ABSTRACT

PARR, MARY CHRISTINE. Nitrogen Fixation and Mineralization Potential of Winter Annual Legume Cover Crops for Reduced-Tillage Organic Corn Production in North Carolina. (Under the direction of Dr. Julie Grossman).

Sixteen winter annual cover crop varieties were grown in North Carolina to determine biomass nitrogen (N) production and N fixation potential by termination dates compatible with a roller-crimper implement, and mineralization potential of residues following roll-kill. Cover crops were tested in a completely randomized block, with termination date as a strip plot, cover crop treatments included hairy vetch (*Vicia villosa*) cv. AU Merit, AU Early Cover, Winter Hardy Early Cover, and Purple Prosperity, common vetch (*Vicia sativa*) variety unstated, crimson clover (*Trifolium incarnatum)* cv. AU Sunrise, AU Robin, Dixie and Tibbee, Austrian winter pea (*Pisum sativum*) cv. Wistler and variety unstated, berseem clover (*Trifolium alexandrinum*) cv. Bigbee, subterranean clover (*Trifolium subterraneum*) cv. Denmark, and narrow leaf lupin *(Lupinus angustifolius*) cv. TifBlue78, and balansa clover (*Trifolium michelianum*) cv. Frontier as well as bicultures of rye (*Secale cereale)* and AU Merit, AU Early Cover, and Austrian winter pea. Termination occurred at in mid April, early May and mid May in 2009 and late April and mid to late May in 2010. Total biomass, N concentration, C:N ratios and ^{15}N natural abundance was determined for biomass at all roll times. Soil N and N flux was determined under rolled AU Early Cover and AU Sunrise with KCl extraction and ion exchange resins at two week intervals for a total of 12 weeks.

Hairy vetch and crimson clover had greatest overall biomass of monocultures. Mixtures had greatest biomass in 2010. Crimson clovers were easiest to roll kill in late April, Hairy vetches and Austrian winter peas didn’t roll kill until early - mid May, berseem clover and common vetch roll killed in late may. All cover crops derived between 70 and 100% of their atmosphere from the air, with the exceptions of lupin and subterranean clover. B-values for vetches and winter peas were significantly affected by nodulating rhizobium strains, with inoculant b-values giving the highest $\delta^{15}N$ values and piedmont b-values being the lowest. Corn response to cover crop mulches was significantly affected by roll time, with corn planting in mulches where kill was attempted too early suffering from competition and poor stands. Crimson, balansa and subterranean clover mulches resulted in poor corn yields despite relatively high levels of biomass N. In 2009, soil extract N and N flux from PRS probes was greater under hairy vetch biomass than under either 0N control or crimson clover, with peak soil N between 4 and 6 weeks after roll kill. Soil N under crimson clover mulches was lower in crimson clover than 0N, suggesting immobilization. In 2010, soil N and N flux was equal to 0N for all cover crop mulch treatments at Caswell. In piedmont, there was considerable variability, with hairy vetch having the greatest overall soil N of the cover crop treatments but this was equal to or lower than the 0N for most of the season. Corn yields were greatest in hairy vetch and 150N treatments in 2009 and in the150N and hv+rye treatment in 2010. Yields in crimson clover were routinely less than or equal to that of 0N. N flux as measured by PRS probes showed decreases during times of low rainfall, whereas soil extract N increased during these periods, indicating that while mineral N was in the soil, dry conditions affected nutrient movement and therefore affected uptake by the ion resin PRS probes.

Nitrogen Fixation and Mineralization Potential of Winter Annual Legume Cover Crops for Reduced-Tillage Organic Corn Production in North Carolina.

by
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DEDICATION

To Clyde L Parr, my grandfather, a surveyor and geologist, and Bobbie Steninger: two people those who inspired me to love nature and the earth, believe in my curiosity, and approach life with perseverance and wonder.

BIOGRAPHY

Mary Parr grew up in Eastern Oregon where she cultivated a love for the outdoors. She completed her undergraduate degree at Fairfield University in Connecticut where she studied French and theatre. Following graduation, she spent 2 years in the Jesuit Volunteer Corps working as a community organizer and a GED teacher to court involved youth. During this time, she cultivated an interest in science and agriculture. Upon completion of her Master's of Science, Mary will continue at NC State University where she will study soybean rhizobia diversity on Malawian smallholder farms. She hopes to combine her love of science with her desire to pursue social justice in the world.

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INTRODUCTION

Organic agriculture continues to grow vigorously, both in quantity of sales and number of farmers converting to organic practices. Farmers choose to adopt organic farming practices for both economic as well as environmental concerns (Khaledi et al., 2010; Stofferahn, 2009). Conventional agricultural practices have long been cited as leading causes of many environmental problems. Increased nitrogen (N) inputs in the form of synthetic fertilizer have resulted in increased concentrations of N_2O in the atmosphere, contributed to acidification of soils and surface waters, and increased N concentrations in both fresh and salt waters, leading to eutrification and loss of marine life (Vitousek et al., 1997). Tillage is linked to soil losses in the form of wind and water erosion, and pesticide and fertilizer use to the contamination of water sources. However, developing sustainable agricultural systems is a difficult challenge as the rejection of one environmentally damaging practice often means increased reliance on another. Organic producers who do not use synthetic fertilizers and pesticides are often reliant on tillage via incorporation of manure and legumes for nutrient additions and cultivation for weed management. On the other hand, no-till systems, which have been shown to benefit soil physical properties and conserve soil organic matter (Aase and Pikul, 1995) rely heavily on herbicides and synthetic fertilizers for weed control and nutrient management. Developing innovative systems that allow farmers – both organic and conventional - to reduce dependence on practices that are environmentally, energetically, and financially costly, is an important step to develop a more sustainable agricultural sector. Of particular interest in current research is to investigate ways to integrate the benefits of a low external input organic system with one that reduces tillage.

ORGANIC AGRICULTURE

Sales and production of organic agriculture in the United States has grown considerably over the last several years, and now represent a considerable proportion of the food industry. Nationwide, organic products now represent a $26.6 billion industry. In 2009, organic food sales grew by 5.1%, while total food sales only grew by 1.6% (Organic Trade Association). In North Carolina, organic agriculture still comprises a very small sector of the total agricultural land, however it is growing rapidly. Between 2006 and 2008, the total organically certified acreage has more than tripled in the state and the number of certified farms had nearly doubled from 86 to 156 (Green and Slattery, 2010). This trend is expected to continue, as demand for local organic product grows.

As in all agricultural systems, organic farmers must manage limitations in order to produce economically viable yields. However, in order to maintain organic certification, they must also follow the National Organic Program Practice Standards, which specifically limit the use of synthetic off farm inputs, such as pesticides and fertilizers (Bellow, 2005), leading to reduced yields relative to conventional systems. A long term study comparing various organic rotation systems to conventional systems found that corn and soybean yields, but not wheat were reduced in organic systems. 70 – 75% of this yield reduction was explained by low N availability, 21 – 25% by weed competition and 3 – 5% by plant population. The study also suggests that in drought years, N mineralization from organic sources is significantly reduced (Cavigelli et al., 2008).

