant, inorganic soil NO_3-N extractions tended to be higher in the hairy vetch and vetch-wheat treatments at corn growth stage V6 (1 July), and at R1 growth stage (8 August), hot KCL extracted NO_3-N concentrations were higher in the vetch and vetch-wheat treatments (Fig. 3.8A,B, Table 3.9).

Post-termination of cover crops, NO_3-N leachate values were greater in the hairy vetch and vetch-wheat treatments than in wheat alone (Table 3.5, Fig. 3.9D). When added to pre-fertilization leachate values, annual NO_3-N leachate was two times greater in hairy vetch than vetch-wheat, and four times greater than that measured in wheat (Table 3.6). During the post-fertilization intensive measurement period, repeated measures tests found that N_2O-N emissions were greater in the hairy vetch and vetch-wheat treatments than the wheat alone, but there were no detectable treatment differences after 12 June 2014 (Fig. 3.5B, C, Table 3.7). However, when summed across the entire post-fertilization period (May 29 – November 1) and across the entire year, total N_2O-N emissions did not differ between treatments (Table 3.5, 3.6). Similarly, no significant differences between treatments were observed for NH_3-N emissions (Figs. 3.6B, C, Table 3.5, 3.6, & 3.8).

3.1.3 N-balance and Yield

Corn yield was significantly greater in hairy vetch ($p<0.0173$) and vetch-wheat ($p<0.0067$) treatments than in wheat alone (Fig. 3.10A). A N balance calculation found that all 0 N cover crop treatments had a positive N balance, with that of the vetch-wheat treatment significantly greater than either the hairy vetch or wheat alone treatments (Fig. 3.9A). A lower net N balance in the hairy vetch treatment compared to the vetch-wheat treatment may be attributed to: 1) greater corn grain N export in hairy vetch (vetch > vetch-wheat > wheat; Fig. 3.9A); 2) greater N leachate loss (NO_3-N and NH_4-N combined) as patterns were similar to those observed in corn grain N (Fig. 3.9D); and 3) as previously mentioned, N_2O-N emissions were greater in the vetch treatment during the period immediately following cover crop termination (Fig. 3.5B). A lower net N balance in the wheat treatment compared to the bi-culture is likely due to an initially low N input, as wheat had a much lower cover crop N content than either of the other N-fixing treatments (Fig. 3.1A, B). The N-balance calculation suggests that by the end of the corn-growing season, more of the cover crop N remained in the vetch-wheat system. This N may be tied up in the remaining cover crop residue, as the %N content of the vetch-wheat residue slightly increased during August and September (Fig. 3.1B).

3.2 Organic Field: Fertilizer Comparisons, Hairy Vetch Cover Crop

3.2.1 Cover Crop Growing Season

In May, at termination, biomass of the hairy vetch cover crop was significantly greater in the N-credit treatment than the unfertilized or organic fertilizer treatments ($p=0.0441$, $p=0.0119$, respectively) with 2791 (0 N, unfertilized treatment), 2306 (168 kg N ha^{-1} organic fertilizer treatment), and 4745 kg ha^{-1} (N-credit treatment after additional vetch was added). Similarly, N content of the hairy vetch biomass in the N-credit treatment was greater than the other treatments with 176 kg N ha^{-1}, 78 kg N ha^{-1} in the unfertilized, and 74 kg N ha^{-1} in the organic fertilizer treatment (Fig. 3.1D, Table 3.1). During cover crop growth (Oct-May), soil moisture and temperature were similar across all treatments (Figs. 3.2D & 3.3D, Tables 3.2 & 3.3), however, temperatures fell lower than the historic average (Fig. 2.2) and hairy vetch biomass production was lower than expected across all treatments. Consequently, to better represent the hairy vetch biomass that may accumulate in an average year, extra hairy vetch biomass from an adjacent field was cut and added to the N-credit treatment. This explains the significantly greater cover crop biomass and biomass N content in the N-credit treatment immediately following termination (Fig. 3.1D).

As all treatments were planted with hairy vetch and fertilizers had not been applied yet, measured soil resin N concentrations (NO_3-N and NH_4-N), N leachate (NO_3-N and NH_4-N), and gaseous N (N_2O-N and NH_3-N) loss parameters did not differ during the cover crop growing season (Figs. 3.4C, D, 3.5D, & 3.6D, Tables 3.4, 3.5, 3.7, & 3.8). Soil resin NO_3-N tended to decrease during the latter half of the cover crop growing season (Feb/Mar-May), but soil resin NO_3-N and NH_4-N concentrations were low during this time of the year (Figs. 3.4C, D). Though there were slight differences in NH_3-N emissions on three dates, treatments were not significantly different across the entire measurement period (Fig. 3.6D, Tables 3.5 & 3.8) as gaseous N losses were low at this time.

3.2.2 Corn Growing Season

Post-termination of cover crops and post-fertilization of the N-credit and fertilized hairy vetch treatments (May-November), there were no soil temperature differences between treatments, but there were significant differences in soil moisture (Figs. 3.2E, F, & 3.3E, F, Tables 3.2 & 3.3). During the post-fertilization intensive measurement period, soil moisture was consistently greater (by 2-6%) in the N-credit treatment than in either the unfertilized or fertilized treatments ($p<0.0075$ and $p<0.0092$, respectively), though means comparisons tests failed to identify specific days where this effect was significant within the time period (Fig. 3.2E, Table 3.2). Additionally, soil moisture differed for several measurement dates during the longer-term post-fertilization measurement period (Fig. 3.2F, Table 3.2).

Nitrogen dynamics in the decomposing cover crop residue were similar across treatments. Although initially N content was greatest in the N-credit treatment, reflecting that additional vetch material had been added to those plots, by June, there was no difference between treatments (Fig. 3.1D, Table 3.1). N content and %N of residue declined in all treatments during decomposition, indicating N mineralization was occurring (Figs. 3.1D, E, Table 3.1). However, the %N of the biomass residue in the organic N fertilized treatment tended to decline less rapidly than the other treatments. Though %N was greatest in the N-credit treatment in May, by June the N-credit and organic fertilizer treatments had equivalent %N, and in July and August %N was greatest in the organic treatment (Fig. 3.1E). Biomass resin NO_3-N concentrations found no significant differences between treatments for any month; however, NH_4-N concentrations reflected trends in biomass %N, with greater N captured in the organic N fertilized treatment (fertilized > N-credit > unfertilized), particularly during the months of July and August when biomass %N was also significantly greater than the other treatments (Fig. 3.7D, Table 3.1).

Post-fertilization, soil N dynamics also differed between treatments. Overall, soil resin NO_3-N and NH_4-N tended to be greatest in the organic N fertilized treatment, followed by the N-credit treatment, and lowest in the unfertilized treatment (Fig. 3.4C, D, Table 3.4). In June, soil resin NH_4-N concentrations were more than 2x greater in the organic fertilized treatment vs. unfertilized (Fig. 3.4D, Table 3.4), but in July, soil resin NO_3-N and NH_4-N concentrations were very low in all treatments, likely due to rapid corn growth, N demand, and N uptake. In August, both soil resin NO_3-N and NH_4-N concentrations were significantly greater in the organic fertilized treatments than the unfertilized (168 organic, N-credit > unfertilized for NO_3-N; 168 organic >N-credit, unfertilized for NH_4-N) (Figs. 3.4C, D, Table 3.4). Across all sampling dates, soil inorganic NO_3-N extractions were greater in the organic fertilized treatment and the N-credit treatment than the unfertilized ($p<0.0223$ and $p<0.0438$, respectively), with the greatest differences occurring at stage R1 and harvest (Fig. 3.8C, Table 3.9).

Despite observed treatment differences in soil resin and soil inorganic extracted N post-fertilization, there were no differences in NO_3-N or NH_4-N leachate values between treatments (Table 3.5, Fig. 3.9E). However, there were differences in gaseous N emissions. Repeated measures ANOVA found that across the post-fertilization intensive measurement period (May 29 – June 12), N_2O-N emissions were greater in the N-credit treatment than the unfertilized treatment ($p<0.0069$) (Fig. 3.5E, Table 3.7). Although means comparisons failed to identify specific dates where this significant difference occurred, N-credit N_2O-N emissions were higher than the other treatments within the first week of fertilizer application, with fluxes from both fertilizer treatments becoming more similar towards the end of the intensive measurement period (Fig. 3.5E). During the remainder of the corn-growing season (June 19 – November 1), there were significantly greater emissions from the organic fertilized treatment compared to the N-credit or the unfertilized treatment ($p<0.0028$ and $p<0.0046$, respectively) (Fig. 3.5F, Tables 3.5 & 3.7). However, when annual N_2O-N emissions were calculated, only marginally significant treatment differences were observed ($p=0.0558$), with organic fertilized having higher fluxes than unfertilized (Table 3.6).

Treatment effects on gaseous NH_3-N emissions were dissimilar to those observed for N_2O-N. During the post-fertilization intensive period (May 29-June 12), repeated measures ANOVA found that the organic fertilized treatment had significantly greater emissions than the N-credit and unfertilized treatments, with the greatest difference occurring on 6 June 2014, but no differences occurred during the remainder of the post-fertilization period (June 19 – November 1) (Fig. 3.6E, F, Table 3.8). In contrast, gaseous NH_3-N emissions calculated across time for the entire post-fertilization period (May 29-November 1) differed between all treatments (organic N > N-credit > unfertilized); however, these post-fertilization differences were not strong enough to significantly influence annual calculations, where no difference between treatments was identified (Table 3.6).

3.2.3 N-balance and Yield

Corn yield and corn grain N export were significantly greater in the organic fertilized ($p<0.0042$) and N-credit treatments ($p<0.0019$) than in the unfertilized treatment (Fig. 3.9B & 3.10B). All treatments had a positive N balance for the corn growing season (Fig. 3.9B), and, though no statistically significant differences in N balance were detected, the N-credit treatment had the largest positive net N gain (on average, 50-91 kg N ha^{-1} more than the other treatments), suggesting that a larger proportion of applied N remained in this system, possibly in either plant biomass (corn plant or weeds) or soil organic matter, as cover crop N content was equivalent across treatments by the end of the corn growing season (Fig. 3.1D). Lower N retention in the organic fertilized treatment may be attributed to greater N_2O-N and NH_3-N emissions following fertilization (Table 3.5) and highest grain N loss, rather than enhanced losses via N leachate (Fig. 3.9E).

3.3 Conventional Field: Fertilizer Comparisons, Winter Wheat Cover Crop

3.3.1 Cover Crop Growing Season

At termination, wheat cover crop biomass was 6572 (unfertilized treatment), 6070 (organic N treatment), and 6590 kg ha^{-1} (urea N treatment). There were no significant differences across treatments in biomass or N content as all were planted with the same cover crop species (Fig. 3.1G, Table 3.1). During cover crop growth (Oct-May), there were no differences in soil temperature, but, on average, soil moisture was 2% greater in the urea N vs. the organic N treatment ($p<0.0080$) (Figs. 3.2G & 3.3G, Tables 3.2 & 3.3), though a means comparisons test failed to identify specific dates where the treatment effect was significant.

Soil NO_3-N and NH_4-N concentrations were similar across treatments during this time period, perhaps reflecting that all treatments were planted with a winter wheat cover crop and fertilization had not yet occurred (Figs. 3.4E, F, Table 3.4). However, during Feb/Mar, soil NH_4-N was slightly lower in the organic N fertilizer treatment (urea N, unfertilized > organic N) (Fig. 3.4F, Table 3.4). There was no difference in N loss via leachate or N_2O-N emissions, but differences in gaseous NH_3-N loss did occur (Table 3.5). NH_3-N emissions tended to be slightly elevated in the urea N and unfertilized treatments, but were only significant on a few dates during the measurement period (Figs. 3.6G, Table 3.8), and may reflect slightly higher soil NH_4-N in these treatments at those times (Fig. 3.4F, Table 3.4). However, when pre-fertilization total NH_3-N emissions were calculated, all three treatments differed from each other (urea N > unfertilized > organic N) (Table 3.5).

3.3.2 Corn Growing Season

Post-termination of the winter wheat cover crop and post-fertilization of the organic N and urea N treatments (May-November), soil temperature and soil moisture differed across treatments (Figs. 3.2H, I, & 3.3H, I, Tables 3.2 & 3.3). During the post-fertilization intensive measurement period (May 29 - June 12), average soil temperature was slightly higher (<1^{o}C) in the organic N treatment than the urea treatment ($p<0.0057$), but there were no treatment differences in soil moisture at that time (Figs. 3.2H & 3.3H, Tables 3.2 & 3.3). During the longer-term post-fertilization measurement period (June 19-November 1), average soil moisture was greater in the unfertilized treatment than in either the organic N or urea N treatments ($p<0.0008$ and $p<0.0023$, respectively), and soil temperature was similar.

Nitrogen dynamics in the decomposing cover crop residue did not significantly differ between treatments. N content of the residue fluctuated over the course of the season, likely due to microbial mediated movement of N in and out of the residue, and the %N of the residue increased in all treatments, indicating N immobilization was occurring (Figs. 3.1G, H, Table 3.1). Though not significantly different, the temporal trends in organic fertilizer residue %N and N content differed somewhat from those observed in the other treatments: the %N and the N content increased in June in the organic fertilizer treatment whereas the other treatments experienced a decrease (Figs. 3.1G, H).

In support of differential patterns of residue decomposition and N release, biomass resin extractions differed between treatments. Overall, significantly more NH_4-N was released from the wheat biomass in the organic N treatment than the unfertilized treatment (Fig. 3.7F). Though there was no overall difference in NO_3-N and NH_4-N extracted from the biomass resins in the urea N and organic N treatments, the timing of greatest seasonal concentrations were dissimilar (Figs. 3.7E, F, Table 3.1). In June, greater concentrations of NO_3-N and NH_4-N were extracted from the resins in the urea N treatment, but in July and August a greater concentration of NH_4-N was extracted from the resins in the organic N treatment (Figs. 3.7E, F).