In general, the most limiting nutrient in organic systems is nitrogen (Gaskell and Smith, 2007; Rosen and Allan, 2007). Sources of N available to organic producers include animal manures, compost (of either manures or other organic residues), commercial sources (such as feather meal, soybean meal, blood meal, fish emulsions etc.), and legume cover crops. Animal manures are useful for a variety of reasons: they contain a wide range of essential nutrients beyond N, much of the N is readily available, and they also provide organic matter and liming properties to the soil. However, because the N:P (phosphorous) ratios in manures are lower than what a crop would normally need, extensive use of manures can cause P loading on soils (Hepperly et al., 2009), contributing to eutrification of fresh waters (Drinkwater and Snapp, 2007). Manures can also contain high levels of heavy metals and arsenic, leading to soil contamination and toxicity (Jackson et al., 2003). Fresh manure provides challenges in that it can contain harmful pathogens. Commercial N sources are formulated to have a more balanced nutrient content than manures, but tend to be prohibitively expensive for use on a large scale (Gaskell and Smith, 2007).

Other challenges faced by organic producers include weed management and pest pressure. Weeds are often managed by cultivation, which can lead to degredation of soil physical properties, loss of soil OM and erosion (Rosen and Allan, 2007). The NOP Soil

Fertility and Crop Nutrient Management Practice Standard, §205.203, defines general management and environmental objectives that producers must work towards. These objectives include: using tillage and cultivation practices that improve soil physical, chemical and biological properties and reduces soil erosion; managing crop nutrients through rotations, cover crops and plant and animal residues; and managing these residues in a way that increases soil organic matter while reducing chances of hazardous biological or chemical contamination (Bellow, 2005).

LEGUME COVER CROPS

Use and Management

Cover crop use is specifically mentioned in the NOP standard because cover crops not only maintain soil structure and prevent erosion, but also can contribute substantially to soil fertility. The use of legume cover crops, specifically is an established and historical practice to increase soil N through biological N fixation (BNF) (Baldock et al., 1981; Harlan, 1899; Kramberger et al., 2009; Lyon and Wilson, 1928). Biological N fixation is defined as the conversion, by either free living or symbiotic microbes, of atmospheric N_2 gas into a plant available form (NH_4^+). Legumes are involved in BNF through their symbiotic association with rhizobia, soil bacteria that form root nodules in legumes and symbiotically fix nitrogen. Managed well, legume cover crops can provide most if not all of the nitrogen needs for an organic farm (Baldwin and Creamer, 2006). Benefits of cover crops include N fixation, (lowering fertilizer needs) increased OM, erosion control, and moisture control (Baldock et al., 1981; Torbert et al., 1996). Historically, N from legume cover crops was not the best economic option in conventional systems (Hoyte et al., 2004), however, as a result of recent volatility in energy and nitrogen prices, conventional farmers are increasingly interested in using cover crops to add N to their soils (Baldwin and Creamer, 2006).

The species and use of a cover crop can vary according to the needs of a particular farm or crop as well as by region. Most commonly, legume cover crops are planted during a fallow season to provide N to a cash crop during the growing season (Sustainable Agriculture Network, 1998). In North Carolina, winter annual legumes are usually planted in early to mid October after a corn crop is removed and killed in late March or early April before planting (Hoyte et al., 2004; Wagger, 1989b). Other uses of legume cover crops include interseeding into a standing crop such as corn, summer legume fallow, and ground cover in orchards or vineyards (Bair et al., 2008; Baributsa et al., 2008). Winter annual legume cover crops best adapted to NC soils and climates are crimson clover, hairy vetch, Austrian winter pea and Cahaba white vetch (Hoyt et al., 2004).

Cover crop termination can be done either chemically, with herbicide, or mechanically, via tilling, undercutting, mowing or rolling. Legumes are shown to have a higher N concentrations during vegetative stages, but will produce more biomass and more total N at flowering (Anugroho et al., 2009; Teasdale et al., 2004); mechanical termination is also more effective at full flower (Mirsky et al., 2009; Mischler et al., 2010). Early flowering varieties if hairy vetch have recently been developed at Auburn University that could allow farmers more flexibility in timing cover crop kill and planting dates (Teasdale et al., 2004).

Managing legumes for maximum N fixation

The estimated amount of nitrogen that cover crops add to a system is variable, and depends on the species of plant and infecting rhizobia, inoculation rate, soil mineral N availability, and biomass accumulation (Amato et al., 2007; Armstrong et al., 1997; Wagger, 1989a). According to Drinkwater (2006), grain legumes can fix up to 200 kg ha^{-1} a year, but much if not all is taken out of the field when the grain is harvested. Green manure legumes are reported to fix up to 300 kg $ha^{-1}yr^{-1}$. A review by Unkovich and Pate (2000), estimates that N_2 fixation for a variety of common crop legumes ranges from 0 to 450 kg ha^{-1}, with estimates for temperate regions between 50 to 200 kg ha^{-1} yr^{-1} (Fageria et al., 2005). Most estimates of nitrogen are based on accumulation in above ground dry matter. Below ground N could almost double this number, depending on root to shoot ratio of specific species (Drinkwater et al., 2008).

Soil nitrate levels have a direct impact on the proportion of nitrogen legumes will derive from the atmosphere. As biological N fixation requires energy, legumes will tend to fix less nitrogen if they have a more readily available N source and will acquire more nitrogen from the atmosphere where the soil nitrate levels are low (Schipanski et al., 2010). A study on irrigated soybean, comparing pre-fallowed soil with soil previously cropped with oats found that both proportion of N derived from the atmosphere and total N accumulated by soybean was greater in pre-cropped soil (Bergersen et al., 1989). Other strategies to reduce soil nitrate levels include double cropping a legume cover crop after a non-legume, using bicultures of legumes and non-legumes (Sainju et al., 2006), and reducing tillage (Herridge and Holland, 1984).

Legumes can differ in their reliance on BNF based on species and even variety within the same species, even when plants accumulate the same total amount of nitrogen. Choosing varieties that are more reliant on BNF will result in overall greater inputs of nitrogen by fixation. Variation can be a result of a legumes sensitivity to nitrate levels (Herridge et al., 1990b), or nutritional and drought stress (Sinclair et al., 2000). In crops with similar nitrogen concentrations, the total kg N fixed contributing to a system is a direct result of the amount of biomass produced. This is often a result of the species and variety selected, planting date, harvest date and weather during the growing season (Peoples et al., 1995; Ranells and Wagger, 1996).

Rhizobia and inoculation

In order for a legume cover crop to effectively add nitrogen to an organic system, they must have rhizobia partners. While native rhizobia populations exist throughout most soils, it is difficult to predict whether there will be significant numbers capable of nodulating a particular legume crop. The issue is further complicated in that while, many hosts can be nodulated by multiple types or strains of rhizobia, and many rhizobia are capable of nodulating multiple host plants (Young and Haukka, 1996), the capacity for nitrogen fixation for differing strains of rhizobia varies. For agricultural use, commercial inoculants tend to contain only a few strains of rhizobia that have been shown to nodulate a particular species and provide adequate nitrogen in greenhouse settings. However, inoculant performance can vary according to competitiveness of indigenous populations and the encountered soil environmental conditions (Materon and Hagedorn, 1982; Thies et al., 1991). Strains of rhizobia have differential competitiveness in their ability to form nodules as well in their saprophytic ability. Laguerre et al (2003) found that while the richness of the combined genotypes detected in nodules of fava (*Vicia faba* L.) and pea (*Pisum sativa* L.) was as high as that in bulk soils, the dominant genotypes in nodules were not necessarily dominant in the bulk soils (Laguerre et al., 2003). This study also found that host species has an affect on the diversity of rhizobia, with diversity being higher in the vetch (*Vicia sativa* L.) nodulating populations than in that of fava or pea drawing from the same soils.

Cropping history and past inoculation can have a significant impact on rhizobial diversity in a soil. In a study of peanut nodulating rhizobium in Cameroon, the highest diversity was found in sites with no history of peanut cultivation, suggesting that the introduction of a legume is capable of promoting particular rhizobium taxa (Nkot et al., 2008). However, In Brazilian soils, genetic diversity of soybean rhizobia (*Bradyrhizobium japonicum*) was found to be great after 18 years of cropping where only 4 strains of bacteria had been introduced. As these soils had previously had no native rhizobia capable of nodulating soybean, this suggests rapid evolution in the harsh Cerrado oxisols (Loureiro et al., 2007). In general, using commercial inoculants can provide enough rhizobia to effectively nodulate a cover crop. The amount of N accumulated in the soybeans has been shown to correlate with inoculation rate of appropriate rhizobia (Bergersen et al., 1989).

N-Fixation assessment:

There are many different ways to assess N_2 fixation in nodulated legumes in the field. The array of methods can be divided into procedures that asses nodule or enzyme activity, such as nodule observation and acetylene reduction assays, and procedures that quantify the total amount of nitrogen fixed at the field scale, such as the total N difference method, and ^{15}N methods including Isotope Dilution and Natural Abundance (Hardarson and Danso, 1993; Herridge et al., 1990a; Unkovich and Pate, 2000).

<u>*Nodule observation:*</u>

The weight and number of nodules on a legume is positively correlated with the amount of N_2 that a legume fixes (Vincent, 1970). However, while this data can be used to compare competitiveness of rhizobial strains, or the ability of a plant to form nodules, it does not give an amount or proportion of N fixation. In addition, nodules formed by inefficient rizobia may erroneously lead one to believe that nitrogen fixation is occurring where it is not (Hardarson and Danso, 1993).

<u>*Acetelyne Reduction Assay:*</u>

In the acetylene reduction assay, acetylene gas is used as an analog for N_2. Nodulated roots are incubated in acetylene gas and the ethylene produced after a specific time is measured. This method can provide a point measurement for nitrogenase activity but does not provide an accurate assessment of the actual N_2 fixation that would occur in the field (Hardarson and Danso, 1993; Unkovich and Pate, 2000).

<u>*Ureide abundance technique:*</u>

Nitrogen exported from actively fixing nodules is exported in the form of ureides, whereas N assimilated from soil ammonium or nitrate typically is exported in the form of amides. The ureide abundance technique estimates BNF based on the proportion of ureides to amides in the ascending xylem stream of an actively growing legume

(Herridge et al., 1990a; Sinclair et al., 2000). This technique can be applied in natural systems as well as in field experiments, but only accounts for the N fixation activity at the time of sampling, and one cannot extrapolate this to the total N fixing activity over the course of a season (Unkovich and Pate, 2000).

Total N Difference method:

The N difference method quantifies BNF in legumes by comparing N concentrations and total biomass N in non-fixing reference species and the legume in question. Although simple to calculate, this method assumes that a fixing and non-fixing species will absorb the same amount of N from the soil, and that any additional N in the legume will have come from the atmosphere. Erroneous estimations are made when non-fixing references are used that have different rooting architecture, and are therefore able to better exploit soil N (Hardarson and Danso, 1993) .

^{15}N Isotope Dilution Method:

In the isotope dilution (ID) technique, ^{15}N enriched fertilizer is applied to the soil of both a fixing legume and non-fixing reference species. The % N derived from the atmosphere (Ndfa) is the determined by analyzing plant material for dilution of the ^{15}N isotope by atmospheric ^{14}N in the legume relative to the reference species (Peoples and Herridge, 1990; Unkovich and Pate, 2000). The ID technique is widely used, but has limitations. First, the cost of labeled fertilizers make it prohibitive to use on a large scale, also such fertilizers cannot be used in an organic system. Secondly, the ^{15}N from applied fertilizer is not uniformly distributed throughout the rooting volume, and declines in the soil mineral N pool over time. Different rooting patterns and growth cycles can have an effect on the isotope dilution of the legumes and reference species, resulting in erroneous data (Mueller and Thorup-Kristensen, 2001; Shearer and Kohl, 1986). Doughton, et al. (1995) found that at low % Ndfa resulting from a large NO_3-N mineral pool in the soil, isotope dilution data tends to be unreliable (Doughton et al., 1995).

15N Natural Abundance Method:

The natural abundance (NA) method uses the natural difference in the atmospheric ^{15}N concentrations to that of the soil to determine the % N derived from the atmosphere (0.3663% ^{15}N) verses the soil. This small difference is the result of microbial preference for ^{14}N in biological process of denitrification, returning more ^{14}N to the atmosphere while ^{15}N accumulates in soil. Detecting differences in these isotope ratios requires very precise mass spectrometry techniques, but the advantages are substantial (Bergersen et al., 1989; Hardarson and Danso, 1993; Peoples and Herridge, 1990; Shearer and Kohl, 1986). Like the ID method, the NA method can provide an integrated measurement of BNF over time, but as it does not require the use of ^{15}N enriched fertilizers, isotope dilution over time is not a factor, and the method can be applied to organic production systems as well be used to measure N fixation in natural ecosystems. And, while the ID method tends to have a larger standard deviation at higher levels of BNF, the natural abundance method provides a realistic estimate of N derived from the atmosphere.

The calculation for the NA method is based on a value known as $\delta^{15}N$ which is the proportion of difference between the atmospheric ^{15}N concentration and the ^{15}N concentration being measured. A $\delta^{15}N$ value of 0 would indicate an identical ^{15}N

concentration in the plant matter as in the atmosphere. Using $\delta^{15}N$ values, the %Ndfa can be calculated using the following equation:

$$p = \frac{(x-y)}{(x-b)} \times 100$$

Here, p is the proportion of N derived from N_2 fixation, x is the $\delta^{15}N$ value for a non-fixing reference species, y is the $\delta^{15}N$ value of the legume grown in the field, and b is the $\delta^{15}N$ value of the legume grown in an N-free medium, deriving all N from BNF (Shearer and Kohl, 1986). The choice of a non-fixing reference species in the NA method is important in determining the $\delta^{15}N$ value of a species grown without N fixation. Pate et al (1994) showed that not only is the species important, as some species discriminate in the isotopic ratio of their root uptake, but also the proximity of the reference to the fixing legume is important. Barley and ryegrass grown with legumes were shown to have lower $\delta^{15}N$ values than those grown outside a legume's rooting boundary (Pate et al., 1994). The cause of this is thought to be related to mycorrhizal transfer of N between species. Weed species *Sida spinosa* and *Senna obtusifolia*, arbuscular mycorrhizae hosts, have been shown to aquire as much as 80% of their N through transfer from legumes, when grown in close proximity, whereas N transfer in non-hosts, *Cyperus esculentus* and *Amarantus palmeri* is minimal (Moyer-Henry et al., 2006).