Soil N dynamics also differed between treatments post-fertilization. In June, soil resin NO_3-N concentrations were significantly different between all three treatments (urea N > organic N > unfertilized), and in July both fertilized treatments had greater NO_3-N concentrations than the unfertilized treatment (Fig. 3.4E, Table 3.4). On average, NH_4-N tended to be greater in both fertilized treatments than in the unfertilized treatment, but means comparisons failed to identify specific months during which soil NH_4-N differed, finding only that urea N was marginally greater than the unfertilized treatment for the month of June ($p=0.0552$). Soil inorganic and hot KCL NO_3-N extractions showed that across time soil NO_3-N was highest in the urea N treatment (Fig. 3.8E, F, Table 3.9), though the effect was not significant on all dates.

Post-fertilization, there were no differences between treatments in NH_4-N leachate, but there were marginally significant differences in NO_3-N leachate ($p=0.0539$), with the urea N treatment having more than 5x the concentrations measured in either the organic N or unfertilized treatments (Fig. 3.9F, Table 3.5). There were also differences in gaseous N loss. A repeated measures ANOVA found that across time during the post-fertilization intensive measurement period (May 29-June 12), N_2O-N emissions were greater in both the fertilized treatments vs. the unfertilized (Fig. 3.5H, Table 3.7). On 6 June 2014, N_2O-N emissions were more than 8x greater in the urea N treatment than the unfertilized treatment, and on 12 June 2014 they were more than 9x greater in the organic N treatment than the unfertilized treatment. On several dates during the remainder of the corn growing season (June 19 – November 1), N_2O-N emissions from the organic N treatment remained significantly higher than the other treatments (Fig. 3.5I, Table 3.7). However, these date-specific trends were not strong enough to significantly influence the entire post-fertilization N_2O-N flux estimates (May 29-November 1), where no differences in N_2O-N emissions between treatments were found (Table 3.5).

Similarly, a repeated measures ANOVA found that during the post-fertilization intensive measurement period, NH_3-N emissions from both fertilized treatments were greater than those from the unfertilized treatment (Fig. 3.6H, Table 3.8). Temporally similar to N_2O-N, the urea N treatment had greater emissions during the first part of the measurement period while the organic N treatment had greater emissions during the latter half of the measurement period. Despite substantial fertilizer effects early on, treatment differences did not persist across the longer-term post-fertilization period (Fig. 3.6I, Table 3.8). When summed across the entire post-fertilization period (May 29-November 1), NH_3-N emissions were significantly greater only in the organic N treatment compared to the unfertilized treatment (Table 3.5). In contrast, when an annual NH_3-N flux was calculated, only the urea N treatment had significantly greater NH_3-N emissions than the unfertilized treatment, which was likely due to higher levels of NH_3-N emissions from the urea N plots prior to fertilization (Table 3.6).

3.3.3 N-balance and Yield

Corn yield was significantly greater in the urea N treatment than the unfertilized treatment ($p<0.0085$), with the organic N treatment falling in between (Fig. 3.10C). Though treatment differences were not significant, the organic N fertilizer treatment had the greatest positive N balance followed by the urea N treatment, while the unfertilized treatment actually had a negative N balance indicating net N export (Fig. 3.9C). Though total N input was the same in both fertilizer treatments, pathways of N removal/loss differed. The urea N treatments lost more N via NO_3-N leachate (marginally significant, p=0.0539) and corn grain N export (p=0.0413), while the organic N treatment loss more N via N_2O-N and NH_3-N emissions (Fig. 3.9C, F, Table 3.5).

Table 3.1: Repeated measures ANOVA results for cover crop biomass residue N content (kg N ha^{-1}), % N, and C:N ratio during the months of May-September 2014 and for cover crop biomass resin NO_3-N and NH_4-N concentrations during the months of June-September 2014. Bolding indicates significance.

Effect		**Biomass N Content**		**Biomass N (%)**		**C:N Ratio**			**NO_3-N Resin Concentration**		**NH_4-N Resin Concentration**	
	Biomass $DF_{n,d}$	F	P	F	P	F	P	Resin $DF_{n,d}$	F	P	F	P
Organic Unfertilized Treatments												
Cover Crop	2,4	34.06	**0.0031**	303.46	**<0.0001**	357.71	**<0.0001**	2,4	33.29	**0.0032**	57.46	**0.0011**
Month	4,24	27.77	**<0.0001**	3.65	**0.0186**	6.37	**0.0012**	3,17	19.65	**<0.0001**	311.41	**<0.0001**
Month*Cover Crop	8,24	1.97	0.0949	4.26	**0.0016**	3.52	**0.0079**	6,17	9.15	**0.0001**	10.83	**<0.0001**
Organic Vetch Treatments												
Fertilizer	2,4	9.18	**0.0320**	8.29	**0.0378**	4.76	0.0874	2,4	0.77	0.5230	18.76	**0.0093**
Month	4,24	24.57	**<0.0001**	30.13	**<0.0001**	7.76	**0.0004**	3,17	14.96	**<0.0001**	190.48	**<0.0001**
Month*Fertilizer	8,24	2.47	**0.0412**	2.56	**0.0355**	1.67	0.1567	6,17	1.65	0.1897	7.11	**0.0005**
Conventional Treatments												
Fertilizer	2,4	3.47	0.1137	2.00	0.2503	1.00	0.4437	2,4	0.58	0.6022	8.70	**0.0349**
Month	4,24	4.79	**0.0055**	12.87	**<0.0001**	17.53	**<0.0001**	3,17	78.43	**<0.0001**	344.75	**<0.0001**
Month*Fertilizer	8,24	0.59	0.7731	1.82	0.1219	1.47	0.2201	6,17	3.26	**0.0240**	13.25	**<0.0001**

Table 3.2: Repeated measures ANOVA results for soil moisture for all treatments during three measurement periods (pre-fertilization only, intensive sampling period immediately following fertilization, and longer-term post-fertilization). Bolding indicates significance.

Effect	**Pre-Fertilization**			**Post-Fertilization Intensive**			**Post-Fertilization**		
	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P
Organic Unfertilized Treatments									
Cover Crop	2,6	2.05	0.2044	2,6	15.84	**0.0040**	2,6	26.19	**0.0011**
Date	12,72	113.49	**<0.0001**	9,54	41.24	**<0.0001**	12,66	290.29	**<0.0001**
Date*Cover Crop	24,72	1.86	**0.0235**	18,54	1.97	**0.0290**	24,66	4.02	**<0.0001**
Organic Vetch Treatments									
Fertilizer	2,6	4.26	0.2097	2,6	9.99	**0.0123**	2,6	4.92	0.0542
Date	12,72	132.51	**<0.0001**	9,54	46.39	**<0.0001**	12,66	490.26	**<0.0001**
Date*Fertilizer	24,72	1.88	**0.0212**	18,54	1.30	0.2233	24,66	3.01	**0.0002**
Conventional Treatments									
Fertilizer	2,6	7.76	**0.0217**	2,6	2.00	0.2048	2,6	23.56	**0.0014**
Date	12,72	78.32	**<0.0001**	9,54	11.52	**0.0001**	12,66	60.60	**<0.0001**
Date*Fertilizer	24,72	1.52	0.0900	18,54	1.40	0.2676	24,66	1.74	**<0.0001**

Table 3.3: Repeated measures ANOVA results for soil temperature for all treatments during three measurement periods (pre-fertilization only, intensive sampling period immediately following fertilization, and longer-term post-fertilization). Bolding indicates significance.

Effect	**Pre-Fertilization**			**Post-Fertilization Intensive**			**Post-Fertilization**		
	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P
Organic Unfertilized Treatments									
Cover Crop	2,6	1.77	0.2489	2,6	0.58	0.5876	2,6	3.25	0.1109
Date	12,72	715.02	**<0.0001**	9,54	44.51	**<0.0001**	12,66	153.43	**<0.0001**
Date*Cover Crop	24,72	4.11	**<0.0001**	18,54	0.85	0.6366	24,66	0.80	0.7236
Organic Vetch Treatments									
Fertilizer	2,6	0.26	0.7802	2,6	2.08	0.2061	2,6	3.03	0.1230
Date	12,72	3558.16	**<0.0001**	9,54	38.53	**<0.0001**	12,66	135.69	**<0.0001**
Date*Fertilizer	24,72	1.04	0.4313	18,54	0.77	0.7276	24,66	1.43	0.1296
Conventional Treatments									
Fertilizer	2,6	2.19	0.1933	2,6	8.92	**0.0159**	2,6	5.08	0.0511
Date	12,72	5118.55	**<0.0001**	9,54	37.74	**<0.0001**	12,68	385.43	**<0.0001**
Date*Fertilizer	24,72	1.53	0.0868	18,54	0.79	0.7036	24,68	1.43	0.1272

Table 3.4: Repeated measures ANOVA results for NO_3-N and NH_4-N concentrations extracted from soil resin bags for two periods (pre-and post-fertilization) from November 2013 to October 2014. Bolding indicates significance.

	Pre-Fertilization					Post-Fertilization				
Effect		NO_3-N (Nov-May)		NH_4-N (Nov-May)			NO_3-N (May-Oct)		NH_4-N (May-Oct)	
Organic Unfertilized Treatments	$DF_{n,d}$	F	P	F	P	$DF_{n,d}$	F	P	F	P
Cover Crop	2,6	13.64	**0.0059**	4.13	0.0744	2,6	8.58	**0.0174**	2.66	0.1487
Time	5,30	28.41	**<0.0001**	7.28	**0.0001**	4,24	15.36	**0.0004**	4.6	**0.0067**
Time*Cover Crop	10,30	1.00	0.4641	0.67	0.7404	8,24	0.49	0.7456	1.48	0.2147
Organic Vetch Treatments										
Fertilizer	2,6	0.21	0.8148	0.31	0.7440	2,6	15.84	**0.0040**	7.92	**0.0207**
Time	5,30	21.25	**<0.0001**	7.40	**0.0001**	4,24	20.65	**<0.0001**	21.32	**<0.0001**
Time*Fertilizer	10,30	1.57	0.1643	0.98	0.4826	8,24	0.63	0.7460	1.75	0.1383
Conventional Treatments										
Fertilizer	2,6	1.64	0.2710	5.35	**0.0464**	2,6	4.37	0.0675	6.04	**0.0366**
Time	5,30	48.29	**<0.0001**	15.16	**<0.0001**	4,24	58.71	**<0.0001**	5.44	**0.0029**
Time*Fertilizer	10,30	1.11	0.3848	1.74	0.1168	8,24	3.82	**0.0051**	0.99	0.4711

Table 3.5: Average (±SE) N_2O-N, NH_3-N, NO_3-N, and NH_4-N lost as gas (as estimated by total flux calculation) or caught in lysimeters from treatments for the entire pre-fertilization and post-fertilization periods, and the corresponding p-values for contrasts comparing the organic, unfertilized treatments, the organic vetch treatments, and the conventional treatments. For significant contrasts, LS-means comparisons across treatments are provided (highlighted in red).

		Pre-Fertilization: October 28 - May 29				**Post-Fertilization: May 29 – November 1**			
Treatments		N_2O-N (kg N ha^{-1})	NH_3-N (kg N ha^{-1})	NO_3-N (ppm)	NH_4-N (ppm)	N_2O-N (kg N ha^{-1})	NH_3-N (kg N ha^{1})	NO_3-N (ppm)	NH_4-N (ppm)
Organic									
	Wheat (0)	1.16 (±0.24)	-4.40 (±3.78)	221.7 (±79.80)	2.85 (±0.39)	1.79 (±0.07)	7.27 (±1.37)	18.97 (±3.41)	8.99 (±0.75)
	Vetch-Wheat (0)	0.65 (±0.27)	28.09 (±35.58)	248.86 (±52.95)	2.09 (±0.09)	2.05 (±0.22)	18.91 (±5.29)	106.17 (±19.73)	13.08 (±2.39)
	Vetch (0)	0.79 (±0.08)	-4.01 (±7.59)	442.82 (±102.19)	2.54 (±0.22)	2.13 (±0.23)	8.71 (±6.91)	217.36 (±70.59)	22.12 (±5.27)
	Vetch (N-credit)	1.99 (±0.30)	22.75 (±9.63)	467.69 (±122.01)	2.53 (±0.10)	3.10 (±0.59)	12.81 (±1.47)	249.41 (±155.81)	50.20 (±30.61)
	Vetch (168 organic N)	1.31 (±0.74)	7.62 (±0.81)	304.01 (±60.95)	2.65 (±0.14)	4.47 (±0.48)	39.09 (±3.35)	307.42 (±6.15)	28.73 (±9.23)
Conventional									
	Wheat (0)	1.97 (±0.23)	13.69 (±2.63)	263.45 (±75.87)	4.04 (±1.43)	1.83 (±0.13)	-5.80 (±6.88)	62.59 (±25.08)	17.39 (±3.16)
	Wheat (168 urea N)	3.04 (±0.77)	36.95 (±9.96)	226.76 (±21.9)	2.11 (±0.13)	3.54 (±0.23)	24.29 (±8.97)	601.59 (±238.11)	43.85 (±21.63)
	Wheat (168 organic N)	0.86 (±0.28)	-8.10 (±6.39)	153.94 (±32.63)	3.42 (±0.40)	5.05 (±1.03)	43.07 (±4.41)	107.66 (±41.32)	24.55 (±3.17)
Contrast		N_2O-N (kg N ha^{-1})	NH_3-N (kg N ha^{-1})	NO_3-N (ppm)	NH_4-N (ppm)	N_2O-N (kg N ha^{-1})	NH_3-N (kg N ha^{1})	NO_3-N (ppm)	NH_4-N (ppm)
Organic Unfertilized Treatments		0.2668	0.5075	**0.0015** V> VW>W	0.1139	0.4637	0.2585	**0.0010** V, VW > W	0.0653
Organic Vetch Treatments		0.2912	0.1475	0.1484	0.8479	**0.0096** 168 > 0 168 > N-credit	**0.0264** 168 > 0, N-credit N-credit > 0	0.5481	0.6923
Conventional Treatments		0.0875	**0.0051** Urea > 0 > Organic	0.8145	0.2223	0.0648	**0.0269** Organic > 0	0.0539	0.6618

"V" = Hairy Vetch, "VW" = Vetch-Wheat mix, "W" = Wheat, "168" = vetch applied with 168 kg ha^{-1} organic N, "N-credit" = vetch N-credit treatment, "0" = unfertilized treatment, "urea" = conventional wheat cover crop treatment with 168 kg ha^{-1} urea N + urease inhibitor, and "Organic" = conventional wheat cover crop treatment with 168 kg ha^{-1} organic N.