NO-TILL SYSTEMS

Reducing tillage in agriculture has been shown to have numerous environmental benefits including conservation of soil carbon through reduced respiration, improved infiltration, and decreased bulk density (Aase and Pikul, 1995; Blevins et al., 1983;

Golabi et al., 1995). No-till systems have also been shown to have improved soil structure, and suppressed weed development (Zougmore et al., 2006).

Reduced and no-till production systems rely on herbicides for weed and cover crop management where organic systems depend on tillage. Because of weed pressure it remains difficult to manage a no-till organic system. A study comparing no-till and organic systems found weed populations in the organic system to be manageable when using a disc and chisel plow prior to planting and cultivation after, but after eliminating pre-plant tillage after five years, weed competition in corn became unacceptable (Teasdale et al., 2007). Many of the benefits accrued in organic management, including organic matter inputs, and increased soil biological activity are lost through the increased tillage necessary to manage weeks. Comparisons between untilled pastures and organic production systems have found that organic matter increases when legumes and manure are used in organic systems but that tillage in these systems can result in lower soil organic matter content, increased mineralization, reduced earthworm activity and aggregation than in pastures receiving no tillage (Pulleman et al., 2003).

Roller Crimper

No-till weed management, however may be possible in organic systems with the use of a tractor mounted implement called a roller-crimper (Davis, 2010). The roller crimper is designed to mechanically kill a cover crop by rolling it down and crimping the stems while leaving the plant attached to the ground (Ashford and Reeves, 2003). Advantages of this system are that it allows for cover crop termination without disturbing the soil, making a no till organic system possible. A recent study in Pennsylvania has indicated that a hairy vetch cover crop is susceptible to the roller-crimper, and that the resulting mulch does provide weed control for a subsequent cover crop. However, the effectiveness of the roller-crimper at terminating the cover crop does depend on age and flowering stage of the hairy vetch. In cases where the hairy vetch was not killed by the roller-crimper, it reduced corn stand count and yields (Mirsky et al., 2009).

DECOMPOSITION AND SOIL N CYCLING

Soil N cycling

Unlike fertilizers or soluble N sources, organic nutrient sources, such as animal manures and cover crops rely on microbial processes in order for the N and other nutrients to be available to a crop. In contrast to conventional systems, where inorganic fertilizer N sources are applied based on the needs of a specific crop, organic systems aim to apply an ecological framework, wherein soil nutrient reserves, or "pools" are maintained while balancing additions and exports of nutrients as much as possible. These nutrient pools include all inorganic and organic forms of N and P with both long and short mean residence times. Availability of these nutrients is based on plant and microbial assimilations and mineralization of N, P and C cycling and storage (Drinkwater et al., 2008; Drinkwater and Snapp, 2007; Gaskell and Smith, 2007). Figure 1-1 shows a schematic of N pools and transformations (or *fluxes*) as would exist in a legume based organic cropping system. Note that in order for this system to be in balance, N input by BNF would have to equal harvested export of the cash crop, but that N availability to the cash crop is a result of soil N transformations between organic matter, microbial biomass and mineral N.

Plant available N

Due to the dynamic nature of soil N pools, plant available N quantification resulting from cover crop residues can be problematic. Various measurement methods have attempted to quantify N release from residues, resulting in widely varying results. Litter bag studies that measure N remaining in residues at various times following termination have found that N is released within 4 weeks of termination (Ranells and Wagger, 1996). However, tracer studies using 15N labeled organic matter found that only 10 – 30% of this nitrogen was recovered by the subsequent crop, despite the fact that these crops achieved yields comparable to fertilized crops (Wagger et al., 1985). Crews (2004) suggests that organic sources of N are subject to 'pool substitution,' meaning that the nutrients in the fresh residues are being immobilized, and at the same time N is being mineralized from the microbial biomass pool. This results in a slower more constant nutrient source from cover crops than from inorganic fertilizers (Crews and Peoples, 2005). Still other researchers have concentrated on soil N, and have attempted to quantify N pools as they change throughout the decomposition of residues. Bair et al (2008) measured both soil extract N as well as N flux on ion exchange resins to track N release from hairy vetch and sweet clover in Concord grape vineyards, and found these measurements to be well correlated with each other.

Nitrogen Mineralization

Timing of N mineralization to coincide with crop needs is critical, and can be altered through residue management choices. In organic production, cover crop termination is mechanical, often involving a combination of methods including tilling, mowing, undercutting and roll-killing. Undercutting and roll killing leave the residues whole and attached to roots, which results in slower decomposition and potential weed suppression from the resulting mulch (Davis, 2010). Mowing or chopping residues increases surface area, which is known to increase microbial decomposition, and reduce the overall biomass bulk (Snapp and Borden, 2005; Swift et al., 1979). Residue soil contact is also a critical factor affecting decomposition and N mineralization. While surface residues can conserve soil water and prevent evaporation from soil surface before canopy closer is achieved (Baldwin and Creamer, 2006), the exposed nature of surface mulches has been shown to slow decomposition as result of reduced water content in the residue. In as little as 3 – 5 days following a rain event, the water limitation coefficient dropped to zero for surface applied rye and oilseed rape residues, but remained high for incorporated residues (Coppens et al., 2007).

Cover crop tissue quality also affects N mineralization rates and varies with species. Indicators of decomposition rates include C:N ratios, cellulose, hemicelluloses, lignin and phenolic content. Ratios of C:N below 25 are assumed to release N soon after termination, but N mineralization slows with increasing hemicelluloses, lignin and phenolic contents regardless of C:N ratio (Palm and Sanchez, 1990). A decomposition study on hairy vetch, crimson clover and rye bicultures showed that hairy vetch residue, having the lowest hemicellulose concentrations and a C:N ratio of 11, released nitrogen faster than other residues. More than 50% of the nitrogen in hairy vetch residue mineralized in the first four weeks following chemical desiccation. Whereas rye residue with high cellulose and hemicellulose content and a C:N ratio of 40 released nitrogen much more slowly. Bicultures of rye + hairy vetch and rye + crimson clover had intermediate release rates (Ranells and Wagger, 1996).

CONCLUSIONS

Organic production systems are the primary income generator for many farmers (Khaledi et al., 2010). Because the number of variables influencing yields in organic systems is greater than in conventional systems, the inherent risk to producers is greater (Cavigelli et al., 2008; Ott and Hargrove, 1989; Teasdale et al., 2007). However, organic systems have been shown to yield as well as conventional systems under optimal circumstances. Treadwell and Creamer showed that organically managed sweet potatoes under various management systems (cover crop, cover crop reduced till and organic, no cover crop) produced similar yields to conventionally managed crops and that under most of these systems nutrients were not limiting when adequate moisture was available. However, when early spring moisture was low, N was limited in reduced tillage systems due to slow mineralization of organic N.

Farmer decisions on cover crop use and residue management strategies must coincide with the needs of the cash crop, and physiological characteristics of the cover crop. For example, roll killing and mowing some cover crops is only effective when the plant is at full maturity (Ashford and Reeves, 2003; Kornecki et al., 2009). If a North Carolina farmer wishes to plant a crop in early April, a later maturing vetch would fail to die and could become a weed in the subsequent crop. Corn is known to yield better when planted earlier (Heiniger et al., 2000), but an early incorporation of a cover crop limit the amount of biomass a cover could accumulate and therefore the nitrogen delivered (Anugroho et al., 2009; Wagger, 1989b).

Continued research in organic agriculture is necessary in order to improve our understanding of soil N dynamics, the use of cover crops as mulches, and how to improve the overall N supply capacity of soils in organic systems. In doing so, we would not only improve the production capacity of organic systems, but we would develop and understanding of how to how to intensify agriculture in a way that would ensure long-term environmental sustainability.

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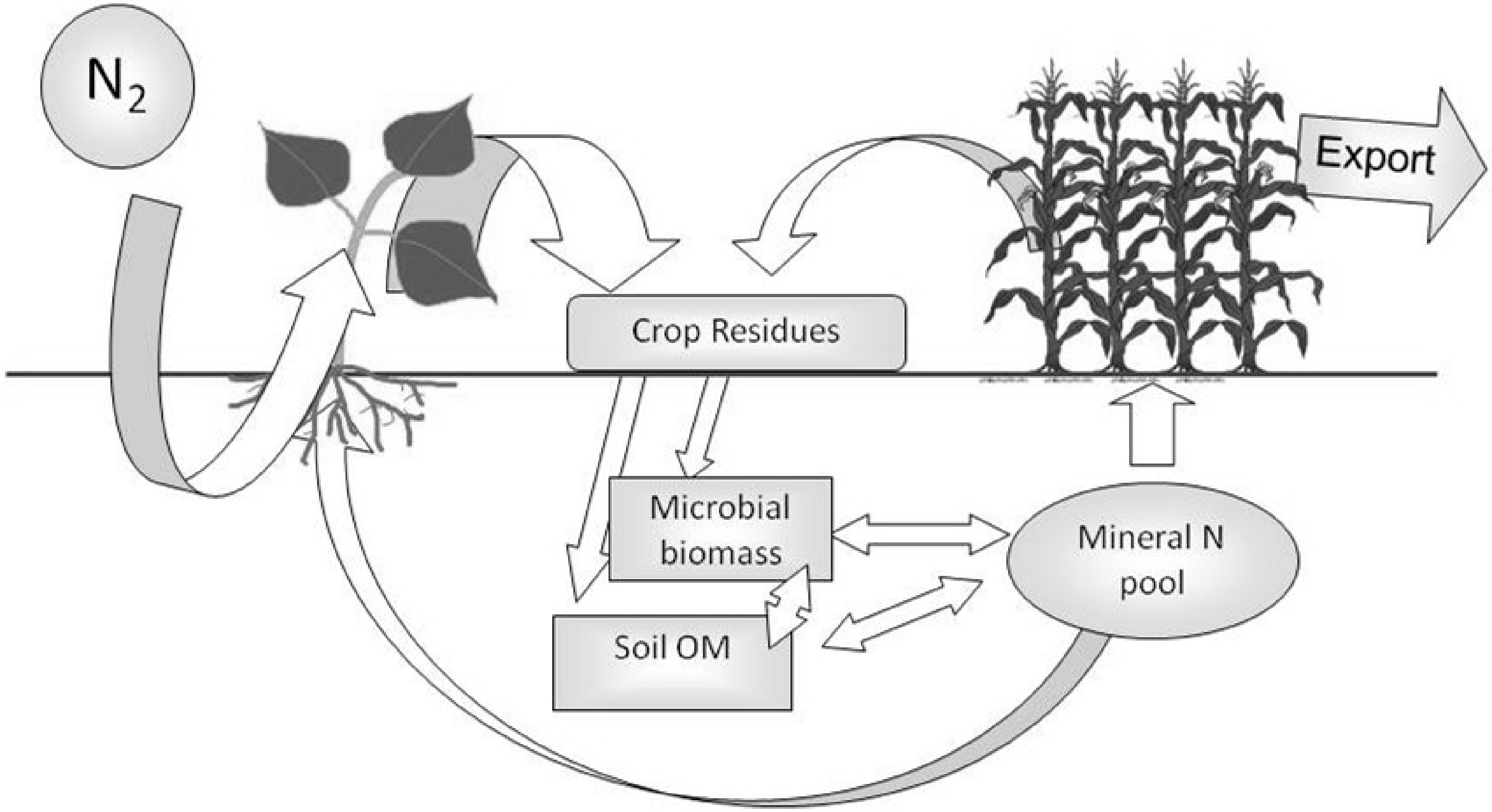

Figure 1-1: Nitrogen transformations in a legume based organic cropping system.

Chapter 2: **Nitrogen fixation and compatibility of winter annual legume cover crops for no-till organic corn production in North Carolina**

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INTRODUCTION

Efficient cover crop management is a vital component of organic production systems. Cover crops have been shown to increase organic matter, reduce erosion and provide moisture control (Baldock et al., 1981; Baldwin and Creamer, 2006; Torbert et al., 1996). Moreover, their use is required in order to maintain organic certification (NOP Standard, USDA). Organic production in North Carolina has increased exponentially in the past decade, with predictions for continued growth in the future (Green and Slattery, 2010). Nitrogen (N) management in organic production is often a costly and uncertain practice. Inputs such as manures, composts, and commercial products derived from various organic sources can be difficult to manage as they often have low available N contents and can provide nutrients in excess of crop needs in the form of phosphorous (P) and other micronutrients (Gaskell and Smith, 2007). The use of legume cover crops to fix atmospheric nitrogen through biological N fixation (BNF) is an established and highly beneficial farming practice (Fageria et al., 2005). Nitrogen benefit to a succeeding cash crop from a leguminous cover crop varies with species, variety, climate, soil N availability, biomass accumulation, plant chemistry and residue management (Bergersen et al., 1989; Peoples et al., 1995; Sarrantonio and Scott, 1988; Wagger, 1989a).

Estimates of N contribution from legume cover crops vary widely, from 0 to more than 300 kg N ha^{-1}, with the majority of estimates falling between 50 and 200 kg N ha^{-1} in the crop residues, and between 5 and 95% of that N derived from the atmosphere (Ndfa) (Drinkwater et al., 2008; Fageria et al., 2005; Herridge et al., 1990). In North Carolina, much research has been conducted on hairy vetch (*Vicia villosa* Roth) and crimson clover (*Trifolium incarnatum* L.) legume cover crops, with total crop N estimated to be between 100 and 230 kg N ha^{-1} for hairy vetch and 100 and 150 kg N ha^{-1} for crimson clover (Wagger, 1989b).