Table 3.6: Average (±SE) N_2O-N, NH_3-N, NO_3-N, and NH_4-N lost as gas (as estimated by total flux calculation) or caught in lysimeters from treatments annually, and the corresponding p-values for contrasts comparing the organic, unfertilized treatments, the organic vetch treatments, and the conventional treatments. For significant contrasts, LS-means comparisons across treatments are provided (highlighted in red).

		Annual Total			
Treatments		N_2O-N (kg N ha^{-1})	NH_3-N (kg N ha^{-1})	NO_3-N (ppm)	NH_4-N (ppm)
Organic					
	Wheat (0)	2.95 (±0.19)	2.86 (±3.31)	162.91 (±3.15)	11.85 (±1.10)
	Vetch-Wheat (0)	2.70 (±0.44)	46.88 (±40.68)	332.93 (±21.89)	15.17 (±2.49)
	Vetch (0)	2.92 (±0.27)	4.69 (±14.05)	660.18 (±121.56)	24.66 (±5.48)
	Vetch (N-credit)	5.08 (±0.66)	35.47 (±10.01)	717.09 (±133.46)	52.73 (±30.71)
	Vetch (168 organic N)	5.77 (±1.22)	46.58 (±3.79)	611.43 (±14.42)	31.38 (±9.34)
Conventional					
	Wheat (0)	3.78 (±0.37)	7.87 (±8.82)	311.45 (±44.68)	21.43 (±4.48)
	Wheat (168 urea N)	6.56 (±0.84)	61.08 (±1.00)	823.31 (±295.03)	45.96 (±21.51)
	Wheat (168 organic N)	5.89 (±1.22)	34.87 (±8.59)	371.11 (±80.40)	27.97 (±2.99)

Contrast	N_2O-N (kg N ha^{-1})	NH_3-N (kg N ha^{-1})	NO_3-N (ppm)	NH_4-N (ppm)
Organic Unfertilized Treatments	0.8365	0.4115	**0.0007** V > VW >W	0.3300
Organic Vetch Treatments	0.0558	0.1101	0.7823	0.9917
Conventional Treatments	0.1792	**0.0222** Urea > 0	0.2939	0.6941

"V" = Hairy Vetch, "VW" = Vetch-Wheat mix, "W" = Wheat, "168" = vetch applied with 168 kg ha^{-1} organic N, "N-credit" = vetch N-credit treatment, "0" = unfertilized treatment, "urea" = conventional wheat cover crop treatment with 168 kg ha^{-1} urea N + urease inhibitor, and "Organic" = conventional wheat cover crop treatment with 168 kg ha^{-1} organic N

Table 3.7: Repeated measures ANOVA results for N_2O-N emissions for all treatments during three measurement periods (pre-fertilization only, intensive sampling period immediately following fertilization, and longer-term post-fertilization). Bolding indicates significance.

Effect	**Pre-Fertilization**			**Post-Fertilization Intensive**			**Post-Fertilization**		
	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P
Organic Unfertilized Treatments									
Cover Crop	2,6	2.12	0.2012	2,6	8.12	**0.0197**	2,6	0.08	0.9278
Date	12,72	10.61	**<0.0001**	9,54	6.49	**<0.0001**	12,66	11.04	**<0.0001**
Date*Cover Crop	24,72	2.28	**0.0039**	18,54	3.42	**0.0002**	24,66	0.60	0.9165
Organic Vetch Treatments									
Fertilizer	2,6	2.44	0.1675	2,6	8.09	**0.0198**	2,6	17.46	**0.0032**
Date	12,72	9.59	**<0.0001**	9,54	5.73	**<0.0001**	12,66	16.93	**<0.0001**
Date*Fertilizer	24,72	1.16	0.3045	18,54	0.94	0.5417	24,66	4.79	**<0.0001**
Conventional Treatments									
Fertilizer	2,6	1.66	0.2667	2,6	20.43	**0.0021**	2,6	7.50	**0.0233**
Date	12,72	13.27	**<0.0001**	9,54	7.56	**<0.0001**	12,66	35.87	**<0.0001**
Date*Fertilizer	24,72	0.67	0.8678	18,54	2.45	**0.0059**	24,66	5.38	**<0.0001**

Table 3.8: Repeated measures ANOVA results for NH_3-N emissions for all treatments during three measurement periods (pre-fertilization only, intensive sampling period immediately following fertilization, and longer-term post-fertilization). Bolding indicates significance.

Effect	**Pre-Fertilization**			**Post-Fertilization Intensive**			**Post-Fertilization**		
	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P
Organic Unfertilized Treatments									
Cover Crop	2,6	0.67	0.5462	2,6	1.86	0.2356	2,6	0.30	0.7500
Date	12,72	3.90	**<0.0001**	9,54	1.43	0.1191	12,66	1.99	**0.0390**
Date*Cover Crop	24,72	1.46	0.1133	18,54	1.11	0.3666	24,66	1.48	0.1068
Organic Vetch Treatments									
Fertilizer	2,6	2.50	0.1625	2,6	10.50	**0.0110**	2,6	2.85	0.1347
Date	12,72	5.80	**<0.0001**	9,54	1.46	0.1852	12,66	4.24	**<0.0001**
Date*Fertilizer	24,72	1.90	**0.0192**	18,54	2.21	**0.0130**	24,66	1.26	0.2250
Conventional Treatments									
Fertilizer	2,6	4.38	0.0673	2,6	14.92	**0.0047**	2,6	0.08	0.9239
Date	12,72	10.65	**<0.0001**	9,54	1.65	0.1254	12,68	11.06	**<0.0001**
Date*Fertilizer	24,72	3.58	**<0.0001**	18,54	3.54	**0.0002**	24,68	1.22	0.2553

Table 3.9: Repeated measures ANOVA results for soil inorganic and hot KCL NO_3-N (μg g^{-1} soil). Bolding indicates significance.

Effect	Inorganic NO_3-N			Hot KCL NO_3-N		
	$DF_{n,d}$	F	P	$DF_{n,d}$	F	P
Organic Unfertilized Treatments						
Cover Crop	2,4	0.32	0.7435	2,4	3.59	0.1282
Date	4,24	3.36	**0.0255**	5,30	16.58	**<0.0001**
Date*Cover Crop	8,24	0.34	0.9421	10,30	2.25	**0.0423**
Organic Vetch Treatments						
Fertilizer	2,4	7.36	**0.0457**	2,4	5.92	0.0638
Date	4,24	9.18	**<0.0001**	5,30	16.75	**<0.0001**
Date*Fertilizer	8,24	0.95	0.4937	10,30	1.31	0.2884
Conventional Treatments						
Fertilizer	2,4	11.98	**0.0205**	2,4	18.00	**0.0100**
Date	4,24	10.67	**<0.0001**	5,30	10.85	**<0.0001**
Date*Fertilizer	8,24	2.27	0.0577	10,30	1.41	0.2254

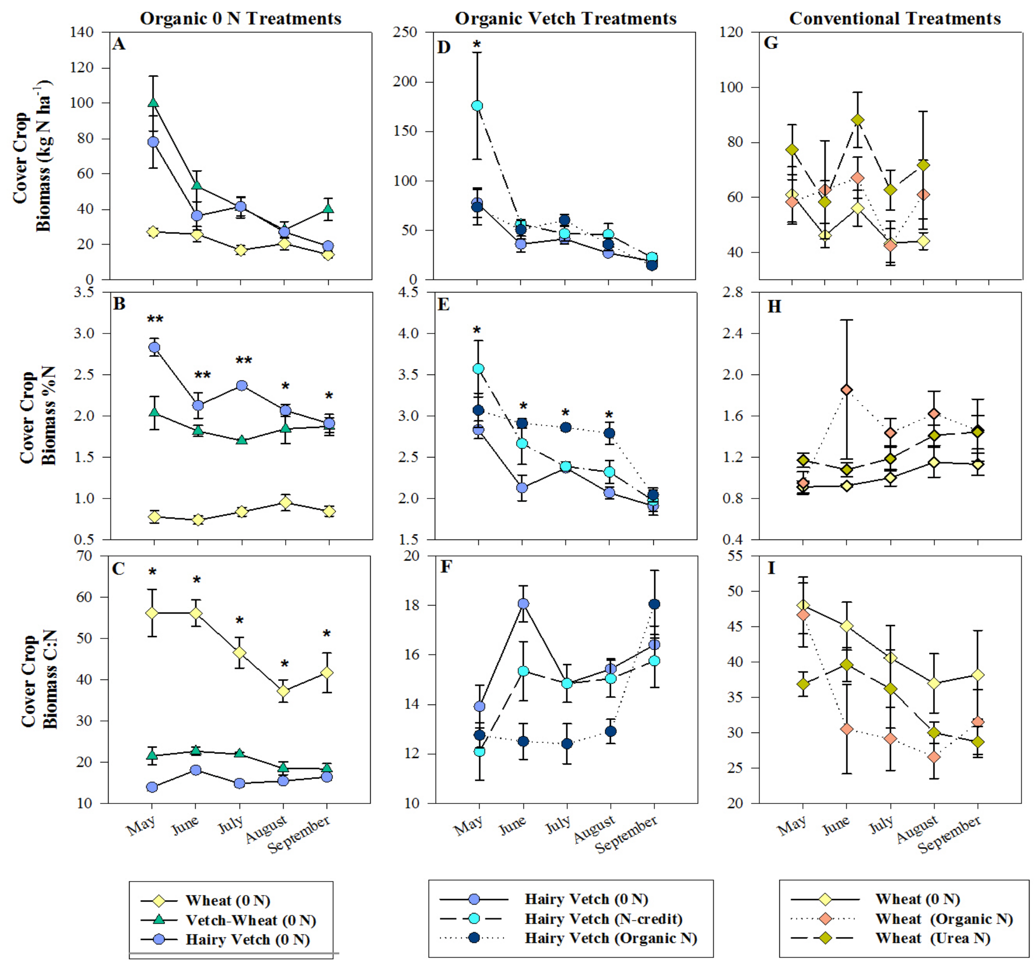


Figure 3.1: Average (±SE) aboveground cover crop biomass residue N content, percent N, and C:N ratio harvested from the soil surface from May to September 2014. Asterisks indicate points in time where significant effects between treatments were observed and double asterisks indicated points in time where all treatments significantly differed from one another. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

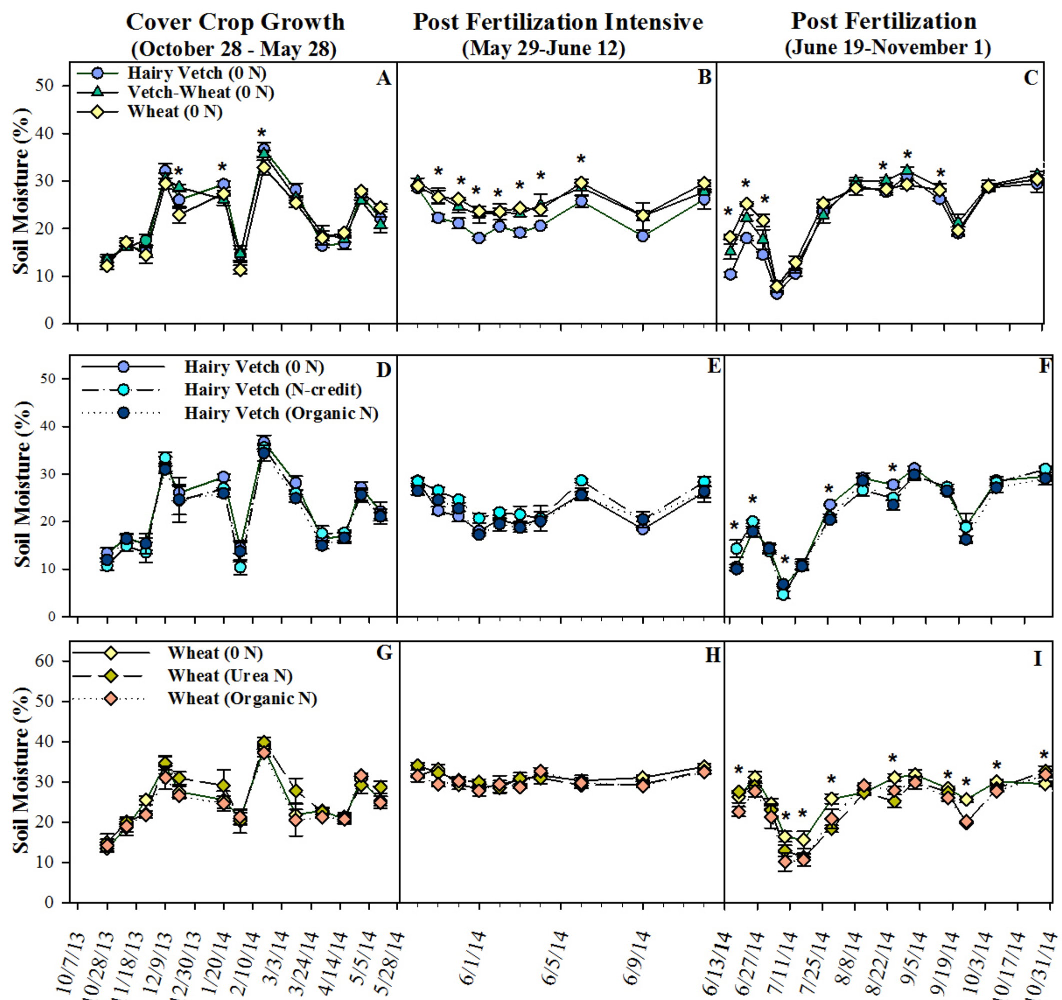


Figure 3.2: Average soil moisture (±SE) at 5 cm depth for organic 0 N treatments (A-C), organic hairy vetch treatments (D-F), and conventional treatments (G-I) across three different time periods from October 2013 to November 2014. Asterisks indicate points in time where significant effects between treatments were observed during the measurement period. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