Many organic farmers incorporate green manures into the soil two or three weeks before planting a cash crop (Baldwin and Creamer, 2006; Hoyt et al., 2004). However, emphasis on reducing tillage and the accompanying negative impacts on soil health has stimulated interest in alternative cover crop management systems and implements. The roller-crimper is a tractor-mounted implement with a water-filled rolling drum that mechanically terminates a cover crop by crimping the stems while leaving the root system and soil undisturbed. Studies on the effectiveness of the roller crimper for managing cereal cover crops such as rye (*Secale cereale*) indicate that effectiveness of roll kill is related to the growth stage of the cover crop, and that roll kill timing for optimal kill corresponds to anthesis (Ashford and Reeves, 2003; Mirsky et al., 2009). Few studies exist evaluating the roller crimper for legume cover crop management in organic systems. The scant literature and anecdotal information that does exist suggest that hairy vetch is best roll-killed at early pod set or later, (Mischler et al., 2010), however nothing is known about its effectiveness in terminating other legume cover crop species.

There are many challenges surrounding the match of winter annual cover crop species with preferred spring planting dates in North Carolina. For optimum yield, corn in North Carolina is usually planted before early April in order to avoid overlap of drought sensitive growth stages with an historical dry period in the end of June (Heiniger et al., 2000). This early planting date is known to prevent most winter annual cover crops from reaching peak biomass production by desired termination time, especially when cover crops are incorporated many weeks prior to planting. New and early flowering cultivars of winter annual legumes have been developed over the past 15 years that could potentially reach maturity early enough in the season to compliment the North Carolina production schedule. The Southern-adapted hairy vetch varieties AU Merit and AU Early Cover have been shown to reach 50% flowering 15 days earlier than common vetch (Teasdale et al., 2004), and crimson clover varieties AU Sunrise and AU Robin are shown to flower earlier than the traditionally used varieties of Tibbee or Dixie (Mosjidis and Owsley, 2002; Teasdale et al., 2004). Other legume cover crops have not been extensively studied in North Carolina, but could be potentially useful for organic farmers. Subterranean and balansa clover, (*Trifolium subterraneum* L. and *Trifolium michelianum* Savi.) are forage legumes used extensively in Australian pasture systems and are known to fix between 30 and 70 kg N ha^{-1} and 70 and 150 kg N ha^{-1}, respectively (Nichols et al., 2007; Ovalle et al., 2006; Rochester and Peoples, 2005). Berseem clover (*Trifolium alexandrinum* L.*)* is a forage legume with potential allelpathic properties, but whose performance in the Southeastern US is not well studied (Maighany et al., 2007).

Few studies have examined the BNF potential of these legumes. Rochester et al (2005) found that in an Australian climate, berseem clover, crimson clover and hairy vetch varieties had derived between 85 and 90% N through BNF. Other studies conducted in Europe have shown BNF accounts for less than 30% of the N in hairy vetch and crimson clover biomass (Mueller and Thorup-Kristensen, 2001). Ovalle et al (2006) found that subterranean clover fixed between 30 and 90 kg N ha^{-1}. However, no studies investigating N fixation by leguminous cover crops has been conducted in the southeastern US.

The ^{15}N natural abundance technique is an established method to measure symbiotic N fixation of leguminous plants by exploiting the naturally occurring difference between $^{15}N/^{14}N$ concentrations in the soil and atmosphere (Bremer and Kessel, 1990; Kerley and Jarvis, 1999; Unkovich et al., 2008). It is preferred in experiments where total quantity of N derived from the atmosphere through BNF is desired, because unlike other BNF measurement methods that, the natural abundance method provides an integrated measurement of N fixation from germination to sampling, and does not require the use of ^{15}N enriched fertilizers (Unkovich and Pate, 2000).

We hypothesized that high %Ndfa and total N in cover crops that are sensitive to termination by the roller-crimper will be associated with high grain yields in a succeeding corn crop. We tested this hypothesis by: (i) measuring biomass production and total nitrogen accumulation at three potential kill dates, (ii) determining %Ndfa at the optimal kill date, and (iii) identifying roller-crimper compatible legume species for North Carolina corn production systems.

METHODS:

Site Descriptions

In 2008/2009 the study was conducted at two sites: Tidewater Research Station in Plymouth, NC on a Portsmouth soil (Fine-loamy over sandy or sandy-skeletal, mixed, semiactive, thermic Typic Umbraquults), and at Piedmont Research Station in Salisbury, NC on a Lloyd soil (Fine, kaolinitic, thermic Rhodic Kanhapludults). In 2009/2010, it was conducted Caswell Research Farm in Kinston NC, on a Pocalla loamy sand (Loamy, siliceous, subactive, thermic Arenic Plinthic Paleudults) and again at the Piedmont Research Station in Salisbury NC on a Mecklenburg Loam (Fine, mixed, active, thermic Ultic Hapludalfs).

Cultural Practices

Cover Crop Planting

A total of fourteen winter annual legume cover crops were planted (Table 2-1). In 2008/2009 the four varieties of crimson cover included AU Sunrise (AUS), AU Robin (AUR), Dixie (DIX), and Tibbee (TIB), populations of hairy vetch, included AU Early Cover (AUE), AU Merit (AUM), a population of AU Early Cover reproduced for many years by a Pennsylvania farmer to achieve winter hardiness (EAR), and Purple Prosperity (PRO), a cultivar recently developed at USDA Agricultural Research Service in Beltsville MD from selectively crossing AU Early Cover with an established winter hardy line (Devine and Maul, personal communication). Additional species included common vetch (*Vicia sativa L.* variety unstated), berseem clover (*Trifolium alexandrinum* L. cv. Bigbee) (BIG), subterranean clover *(Trifolium subterraneum,* cv. Denmark*)*(DEN), two populations of Austrian winter pea, (*Pisum sativum subsp. Arvense* cv. Whistler (WHI) and variety unstated (PEA)), and narrow leaf lupin (*Lupinus angustifolius* L, cv. TifBlue78 (TIF)). In 2009/2010 balansa clover (*Trifolium michelianum* cv. Frontier

(FRO)) was added, as well as 3 legume/rye mixtures. The mixtures consisted of rye and AU Early Cover hairy vetch (MXE), rye and AU Merit hairy vetch (MXM), and rye and Austrian winter pea (variety unstated)(MXP).

Cover crops were planted in a randomized complete block design using a a clean-till Tye grain drill with 11 drill lines at 14 cm spacing at Tidewater and Caswell and a Great Plains no-till drill with 8 drill lines at 19 cm spacing, both equipped with a Hege 80 cone. Additional control plots included 1 weedy check, and rye (*Secale cereale* cv. Wrens Abruzzi) as non-N fixing controls, and 4 conventionally-managed control plots with no cover crops. All legume seeds were inoculated with peat based commercial inoculants, Nitragin “C” for hairy and common vetch and Austrian winter pea, “R/WR” for crimson berseem and subterranean clovers, and “H” for lupin (EMD Crop BioScience) at recommended rates prior to planting. Blocks were replicated 4 times at each site in 2008/2009 and 6 times at each site in 2009/2010. In 2008, cover crops were planted at Tidewater Research Station on October 3, 2008 at Piedmont Research Station on September 25, 2008. In 2009, planting occurred on October 8, at Piedmont Research Station and on October 28 at Caswell. Clovers were planted at a density of 22.4 kg ha^{-1}, vetches at 28 kg ha^{-1}, winter peas at 67.2 kg ha$^{-1,}$ lupin at 134 kg ha^{-1} and rye at 117.6 kg ha^{-1}. In 2009, bicultures MXE and MXM consisted of 28 and 56 kg ha^{-1} hairy vetch and rye respectively, and 50.4 and 56 kg ha^{-1} Austrian winter pea and rye respectively for MXP. On both sites in 2008 the previous crop had been corn (*Zea mays* L.). In 2009, the previous crop at the Piedmont site had been soybean, and at the Caswell site sweet potato.