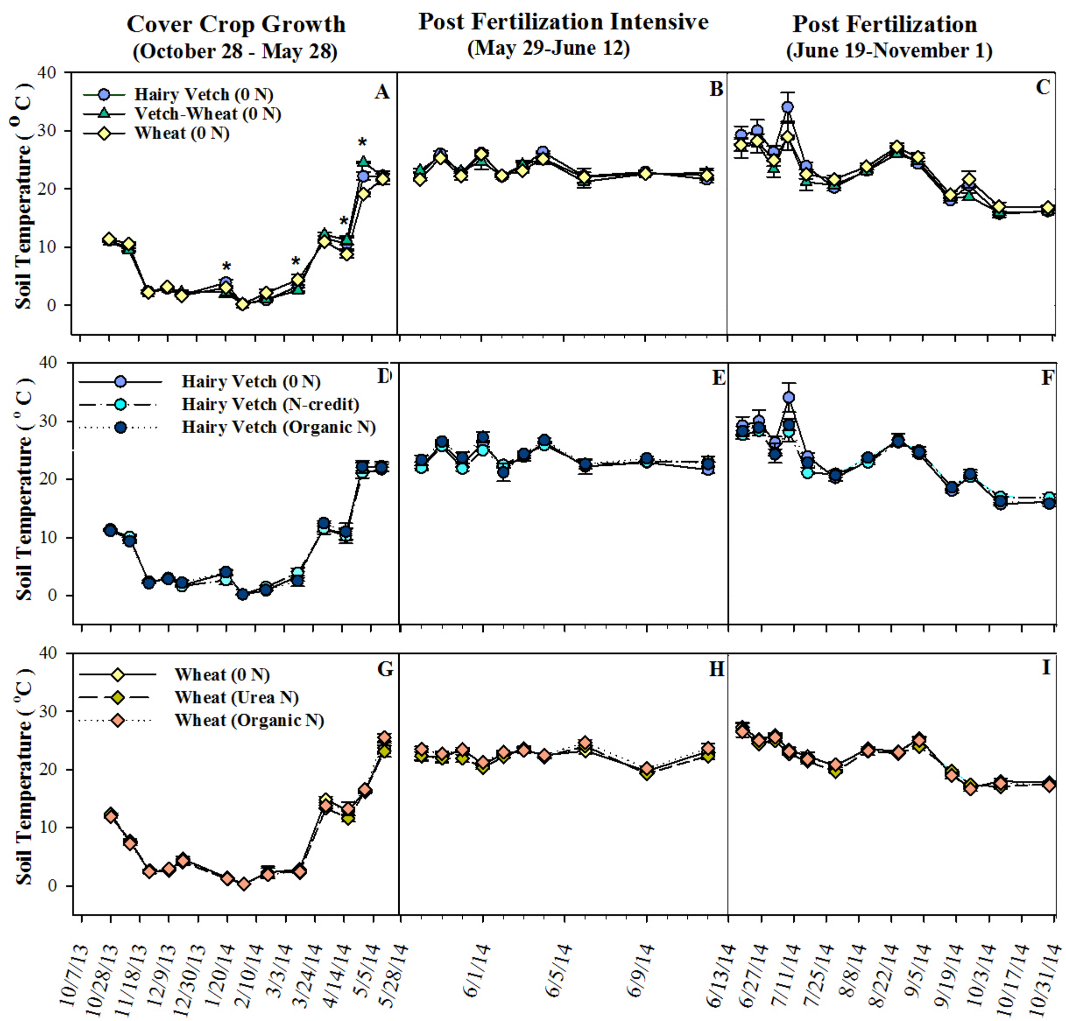


Figure 3.3: Average soil temperature (±SE) at 5 cm depth for organic 0 N treatments (A-C), organic hairy vetch treatments (D-F), and conventional treatments (G-I) across three different time periods from October 2013 to November 2014. Asterisks indicate points in time where significant effects between treatments were observed during the measurement period. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

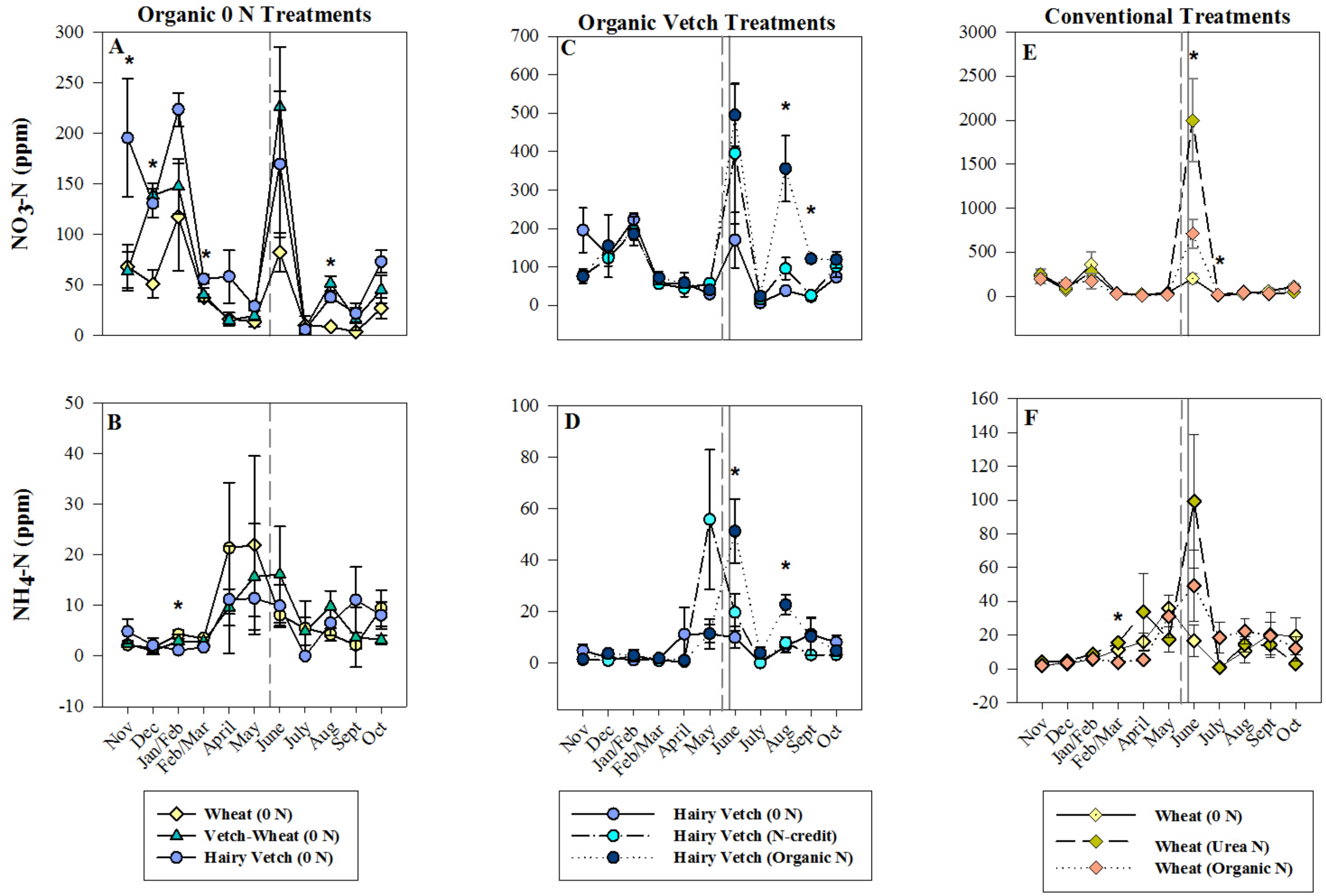


Figure 3.4: Average (±SE) NO_3-N and NH_4-N concentrations extracted from soil resin bags (15 cm depth) from November 2013 until October 2014 in the organic 0 N treatments (A & B), the organic hairy vetch treatments (C & D) and the conventional treatments (E & F). Asterisks indicate points in time where significant effects between treatments were observed and the panels are split to indicate pre and post-fertilization. Note that scales differ across graphs. The dotted grey line illustrates that cover crops were terminated 20 May 2014 and the solid gray line illustrates that fertilizers were applied on 28 May 2014. 0 N = no applied fertilizer, Organic N = 168 kg ha^{-1} organic fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

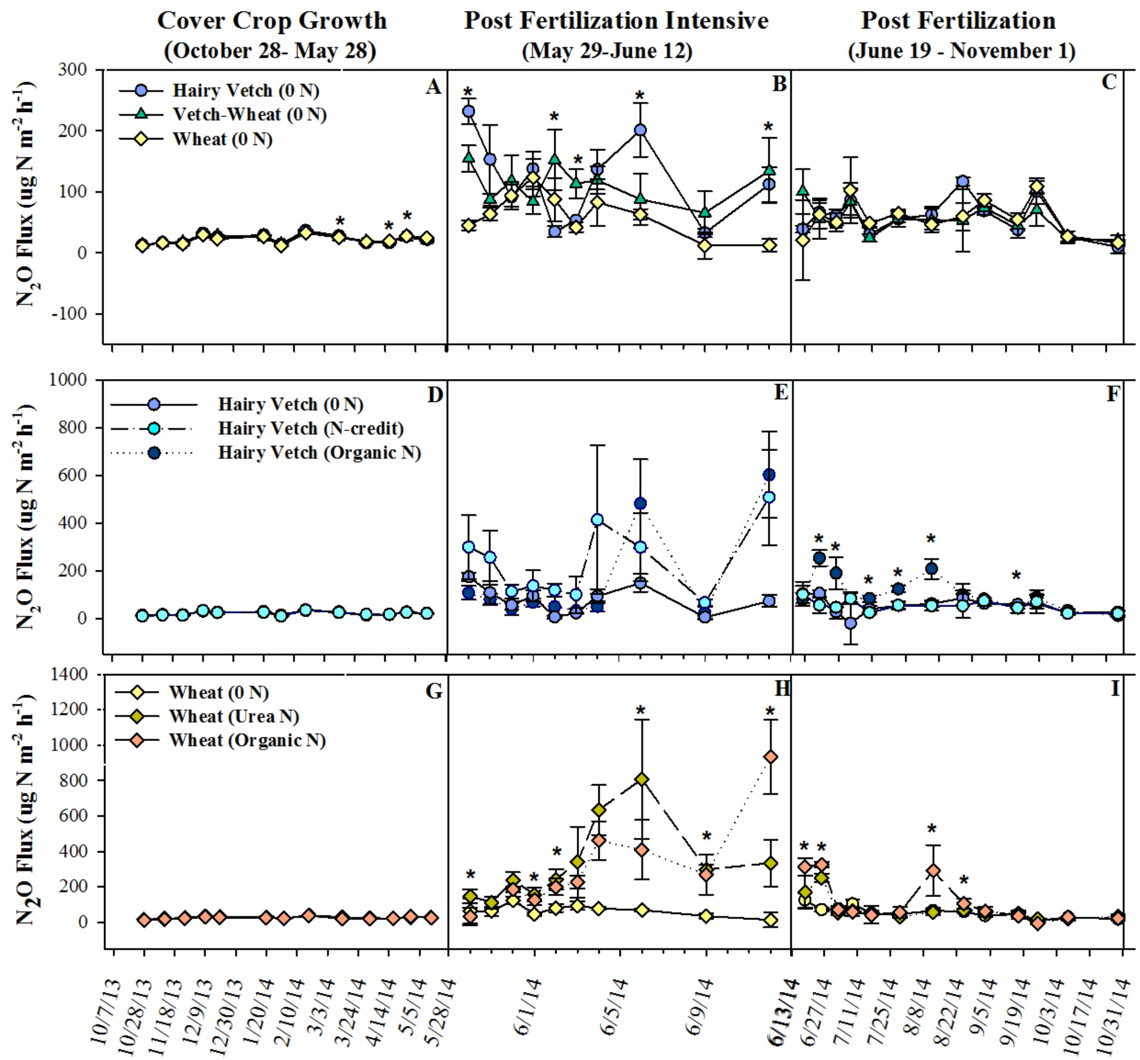


Figure 3.5: Average (±SE) N_2O flux for organic 0 N treatments (A-C), organic hairy vetch treatments (D-F), and conventional treatments (G-I) across three different time periods from October 2013 to November 2014. Asterisks indicate points in time where significant effects between treatments were observed during the measurement period. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

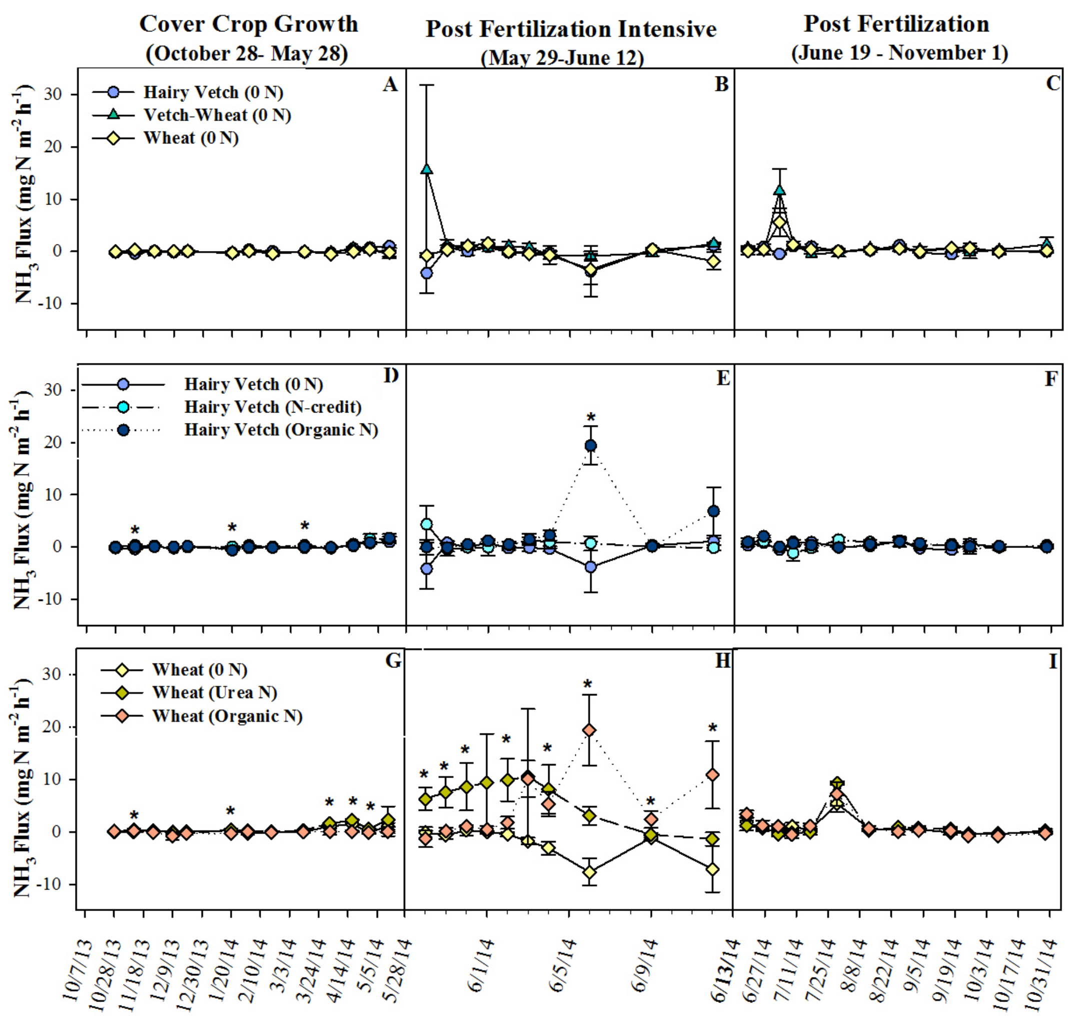


Figure 3.6: Average (±SE) NH_3 flux for organic 0 N treatments (A-C), organic hairy vetch treatments (D-F), and conventional treatments (G-I) across three different time periods from October 2013 to November 2014. Asterisks indicate points in time where significant effects between treatments were observed during the measurement period. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

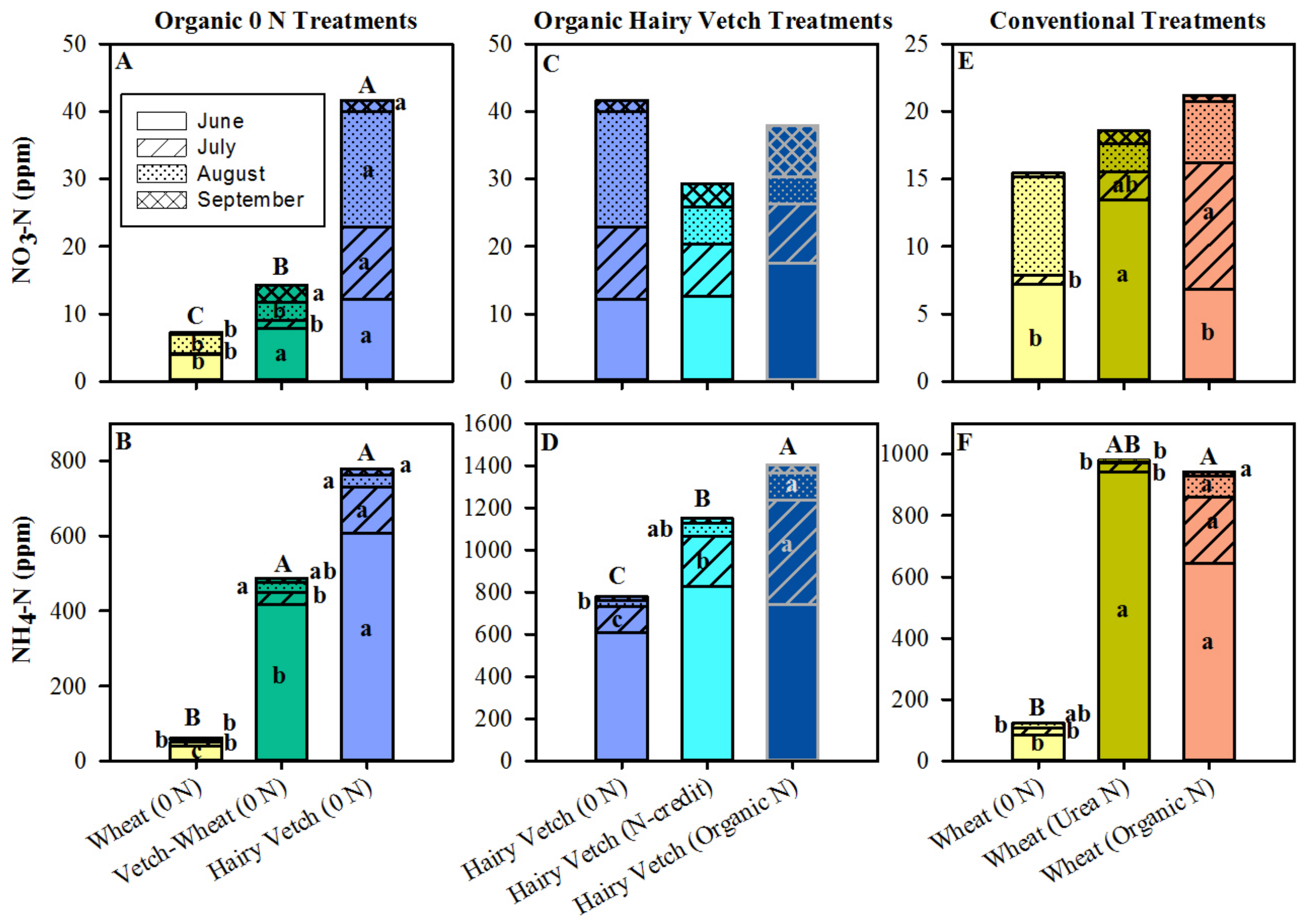


Figure 3.7: Average (±SE) NO_3-N and NH_4-N concentrations extracted from biomass residue resin bags during June-September 2014 for the unfertilized organic treatments (A & B), the organic hairy vetch treatments (C & D), and the conventional treatments (E & F). Upper case letters signify treatment differences across all months, lower case letters signify treatment differences within each month, and no letters indicate that significant differences were not found. In panel F, the urea N treatment appears to have greater NH_4-N release than either of the other two treatments across the entire period; however, the comparison was not significant as the error was very large. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

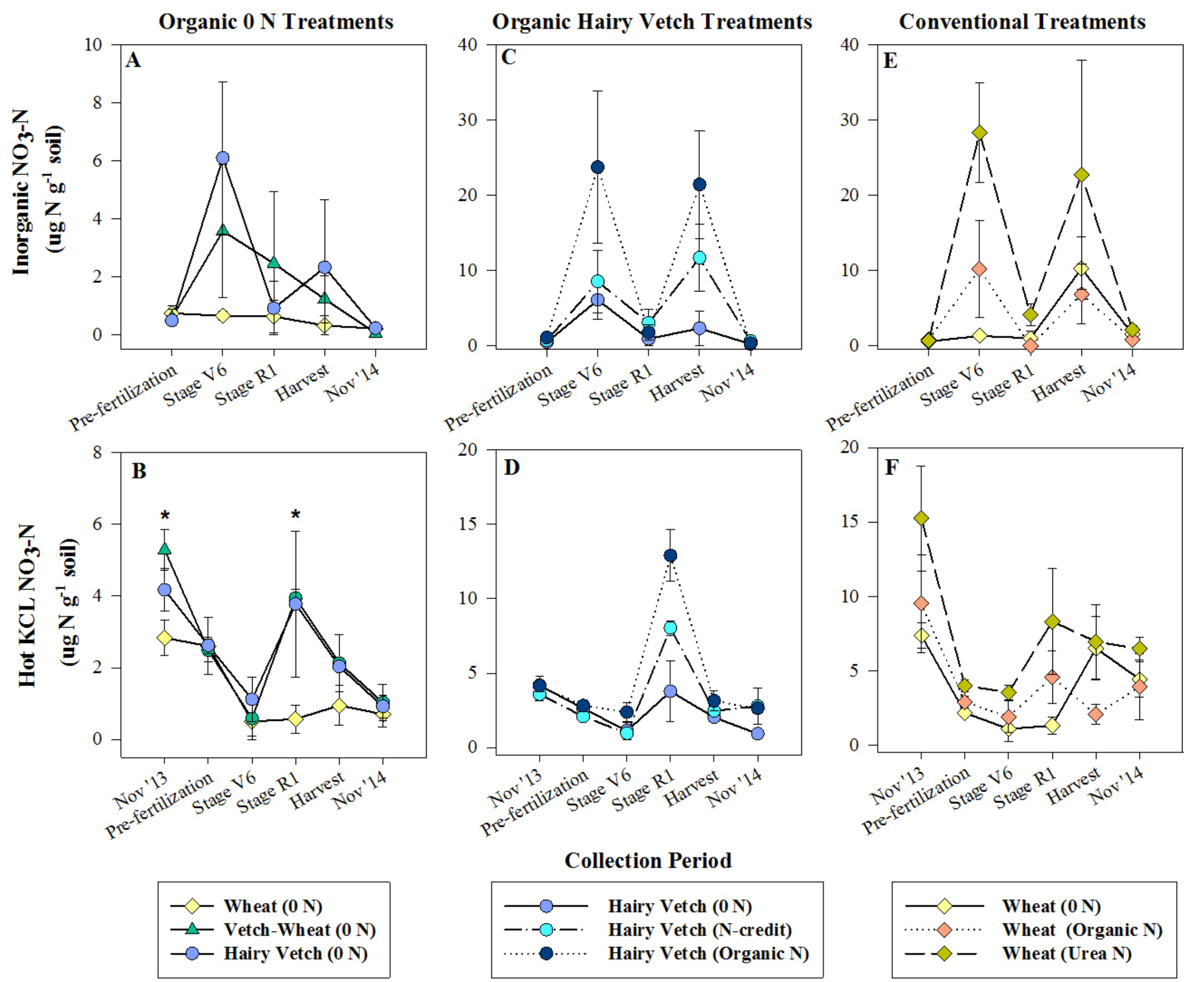


Figure 3.8: Average (±SE) inorganic and hot KCL extracted soil NO_3-N from the organic 0 N treatments (A & B), the organic hairy vetch treatments (C & D), and the conventional treatments (E &F). Asterisks indicate points in time where significant effects between treatments were observed. Note differences in scale across graphs. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

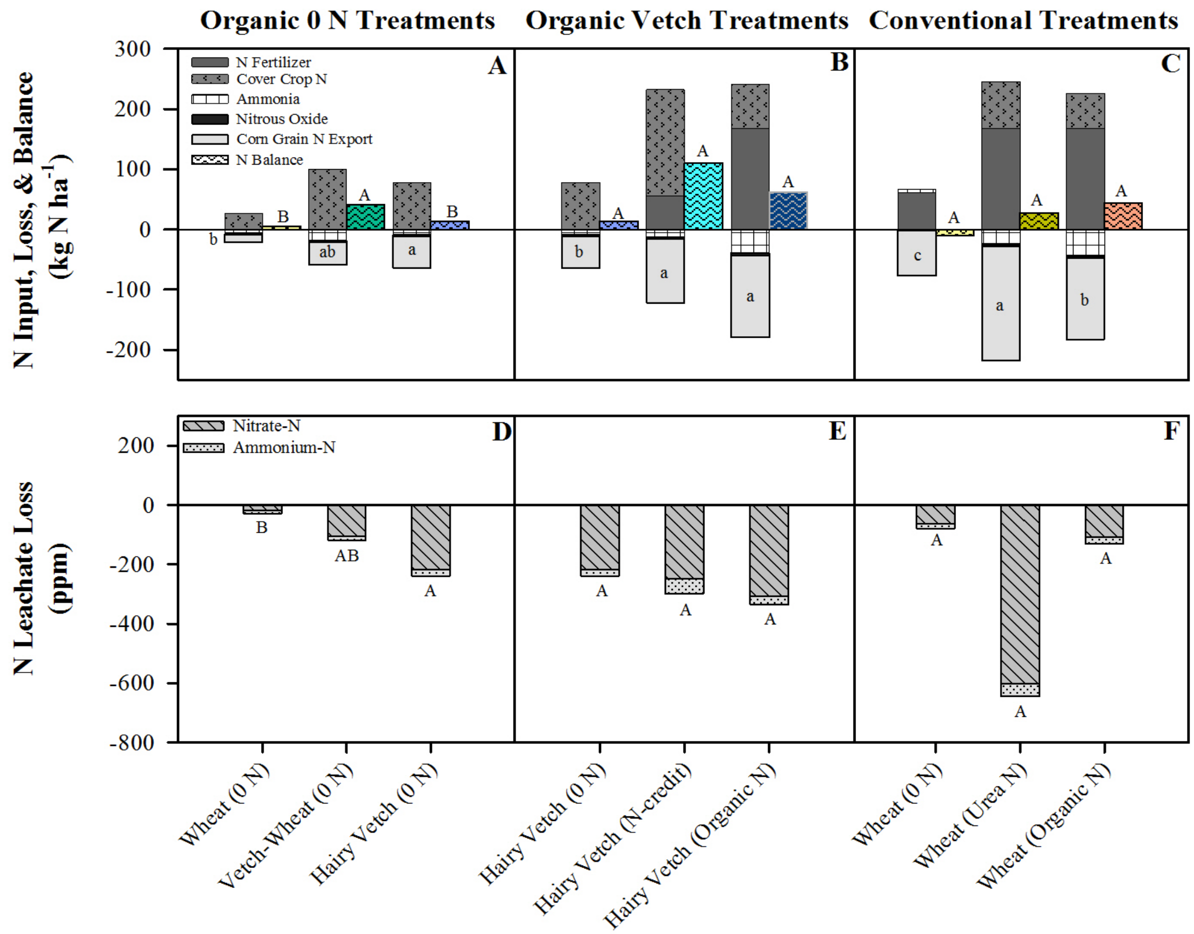


Figure 3.9: Average N input (cover crop N + fertilizer N), gaseous N loss as N_2O and NH_3, corn grain N export, N Balance (N input-corn grain N export-gaseous N losses) (A, B, C) and N leachate loss (D, E, F) post-fertilization for all treatments. Uppercase letters indicate significant differences for N balance and total N leachate calculations, while lowercase letters indicate differences in corn grain N export. Other significant treatment effects for the individual parameters are shown in Table 3.5. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