Cover Crop Termination / Corn Planting:

A strip plot design was used for each of the three treatment dates on which cover crop termination and corn planting took place. Three treatment termination dates in spring 2009 represented appropriate early, mid and late corn planting for the region and included April 16, April 29 and May 13 (Tidewater) and April 23, May 8 and May 21 (Piedmont). In 2010, cover crops were terminated on two dates only due to lack of reliable kill of any of the tested cover crops on the early April dates in 2009, dates included April 23 and May 6, (Caswell) and April 30 and May 28 (Piedmont). On each date, 3 m x 7.6 m strips of each cover crop was mechanically killed with a roller-crimper and corn planted in 76 cm row spacing at a density of 90,000 seeds ha^{-1}. Corn was planted using a Monosem NG Plus Pneumatic Planter, outfitted with trash clearers and iron closures to better handle the cover crop mulch (Tidewater and Caswell), and a John Deere 7200 MaxEmerge Conservation planter, also pneumatic, in Piedmont. Roll killing and corn planting was carried out perpendicular to cover crop planting direction in order to maximize cover crop mulch coverage of soil surface. Weedy and rye check plots and N rate controls received an application of 0.84 kg a.i. glyphosate to control weeds. Nitrogen rate controls received a pre-emergent herbicide in the form of 1.41 kg a.i. ha^{-1} metolachlor and 1.82 kg a.i. ha^{-1} atrazine, as well as urea ammonium nitrate at rates of 0, 56, 112 and 168 kg N ha^{-1}, 39.2 kg ha^{-1} were applied at planting with the rest applied 4 weeks after (Table 2-2). Two weeks following cover crop termination, kill rates were assessed for each species at each date based on visible percentage of living to dead material (data not shown).

Sampling and Analysis

Biomass Sampling:

On each termination date, a 0.5 m^2 biomass sample was collected from each plot immediately following rolling. Where weeds constituted a significant portion of the biomass, they were removed from the sample and weighed separately. Biomass samples were transported to North Carolina State University (NCSU), dried at 65°C until they achieved a constant weight, and final dry weights recorded. Samples were then ground to pass through a 2mm sieve for analysis.

Corn Yields

In 2009, corn was hand harvested at both sites by removing ears from two 3 m sections measured from the center two rows of each plot. Ears were counted, dried and shelled to remove grain from the cob, grain weights were recorded on shelled corn and moisture contents were re-adjusted to reflect 15.5% moisture. In 2010, plots were trimmed to 6 m in length, outer edge rows removed, and grain harvested from the center two rows using an Allis Chalmers K2 Gleaner combine. Final plot dimensions and grain weights were taken in the field; moisture content was determined using a Dickey John miniGAC Plus Moisture Tester, and re-adjusted to reflect 15.5% moisture.

Sample Analysis

N and C Elemental Analysis: Ground biomass was submitted to the Environmental and Agricultural Testing Services Lab (NCSU, Raleigh NC) and analyzed for elemental carbon and nitrogen using a Perkin Elmer 2400 CHNS/O Elemental Analyzer.

^{15}N Natural Abundance determination of %Ndfa

^{15}N Natural Abundance calculations

The natural abundance method calculates %Ndfa based on three values: the proportion of ^{15}N from a non-fixing reference plant grown in the same field and conditions as the legume and assumed to access the same soil N pool, the field grown legume, and the isotopic fractionation value (B-value) of shoot tissue from the same species grown entirely dependent on BNF. The %Ndfa is then calculated using the following equation (Shearer and Kohl, 1986):

$$\%\text{Ndfa} = \frac{\delta^{15}N_{ref} - \delta^{15}N_{fix}}{\delta^{15}N_{ref} - B}$$

Reference plant δ^{15}N is assumed to provide an integrated measurement of the soil δ^{15}N available to the legume over the duration of the study, for this experiment separate plots of rye (*Secale cereale*) and winter weeds were integrated in the randomized complete block.

^{15}N Natural Abundance sample preparation:

Sub-samples of ground biomass were further ground and homogenized using a 2000 Geno/Grinder Tissue Homogenizer (Spex Certiprep Ltd., Metuchen, NJ) and sub samples of 3.25 to 3.75 mg were weighed into ultra-light weight tin capsules (Elemental Microanalysis Limited, Cambridge UK) for isotopic analysis. Samples were sent to the Stable Isotope Facility (UC Davis, Davis CA) for $\delta^{15}N$ analysis.

Isotope Fractionation (B value) determination:

Baseline $\delta^{15}N$ values for nodulated legumes 100% dependant on BNF for nitrogen (B-value) was determined through growth of inoculated treatment legumes in a sterile, N free environment in the NCSU phytotron growth-chamber facility (Environmental Growth Chambers, Houston, TX). Briefly, hairy vetch and common vetch seeds were sterilized by immersing in 95% ethanol for 10 s, followed by 3% hypochlorite for 3 minutes and then 5 immediate rinses of sterile water. The seeds were then soaked in sterile water for 36 hours, and then placed on sterilized, moistened germination paper in glass Petri dishes in a 26°C incubator for 48 hours until both radical and shoot were visible. Berseem clover, crimson clover, subterranean clover and sweet clover seeds were sterilized by immersing in 95% ethanol for 30 s, followed by 0.1% hypochlorite for 3 minutes and then 5 rinses of sterile water. Seeds were then placed on sterilized, moistened germination paper in glass Petri dishes in a 26 C incubator for 48 hours until both a radical and a shoot were visible. Lupin and winter pea seeds were immersed in 95% ethanol for 10 s, followed by 4% hypochlorite for 3 minutes and 5 rinses of sterile water. Seeds were then placed on sterilized, moistened germination paper in glass Petri dishes at room temperatures in ambient light for 72 hours until both radical and shoot were visible.

Two seedlings of all species were individually sown in sterile modified Leonard jars constructed from Magenta culture boxes (PlantMedia, Dublin Ohio) (Tlusty et al., 2004) with total rooting volume of 300 ml containing a 1:1 mixture of sand and vermiculite. Plants were bottom watered with a dilute nitrogen-free plant nutrient solution (Broughton and Dilworth, 1971), growth chamber was set at 22C day and 18C night with 650 µmoles m^{-2} sec^{-1} light for 9 hours per day.

At planting, all species were inoculated with 1 ml of a 1:100 suspension of commercial innoculant “Nitragin C” (EMD CropBioScience) to sterile H_2O. At 10 days after transplanting, plants were thinned to 1 plant per pot and, in response to the growth habit of the vetch and winter pea, the tops of the magenta units were removed and sterilized trellises consisting of a 2” x 8” piece of hardware cloth and a bamboo skewer inserted. Tops were removed from all pots after 10 days to prevent leaf moisture accumulation and possible fungal growth. Plant shoots were harvested at 50 days after planting, dried in a 60 C oven and ground for ^{15}N natural abundance analysis, as described above for field grown legumes.