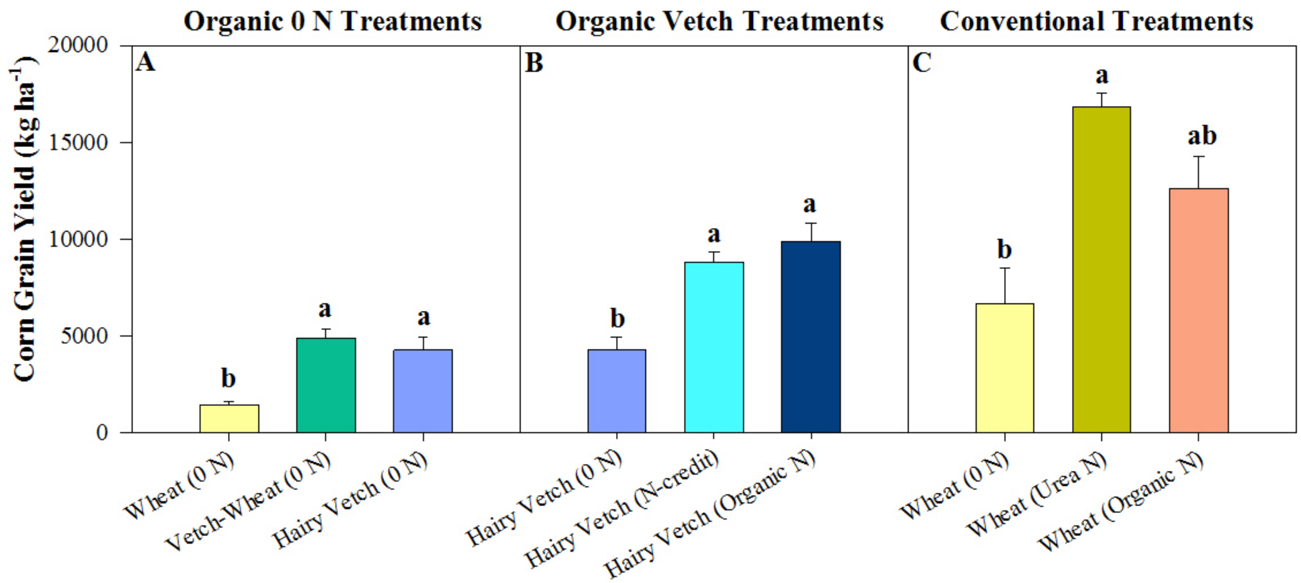


Figure 3.10: Average (±SE) corn grain yield of organic 0 N treatments (A), organic hairy vetch treatments (B), and conventional treatments (C). Letters indicate significant differences between treatments. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

Chapter 4 : Discussion

This study found that agroecosystem management, specifically cover crop choice and fertilization approach, can be utilized to reduce N loss and improve N balance in organic and conventional systems. While these results are consistent with prior work (e.g. Vitousek *et al.*, 1997; Smil, 1999; Galloway *et al.*, 2004), few studies have looked simultaneously at leaching and gaseous N loss in no-till corn systems; thus, this research improves understanding of N loss pathways in conservation agriculture systems and of the management strategies that may be implemented to mitigate these losses.

4.1 Organic Field: Cover Crop Comparisons, 0 N Treatments

My first hypothesis, that N loss would be greatest in systems using a legume only (hairy vetch) cover crop as opposed to a grass only (wheat) or bi-culture (hairy vetch-wheat), was supported by the data. N loss was greatest in the hairy vetch system during both the cover crop growing season and the corn-growing season.

During cover crop growth, low soil temperatures and low N availability likely contributed to low gaseous loss for all treatments especially as temperatures were even lower than the historic average for the site. Parsons *et al.* (1991) also found that N_2O-N emissions from a Kentucky Maury Silt Loam were low during the fall and winter. Thus, the primary N loss pathway during this time was NO_3-N leaching, and the hairy vetch treatment had the greatest quantity of NO_3-N captured in leachate, which is consistent with other work evaluating legume vs. grass cover crop effects on N leaching (McCracken *et al.*, 1994; Ranells and Wagger, 1997). Soil resin data showed that NO_3-N concentrations were greatest in the vetch treatment throughout the winter growing season, suggesting vetch had lower N uptake than the wheat or bi-culture treatments. Biomass produced by the cover crops during this time period also supports lower N demand in the vetch treatment: vetch produced only 56-78% of the biomass of the other treatments. Additionally, vetch's ability to fix N may have lowered its soil N demand, but it is unlikely that N fixation was the only source of N used for vetch growth and prevented soil N uptake, as other studies have found that vetch N fixation does not account for total plant N (Acosta *et al.*, 2011). In support of the existing body of research, my data indicate that a hairy vetch monoculture may not be effective for retaining soil N, and preventing NO_3-N leaching loss, during winter growth.

Also, consistent with the literature, this study found that growing cover crops in bi-culture increased biomass production and the winter hardiness of the legume (Teasdale and Abdul-Baki, 1998; Snapp *et al.*, 2005; Teasdale, 2012). Biomass accumulation in the vetch-wheat treatment was 4968 kg ha^{-1} (vs. 2791 and 3590 kg ha^{-1} for vetch and wheat alone, respectively), and the bi-culture had the highest N content. Indicative of higher N demand and uptake in the bi-culture, less NO_3-N leachate was measured in the vetch-wheat treatment than in hairy vetch alone; however, more NO_3-N leachate was captured in the bi-culture than in the wheat alone treatment. Higher soil resin NO_3-N concentrations in the bi-culture than the wheat monoculture from December – February indicate less N uptake occurred in the bi-culture during this time (Fig. 3.4A). From field observations, it was clear that the bi-culture grew less vigorously than wheat alone at the beginning of the season, accumulating most of its biomass in April and May (Appendix 2). Reduced winter growth, resulting from the unusually cold temperatures, most likely reduced the bi-culture's ability to capture and retain N at that time. Overall, it seems possible that measured N leaching loss was high in this study, as cover crop growth and biomass across all treatments was low due to historically low temperatures that induced cover crop kill and reduced growth. Unfortunately, I was not able to compare my leaching data to that of similar systems, as the resin lysimeter protocol that was employed is not yet widely used.

During the corn-growing season, N leachate concentrations continued to be significantly higher in the vetch and vetch-wheat systems, but overall, were lower than during the cover crop growing season. However, N_2O-N emissions were greater than during cover crop growth, likely due to a combination of increased N availability in the system after cover crop termination in combination with warmer soil temperatures and adequate soil moisture (Colbourn, 1993; DeKlein and VanLogtestijn, 1996). Similar to the research of Wisal *et al.* (2011), who found that in soils amended with residues of varying C:N ratios lower C:N residue increased N_2O-N emissions and decreased N immobilization into the residue, cover crop C:N appeared to influence gaseous N emissions.

For example, N_2O-N fluxes increased from 13 to 29 May 2014, after cover crop termination, for the vetch (from 57 to 232 μg N m^{-2} h^{-1}) and vetch-wheat treatments (62 to 155 μg N m^{-2} h^{-1}), but emissions decreased in the wheat system at this time (from 70 to 46 μg N m^{-2} h^{-1}) , as the wheat cover crop biomass residue induced N immobilization rather than mineralization (Fig 3.1B,C). These large differences in emissions occurred even though differences in soil temperature between these two dates was $<1^{o}$C. Similarly, although others have shown that soil moisture is important in controlling N_2O-N emissions (Rice and Smith, 1982; Colbourn, 1993; Chen *et al.*, 2011), and moisture may have been important during the post-fertilization intensive measurement period of this study, it does not appear to explain the pronounced cover crop effects we observed during the three weeks following termination, as the wheat cover crop tended to have the greatest soil moisture, but the lowest N_2O-N emissions.

Although the wheat cover crop had the lowest N loss (both via N leaching and gaseous loss), its high C:N ratio caused N immobilization after termination (Wagger, 1989; Wyland *et al.*, 1995; Baldwin and Creamer, 2009) and resulted in corn yields that were significantly lower than either of the other treatments. As N was immobilized in the cover crop residue and little N was exported in the corn grain, the net N balance for the wheat cover crop was small, but positive. Calculated N gain was likely attributable to pre-existing soil N pools that were accessed by the wheat during growth and that were moved into the residue by microbial activity. Overall, using a wheat monoculture cover crop reduced N loss in this study (gaseous and leaching); however, it would require significant quantities of N fertilizer applied during the corn growing season to obtain the yields necessary for a viable production system, perhaps negating its N loss environmental benefit.

Though there were no differences in post-termination leaching, gaseous N loss, corn grain N, or yield between vetch and the vetch-wheat bi-culture, there were significant differences in N balance: less N remained in the vetch system. This difference may be attributed to dissimilar patterns of cover crop decomposition and corn N uptake (Schomberg *et al.*, 1994; Clark *et al.*, 1997; Rosecrance *et al.*, 2000; Zhou *et al.*, 2012). Biomass resins indicated that more total N was released from the hairy vetch cover crop than the bi-culture, especially in June and July. The fact that the hairy vetch cover crop released N more rapidly at the beginning of the growing season than the bi-culture is supported by studies that have found vetch capable of releasing more than half of its total N content within the first 30 days following its termination (Aita and Giacomini, 2003; Acosta *et al.*, 2011). Greater N availability during this time may have provided the corn plants with a greater quantity of "starter" N when they emerged, resulting in increased uptake and corn plant biomass N content that remained greater than the vetch-wheat treatment for the entirety of the growing season (Appendix 3) (Hadas *et al.*, 2002; Pearson *et al.*, 2004).

In addition to disparate N release at the beginning of the corn-growing season, decomposition patterns during the latter half of the season also differed between cover crops. The N content of the hairy vetch only treatment decreased over the entire corn-growing season, indicating N was being mineralized and lost from the material, but in the vetch-wheat system, residue N content began to increase in August, indicating microbial-mediated immobilization was occurring (Clark *et al.*, 1997; Rosecrance *et al.*, 2000). Soil inorganic N levels in August were similar between the two systems, but by September and October inorganic N was lower in the vetch-wheat bi-culture than the vetch (Fig. 3.4 A, B). The higher residue C:N ratio of the bi-culture residue may have stimulated competition between plants and microbes for available N, as microbes required it for continued decomposition of the cover crop residue (Han *et al.*, 2007; Kuzyakov and Xu, 2013). Because less N was made available to the corn plants in the bi-culture system, both early and late in the growing season, the vetch-wheat system had a greater net N balance than vetch alone.

This research indicates that the bi-culture, legume-grass cover crop mixture has potential for reducing N loss in conservation agriculture systems in Kentucky. The bi-culture grew more vigorously than the legume monoculture during the winter months, which resulted in reduced leaching. Post-termination, it released N, making N available to the summer corn plants, albeit less effectively than vetch alone. Although grain N content was less in the bi-culture than the vetch alone treatment, corn yields were equivalent in the two treatments, suggesting that attributes other than N retention and release helped sustain yields in the bi-culture. The bi-culture had greater cover crop biomass which increased soil moisture retention (Fig. 3.2B, C), and, based on field observations, provided superior weed suppression compared to the vetch treatment (Sharma *et al.*, 2010; Das *et al.*, 2015). However, further cover crop management research is essential as all treatments in this system were unfertilized and most likely N limited, as corn yields were half of those produced in the fertilized organic treatments also evaluated in this experiment.

4.2 Organic Field: Fertilizer Comparisons, Hairy Vetch Cover Crop

For these treatments, I hypothesized, and the data support, that as the quantity of organic N fertilizer applied increased, N loss would also increase. While N loss as leachate was not affected by fertilizer approach, N_2O-N and NH_3-N emissions were greater in the organic fertilizer treatment (168 kg N ha^{-1} applied as organic fertilizer, 242 kg N ha^{-1} total) than the N-credit treatment (only 56 kg N ha^{-1} applied as organic fertilizer, 232 kg N ha^{-1} total), and both fertilized systems had greater gaseous emissions than the unfertilized treatment (78 kg N ha^{-1} total).

During the cover crop growing season, N leaching was the dominant N loss pathway, but there were no differences between treatments. It is likely that the majority of leaching occurred from November to January/February as soil NO_3-N levels began to decrease in February/March (Fig. 3.4C, D). Additionally, there were no differences in N gaseous emissions, during this time, as low soil temperatures kept fluxes low (Parsons *et al.*, 1991; Colbourn, 1993; DeKlein and VanLogtestijn, 1996)

During the corn-growing season, N loss pathways were influenced by the form (vetch biomass vs. organic fertilizer) and quantity of applied N. By subtracting the unfertilized gaseous N emissions calculated over the corn growing season from the fertilized treatment and dividing by the total amount of N applied in the fertilized treatments, I estimated the percentage of the applied N that was diverted into N_2O-N or NH_3-N loss pathways (Parkin *et al.*, 2006) (Table 4.1). This approach estimated that 1.4% of the applied N in the organic N treatment was lost as N_2O-N, whereas only 0.6% of applied N was lost in the N-credit treatment via this pathway (Table 4.1). However, the N-credit treatment had higher initial N_2O-N emissions (Fig. 3.5E), likely due to greater quantities of biomass residue being present that prevented evaporative loss from the soil surface, thus creating conditions of increased soil moisture, microbial activity, and N mineralization (Colbourn, 1993; Schomberg *et al.*, 1994). N released during moist soil conditions is likely to result in denitrification as anaerobic microsites are present (DeKlein and VanLogtestijn, 1996; Dobbie *et al.*, 1999; Hu *et al.*, 2013).

After June, soil moisture was not consistently higher in the N-credit treatment, as the hairy vetch biomass underwent rapid decomposition (Hadas *et al.*, 2002; Acosta *et al.*, 2011). However, periods of high soil moisture continued to induce denitrification throughout the growing season, as N_2O-N gas flux peaks in all treatments were often linked with rain events, particularly in the organic N treatment (Figs. 3.2 E, F, & 3.5 E, F). Following a rain event on 4 June 2014, emissions increased dramatically in the organic N treatment and then remained greater than emissions from the N-credit treatment for the entirety of the season. N in slow release, organic fertilizers, such as that applied in this study, commonly undergo mineralization followed by denitrification after rain events (Sistani *et al.*, 2011; Venterea *et al.*, 2011; Halvorson and Del Grosso, 2012, 2013). Nitrifying microbes compete with other microbes for NH_4 and rapidly convert it to NO_3, thus contributing to the production of N_2O-N via both nitrification and denitrification (Inselsbacher *et al.*, 2010). While maintaining a source of available N over the course of the growing season is ideal for plant growth, temporal asynchrony between microbial activity and plant N uptake, such as can occur during wet-dry cycles, can result in significant N_2O-N loss (Augustine and McNaughton, 2004; Schwinning and Sala, 2004; Dijkstra *et al.*, 2012; Parkin and Hatfield, 2014).

Additionally, NH_3-N emissions were greatest in the organic fertilizer treatment. I estimated that approximately 18.5% of the applied N was lost as NH_3-N from the organic N treatment, whereas <3% of applied N was lost from the N-credit treatment (Table 4.1). Significant NH_3-N emission fluxes occurred on 6 June and 12 June 2014, following rain events, in the organic fertilizer treatment. Similar to rainfall effects on N_2O-N fluxes, precipitation events most likely induced fertilizer mineralization, increasing soil NH_3 concentrations and resulting in volatilization, as soil moisture declined following the event (Rochette *et al.*, 2013).

Interestingly, according to soil resins, residue biomass resins, and gaseous N loss data, more inorganic N was released in the organic fertilizer vs. the N-credit treatment, but yields, corn grain N export, and N balance of these two systems were equivalent. Though more inorganic N was released in the organic fertilizer treatment, it may not always have been accessible for corn plant uptake or, as other studies have shown, at a higher level of N supply the corn in the organic fertilizer treatment may have used N less efficiently (Moll *et al.*, 1982).

For example, although the C:N ratio of the vetch cover crop in both treatments was approximately 13:1 (a ratio low enough that mineralization and N release should occur (Wagger, 1989)) and, according to the biomass resins, N moved from the residue to the soil surface in both treatments, the residue %N in the organic fertilizer treatment did not significantly decline between June and August (Fig. 3.1E & 3.7C, D). It is possible that N captured in the biomass resins came directly from mineralization of the organic fertilizer, which was applied on top of the cover crop biomass, and that the presence of the N fertilizer increased N availability and may have inhibited residue decomposition, as previous research has shown (Craine *et al.*, 2007; Milcu *et al.*, 2011), causing residue %N to remain stable during this time period. Alternatively, N released from the fertilizer may have stimulated microbial activity such that vetch N was mineralized and released while simultaneously available N from the soil and the fertilizer was immobilized in the residue, maintaining %N at relatively constant levels (Gentile *et al.*, 2008). Additional research would be required to evaluate which of these scenarios was occurring, but my data suggest some N, originating from either the vetch residue or fertilizer, was retained by the biomass residue in the organic fertilizer treatment during this time period, reducing N available for corn uptake.

Later in the season (i.e. late July/August) fertilizer mineralization continued, but there was less residue to retain/immobilize N and soil inorganic N pools increased in the organic fertilizer treatment (Fig. 3.4C, D). Theoretically, corn plant N uptake should have been stimulated at this point in the growing season as the R1 physiological growth stage was reached in early August, and approximately 50% of corn N uptake occurs after this stage in development (Moll *et al.*, 1982); however, as corn biomass N content and corn grain N were not different between treatments, greater inorganic N appeared to enhance N_2O-N emissions rather than corn plant N uptake (Fig. 3.5F & 3.9B, Appendix 3). Overall, it appears that greater N availability in the organic fertilizer treatment later in the season stimulated gaseous emission losses and did not result in greater yield or corn N uptake and grain N export (Fig. 3.9B; 3.10B). However, late season N release and emissions may be seasonally atypical in Kentucky as rainfall in August was nearly twice that of the historical average (Fig. 2.2).

This research suggests that accounting for the N-contribution of the cover crop via an N-credit fertilization approach may decrease N loss and promote more efficient N use by better timing N release with corn plant demand compared to the organic fertilizer applications. However, though potentially more environmentally beneficial from an N perspective, the N-credit technique requires that spring cover crop grab samples are taken and analyzed, N content calculations made, and fertilization application rates modified. These additional tasks, at a busy time of year, may limit widespread producer acceptance of this technique. Yet, if fertilizer costs continue to rise and/or other N loss reduction incentives appear, producers may be motivated to conduct the extra analysis required to implement an N-credit cover crop approach.

4.3 Conventional Field: Fertilizer Comparisons, Winter Wheat Cover Crop

In the conventional system, I hypothesized that N loss would be greater in treatments receiving fertilizer than without and that gaseous N loss pathways would differ between urea and organic fertilizer treatments. Data supported both hypotheses. N_2O-N and NH_3-N emissions were greater in the fertilized treatments compared to the unfertilized treatment, and gaseous N loss pathways in the fertilized treatments differed both temporally and in magnitude.

During the cover crop growing season, there were no differences in N leachate between treatments, as all treatments were planted in wheat and no fertilization had occurred. However, differences in NH_3-N loss occurred at the end of the cover crop growing season. Though soil NO_3-N and NH_4-N concentrations were equivalent at cover crop planting, soil resin data shows an increase in NH_4-N concentration in all treatments prior to cover crop termination, but particularly in the urea N treatment in April and the unfertilized and organic N plots in May. Increasing soil temperatures during this time most likely stimulated wheat growth and microbial activity in the soil, which may have affected NH_4-N concentrations; however, these effects should have been consistent across the treatments. The only visual difference apparent between treatments was the presence of volunteer big flower vetch: it was greatest in the urea treatment (average percent cover ~7%), followed by the organic fertilizer (~5%), and the unfertilized (~4%). Perhaps the presence of this weed or other unknown factors contributed to treatment differences at this time.

Increased soil NH_4-N concentrations may explain elevated NH_3-N emissions at this time, particularly in the urea N treatment. NH_3-N emissions in the urea N treatment during the cover crop growing season were relatively high at 37 kg N ha^{-1}, perhaps suggesting that NH_3-N loss in wheat cover crops deserves more exploration, as it may not be typically accounted for in agroecosystem N balance calculations. However, it seems unlikely that the wheat cover crop was directly responsible for stimulating these emissions, as NH_3-N emissions from other similar treatments (e.g., the conventional organic N plots, and from the wheat treatment in the organic field) were negative or near zero over the same time period (Table 3.5).

Post-fertilization, gaseous N loss and leaching were affected by the quantity and type of fertilizer. Though soil moisture was slightly increased in the unfertilized treatment (likely due to decreased corn plant water demand as corn plant biomass trended lesser compared to fertilized treatments (Appendix 3)), gaseous and leaching losses were minimal (Tables 3.5, 4.1) compared to those that received fertilizer. Amongst fertilized treatments, gaseous N_2O-N losses were significantly greater on several dates in the organic N treatment, gaseous NH_3-N losses were similar in magnitude between the treatments but differed temporally, and leaching losses were greater (~5x) from the urea treatment (Table 3.5, Fig. 3.9F). Some urea hydrolysis likely occurred prior to corn plant germination on 3 June 2014; thus, N may have leached down into the soil profile prior to corn plant establishment (Quisenberry and Phillips, 1976). To reduce NO_3 leaching loss associated with urea N applications, a split application or application after corn plant establishment might be necessary (Meisinger and Delgado, 2002).

Calculations assessing how much fertilizer N was lost as NH_3-N emissions, made by comparing emissions from fertilized and unfertilized plots, indicated that only 17.9% of urea N was lost as NH_3-N volatilization, whereas 29.1% of the organic fertilizer N was lost as NH_3-N (Table 4.1). Christianson *et al.* (2012) reported that up to 30% of N can be volatilized after broadcast urea N applications, and, in systems where organic residue is present, broadcast urea may be particularly vulnerable to volatilization as the residue acts as a barrier between the soil surface and the fertilizer (Mohr *et al.*, 1998; Rochette *et al.*, 2009; Francisco *et al.*, 2011). The urease inhibitor was likely responsible for mitigating volatilization prior to precipitation events that incorporated the fertilizer into the soil profile (a small rain event occurred the night following application, ~0.25 cm, in addition to a substantial rain event on 4 June 2014, ~4.72 cm). After contact with the soil, the soil environment likely prevented volatilization, as the chemistry at this field site is typically not considered particularly susceptible to volatilization. It has a pH less than 7 that can provide hydrogen ions to transform gaseous NH_3 into its more stable aqueous form, NH_4 (Bitzer *et al.*, 2000; Rochette *et al.*, 2013), and it has a high cation exchange capacity (15-23 cmol (+) kg^{-1}), making it more capable of fixing NH_4 (Schnitze.M, 1965; Hunt, 1981; Evangelou *et al.*, 1986; Harrison and Webb, 2001) (Appendix 1).

Similarly, the organic fertilizer treatment lost a greater proportion of applied N (1.9%) as N_2O-N emissions than the urea N treatment (1.0%) (Table 4.1). This was not unexpected as the fertilizer was not only 13% N, but 40% C (Kirk Carls, Nature Safe Natural and Organic Fertilizers, personal communication, 14 March 2015). Numerous studies have found that fertilizers that provide a C source in conjunction with N stimulate microbial activity and, subsequently, N loss (Limmer and Steele, 1982; Cavigelli *et al.*, 2009; Hayakawa *et al.*, 2009; Chantigny *et al.*, 2010; Sistani *et al.*, 2011; Mitchell *et al.*, 2013). N_2O-N emissions calculated for this study in the organic fertilizer treatment were similar to those calculated by Sistani et al. (2011) in a Kentucky no-till corn system applied with poultry litter (Table 4.2).

Gaseous fluxes also differed temporally between treatments (Hayakawa *et al*., 2009). As previously mentioned, NH_3-N volatilization of urea N subsided after the rain event on 4 June 2014, whereas this same event caused a spike in NH_3-N emissions in the organic N treatment. However, NH_3-N emissions in both fertilized treatments decreased after 12 June 2014. As urea hydrolyzes more readily than organic fertilizer sources, the soil in the urea N treatment was likely more enriched with N prior to the rain event on 4 June; thus, urea N_2O-N emissions were higher on 6 June 2014 than those from the organic fertilizer treatment as there was more inorganic N in the soil (Parkin and Hatfield, 2014), but, after this date, the slower N release nature of the organic fertilizer caused N_2O-N emissions from the organic fertilizer to remain higher than that of the urea treatment for the rest of the corn-growing season.

Despite differences in N loss, corn yield and N balance were statistically equivalent between fertilized treatments. Less N was exported in corn grain in the organic treatment, most likely due to increased gaseous loss (both NH_3-N and N_2O-N) and slightly greater N immobilization in cover crop residue between May and August in this treatment. My study illustrates that significant amounts of N can be lost to the environment in systems using both urea and organic fertilizers, but N loss pathways differ. It is difficult to suggest that one fertilizer treatment is more environmentally beneficial than the other as the organic fertilizer treatment had increased gaseous loss while the urea treatment had greater N leaching. A nitrification inhibitor may be advantageous in both systems as it could reduce the quantity of NO_3 vulnerable to leaching in the urea treatment and to denitrification in the organic fertilizer treatment. Understanding which fertilizer approach is more environmentally beneficial would require a full life cycle analysis of fertilizer production and corn grain N utilization, and will likely depend on how specific site factors, such as climate and soil conditions, impact N release, transformations, plant uptake and loss pathways (Skowronska and Filipek, 2014).

Table 4.1: Post-fertilization gaseous NH_3-N, gaseous N_2O-N, NO_3-N leachate, and NH_4-N leachate loss compared between fertilized and unfertilized treatments in the conventional and organic hairy vetch treatments. The N loss of fertilized treatments was compared to the respective, unfertilized control treatment in order to estimate: 1) the percent increase of leachate loss when fertilizer N was applied, and 2) the percentage of the added N that was diverted into gaseous loss pathways as calculated by (emissions from fertilized treatment - emissions from unfertilized treatment)/fertilizer N added.

Post-Fertilization: May 29 – November 1										
Treatments			**Gaseous Loss**				**Leachate**			
Field	**Cover Crop**	Fertilizer (kg N ha^{-1})	NH_3-N (kg N ha^{-1})	Percentage of fertilizer lost as NH_3-N	N_2O-N (kg N ha^{-1})	Percentage of fertilizer lost as N_2O-N	NO_3-N (ppm)	Percent NO_3-N increase with fertilizer	NH_4-N (ppm)	Percent NH_4-N increase with fertilizer
Organic										
	Vetch	0	8.71	-	2.13	-	217.36	-	22.12	-
	Vetch	154* (extra vetch + organic N)	12.81	2.66	3.11	0.64	249.41	14.74	50.20	126.97
	Vetch	164* (organic N)	39.09	18.52	4.47	1.43	307.42	41.43	24.55	10.99
Conventional										
	Wheat	0	-5.80	-	1.83	-	62.59	-	17.39	-
	Wheat	168 (urea N)	24.29	17.92	3.54	1.02	601.59	861.22	43.85	152.07
	Wheat	168 (organic N)	43.07	29.09	5.05	1.92	107.66	72.02	24.55	41.12

*Calculated by subtracting the N content of the hairy vetch cover crop in the unfertilized treatment (78 kg N ha^{-1}) from the total N added (cover crop N + fertilizer N) of each fertilized system (N-credit : 232 kg N ha^{-1}; Organic fertilizer: 242 kg N ha^{-1}).

Table 4.2: A comparison of N_2O and NH_3 emissions from conventional and no-till corn systems throughout the United States.

Author	Location	Tillage/Cover Crop	Crop	Fertilizer (kg N ha^{-1})	N_2O (kg N_2O-N ha^{-1} yr^{-1})	NH_3 (kg NH_3-N ha^{-1})
Shelton (this study)	Lexington, KY	No-Till, Hairy Vetch	Corn	0	2.92 (±0.27)	4.69 (±14.05) yr^{-1}
				N-credit	5.08 (±0.66)	35.47 (±10.01) yr^{-1}
				168 organic N	5.77 (±1.22)	46.58 (±3.79) yr^{-1}
		No-Till, Wheat		0	3.78 (±0.37)	7.87 (±8.82) yr^{-1}
				168 urea + Agrotain®	6.56 (±0.84)	61.08 (±1.00) yr^{-1}
				168 organic N	5.89 (±1.22)	34.87 (±8.59) yr^{-1}
Venterea et al. (2011)	Rosemount, MN	Conventional Tillage	Corn	146 urea	0.63	-
		No-Till		146 urea	0.75	-
Smith et al. (2013)	Urbana, IL	Conventional Tillage	Corn	168 UAN	7.7	-
Parkin and Kaspar (2006)	Ames, IA	Conventional Tillage (Chisel Plow)	Corn	13 starter N + 202 UAN (split application)	10.2 (±5.80)-11.3 (±3.73)	-
		No-Till			7.87 (±3.87)- 11.3 (±2.41)	-
		No-Till, Rye			7.62 (±2.17) -15.4 (±7.33)	-
Campbell et al. (2014)	Wooster, OH	Minimum Tillage (Chisel Plow)	Corn	202 UAN	~10.95	-
		No-Till for 50 years			~1.83	-
Sistani et al. (2011)	Bowling Green, KY	No-Till	Corn	168 Urea	1.70-3.31	-
				168 Poultry litter	5.81-10.85	-
Keller and Mengel (1986)	City, IN	No-Till, corn residue on soil surface	Corn	168 granular urea		50.9 in 120 hrs
				168 UAN		14.6 in 120 hrs
Thompson and Meisinger (2004)	Beltsville, MA	No-Till, 88% of soil surface covered with maize residue	No crop	137 cattle slurry	-	36 over an eight day period following slurry application
Powell et al. (2011)	Prairie du Sac, WI	No-Till	Corn	187 Dairy slurry, surface applied	-	55 after 120 hours

Chapter 5 : Conclusions

This thesis assessed the effects of cover crop species and fertilizer approach on N loss and dynamics in Kentucky corn conservation agriculture systems across an entire year. I found that dominant N loss pathways varied by season, and that both cover crop species and type of fertilization affected N loss and availability. During the cover crop growing season, NO_3-N leaching was a dominant loss pathway, especially in treatments using leguminous monocultures, but during the corn-growing season, N_2O-N and NH_3-N emissions became the dominant N loss pathways, increasing after cover crop termination, following fertilizer application, and in conjunction with rain events. Additionally, not only was gaseous N loss greater in fertilized treatments, but the type of N fertilizer (organic N vs. N-credit and urea N vs. organic N) also affected flux magnitude and temporal trends. These results suggest that specific management strategies can be employed within both organic and conventional conservation agriculture systems to reduce N loss.

Bi-culture cover crop systems warrant further investigation as this study indicates that they may be the most effective in preventing N loss while also contributing N to the summer crop. Experiments designed to examine other legume and grass species in bi-culture would help determine those with the most vigorous winter growth and greatest N contribution. Importantly, the effect of growing season fertilizer applications on N loss pathways should be examined in bi-culture cover crop systems, as these systems will require fertilizer to improve yield, but this was beyond the scope of my study.

My results support using an N-credit fertilization strategy with hairy vetch to minimize N loss while sustaining corn yields, but, because hairy vetch is not the only leguminous cover crop utilized in this region, research that explores N loss in other leguminous cover crop systems that employ an N-credit fertilization technique is needed (e.g. Crimson clover, Austrian Winter Pea). The results from the conventional system indicate that strategies to reduce N loss from organic and urea fertilizers require further investigation. These strategies may include the application of a nitrification inhibitor, the evaluation of different corn varieties' uptake and assimilation of NH_4-N and NO_3-N, or the assessment of fertilizer placement beneath cover crop residue rather than broadcast on top so that it is more readily incorporated into the soil profile.

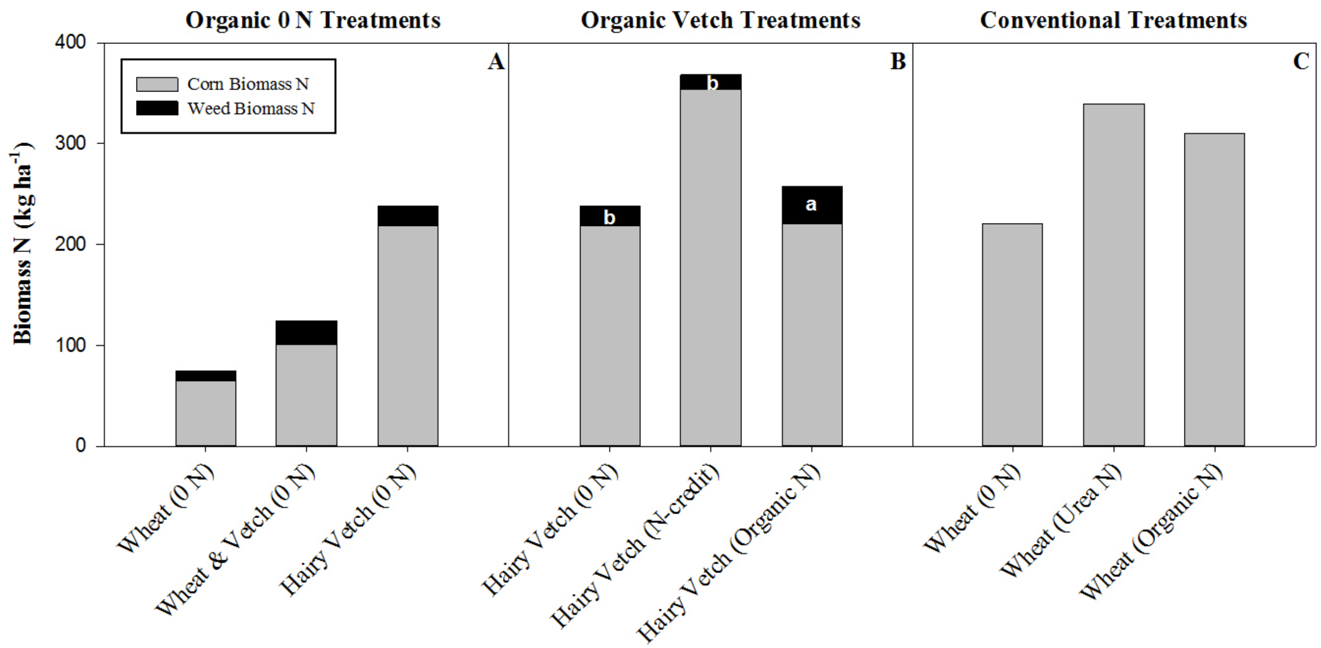
This study is unique as there is little research that simultaneously investigates NH_3-N and N_2O-N emissions in field studies, particularly in conservation agriculture systems or, in Kentucky (Table 4.2). This research may be used to help guide future agroecosystem management options and recommendations for existing conservation agriculture systems. Furthermore, they represent some of the first gaseous N loss data for organic, no-till systems. While this type of agriculture is not yet common in Kentucky, it is increasingly important across the nation (USDA-ERS, 2013a), and research illustrating how organic and conventional N loss pathways differ is needed to help improve N efficiency across agricultural sectors. More research is needed to determine cover crops that will provide both fertility benefits and weed control because ineffective weed management, in organic systems in particular, can prevent desired yields. Currently, cultivation in organic systems is crucial for weed suppression and prevents no-till implementation, but yields from this study suggest that, with further refinement, organic no-till systems may be achievable.

While the annual gaseous emissions measured in the organic and conventional corn systems in this study are well within the range of N_2O-N and NH_3-N emissions found within the literature for similar systems (Table 4.2), significant variability exists, which highlights the need for this type of research across many geographic regions as site-specific factors play a critical role in N loss. Although this research was an important first step in developing management strategies that reduce N loss, further investigation is needed because my data are limited to one site and one measurement year. It would have been interesting to follow my study through several cover crop growth cycles to assess how N dynamics develop with time in the various systems and to have implemented my treatments at additional field sites, because understanding how N loss is influenced by climate, soil texture, pH, cation exchange capacity, organic matter content, and aggregate stability will contribute to more informed regional recommendations (Harrison and Webb, 2001; Six *et al.*, 2002; Francisco *et al.*, 2011; Al-Kaisi *et al.*, 2014; Bandyopadhyay and Lal, 2014). Lastly, when considering the environmental impact of agriculture-associated N, it is critical that the N dynamics observed in a field study are not evaluated alone, but are contextualized within the global N cycle so that the origin and fate of N is understood.

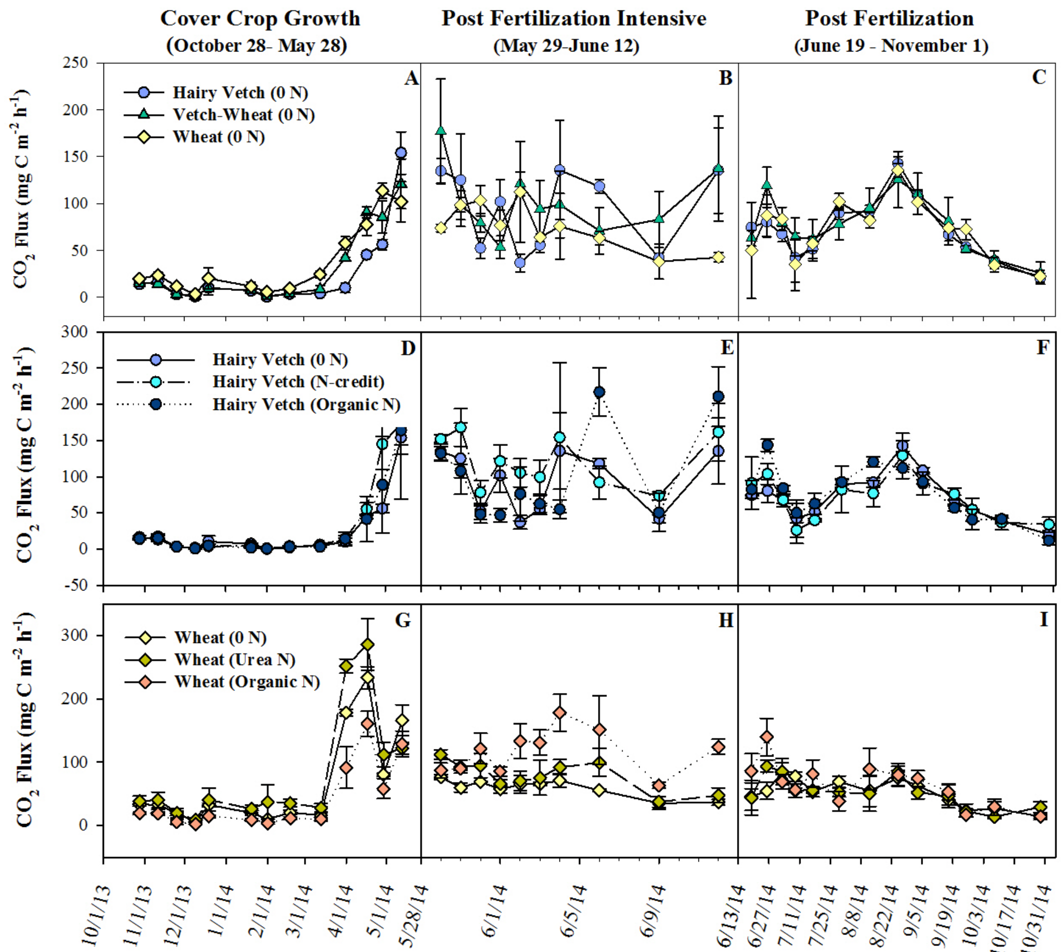
Appendix 1: Average (± SE) soil nutrients, texture, structure, and pH of the organic and conventional fields prior to treatment establishment.

Field	Soil-Water pH	P	K	Ca	Mg	Zn	Total N %	Total C %	Organic Matter %	Stable Aggregate Fraction %	Bulk Density (g cm^3)	Soil Texture		
		kg ha^{-1}										Sand %	Silt %	Clay %
Organic	6.84 (±0.06)	187.01 (±3.64)	553.08 (±12.08)	4580.07 (±127.13)	397.31 (±4.42)	6.05 (±0.44)	0.12 (±0.00)	1.25 (±0.01)	2.16 (± 0.02)	74.49 (±3.13)	1.35 (±0.02)	8.87 (± 0.11)	72.82 (±0.21)	18.31 (±0.17)
Conventional	6.33 (±0.57)	183.99 (±16.56)	570.10 (±51.78)	4292.61 (±409.13)	322.06 (±32.07)	4.6 (±0.54)	0.18 (±0.02)	1.79 (±0.17)	3.08 (±0.29)	81.81 (±2.67)	1.35 (±0.01)	10.66 (±0.94)	72.75 (±6.26)	16.59 (±1.57)

Appendix 2: Progression of cover crop growth in organic field plots: A = 1 December 2013;B = 1 April 2014;C = 29 April 2014; D =16 May 2014. Cover crop biomass accumulation occurred primarily within the last two months of growth (April and May).



Appendix 3: Total corn plant and weed biomass N content at harvest in the organic, unfertilized treatments (A), organic hairy vetch treatments (B), and conventional treatments (C). Lower case letters signify significant differences between treatments ($P < 0.05$). 0 N = no applied fertilizer, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.



Appendix 4: Average carbon dioxide fluxes (±SE) for organic 0 N treatments (A-C), organic hairy vetch treatments (D-F), and conventional treatments (G-I) across three different time periods from October 2013 to November 2014. 0 N = no applied fertilizer, N-credit= 56 kg ha^{-1} organic fertilizer + additional hairy vetch, Organic N = 168 kg ha^{-1} organic fertilizer, Urea N = 168 kg ha^{-1} urea + urease inhibitor.

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Published Abstracts
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Rebecca Shelton and Achim Häger. A comparison of aboveground carbon storage in agroforestry systems and in a secondary forest in Costa Rica. Poster presentation at the 2012 Geological Society of America Southeastern Section- 61st Annual Meeting. 1 April 2012 in Asheville, NC. Available online: https://gsa.confex.com/gsa/2012SE/finalprogram/abstract_202414.htm.