B-Value determination for hairy and common vetch, and Austrian winter pea

Original B-value determinations for hairy vetch, common vetch and Austrian winter pea resulted %Ndfa values greater than 100%, particularly for samples originating from the Piedmont site when compared to in-field ^{15}N natural abundance measurements. Subsequent molecular identification of rhizobia strains from root nodules of Piedmont and Tidewater hairy vetch showed rhizobia occupants to derive from native rhizobia and not from inoculant, thus B-values were corrected using sets of native rhizobia isolates from Tidewater and Piedmont to better represent the nitrogen fixation in field sites. To this end, three representative strains per site were selected and grown in a mixed culture in 200 ml of Tryptone Yeast broth (Somasegaran and Hoben, 1994), for 4 days to a density of 1 x 10^8 cells ml^{-1} and each seedling inoculated with one ml of culture. Growth conditions, biomass harvest and sample preparation were repeated as described above.

Statistical Analysis:

Data were analyzed using SAS software (SAS Institute, Cary, NC). All data for cover crop biomass, biomass N, C:N and grain yields were analyzed separately by site year, using the proc mixed procedure. Experimental design was a stripped block design with two fixed factors, termination date and cover crop species, stripped across each other. Block and the resulting interactions were specified as random factors. In order to fit the assumptions of equal variance, biomass and total N data were square-root transformed for analysis and back transformations of least square means reported in tables and graphs. Data for C:N ratios were log transformed, while grain yield data received no transformation. Means separations were performed using a protected LSD on least squared means.

A combined analysis was performed on treatments present in all four site years, excluding Winter Hardy Early Cover hairy vetch, Tibbee (crimson clover), balansa clover, Austrian winter pea Variety Unstated as well as the vetch rye mixes not repeated at all sight years. Site years were treated as a single random variable, and blocks as random parameters nested within site years.

^{15}N isotope data from the field experiments were analyzed using the mixed procedure, and B-value data using Proc GLM.

RESULTS AND DISCUSSION

Least squared means for cover crop biomass, biomass N, C:N ratios, as well as significance of relevant contrasts are shown in Tables 2-3 through 2-6.

Total biomass and nitrogen content of cover crops

Across all species treatments, biomass and biomass nitrogen varied widely, from 0.5 to 9.6 Mg ha^{-1} of biomass and from 10 to 217 kg ha^{-1} of N across all sites and termination dates, with significant variety×termination date interactions (Tables 2-3 through 2-6). The range of values for both biomass and biomass N differed by site year, with Piedmont 2010 having the greatest values of 9.6 Mg ha^{-1} in MXP and 217 kg N ha^{-1} for MXM. Out of all species evaluated, lupin produced the overall lowest mean biomass and N in Tidewater, producing only 0.3 Mg ha^{-1} biomass and 10 kg ha^{-1} N, with mean lupin biomass across all sites approximately 1.2 Mg ha^{-1}. However, across all site years the lowest biomass and biomass N was found at Caswell in 2010 where hairy vetch and crimson clover produced a mean of only 3.0 and 3.5 Mg ha^{-1} N, respectively, resulting in 70 and 60 kg N ha^{-1} returned to the site in biomass N, 50% lower than the other site years. This is contrasted with Piedmont 2010 where hairy vetch and crimson clover varieties produced 6.5 and 7.5 Mg ha^{-1} biomass resulting in 200 and 150 kg N ha^{-1}, respectively.

For between-species comparisons, biomass production there was a significant cover crop×termination date interaction, ($p<0.05$, Tables 2-3 – 2-6), with crimson clover varieties reaching their peak biomass production earlier in the season than hairy vetch (“Mid” v “Late” termination date, respectively, Figure 2-1). For total N, both the main effect and cover crop×termination date interaction were significant between hairy vetch and crimson clover ($p<0.0001$ and $p<0.05$ for main effect and interaction, respectively) with hairy vetch containing significantly higher mean N concentrations than crimson clover for all site years (~3.5% in hairy vetch vs. ~2.5% in crimson clover (Tables 2-3 – 2.6). Results are consistent with previous literature demonstrating hairy vetch to consistently have lower C:N ratios and higher biomass N content than crimson clover (Hepperly et al., 2009; Teasdale et al., 2004; Wagger, 1989a; Zougmoré et al., 2006).

Among the remaining species, performance varied considerably. Lupin consistently produced the lowest amount of biomass and N relative to other species. Winter kill drastically affected survival of lupin stands in most site years, except in Caswell 2010, where biomass N content equal to that of the poor stands of hairy vetch and crimson clover (Table 2-5). Subterranean clover biomass ranged from 7.6 to 0.7 Mg ha^{-1}, with highest biomass at earlier termination dates. Berseem clover consistently increased in both biomass and total N from approximately 2.5 Mg ha^{-1} at early termination dates to as much as 7.7 Mg ha^{-1} at later dates production at later termination dates.

In 2010, the rye + legume mixtures produced significantly more biomass than their sole-cropped legume counterparts; however there was no difference in the total amount of N produced. This is consistent with previous work comparing bicultures of rye+vetch with monocropped hairy vetch (Ranells and Wagger, 1997).

Within-species comparisons were made for all varieties of hairy vetch, crimson clover and Austrian winter pea. For hairy vetch, AUM produced significantly more biomass and N than the other cultivars at the Caswell site in 2010. This can be attributed to a lack of rainfall in early spring when the early maturing AUE cover was maturing; beginning in May, this site began receiving modest irrigation, which may have benefited the later maturing AUM (Figure 2.3). Within crimson clover, early-maturing AUS, the variety × termination date interaction produced a significant response in total N in Piedmont 2009 ($p = 0.026$), indicating an earlier accumulation of N. Although this trend was observed in other site years, it was non-significant. A similar comparison between early maturing AUE and other hairy vetch varieties was found to be non-significant. In Austrian winter pea cultivars, biomass or total N was found to be non-significant both in main effects, and interaction by termination date.

Nitrogen concentrations (%N) decreased with increasing C:N ratios in later termination dates for all cover crops. This was particularly evident in 2010 in Piedmont, where the "late" termination date and biomass harvest was on May 28; earlier maturing crimson clover, balansa clover, and subterranean clover, had unusually high C:N ratios (25 to 30 , as compared to 17 to 19 at "mid" termination date) and were past senescence at the time of biomass sampling (Table 2-6). Balansa Clover, only included in 2010, reached its peak biomass of 5.8 Mg ha^{-1} by the "mid" 2010 termination date. Although it accumulated comparatively less total biomass than other species, this early flush of biomass production may make it particularly attractive to producers who wish to plant in early to mid April.

^{15}N natural abundance and %Ndfa

$\delta^{15}N$ of field grown legumes and reference species

All $\delta^{15}N$ values observed in this experiment were within the range of previously published for temperate cover crop legumes (Ovalle et al., 2006; Rochester and Peoples, 2005). In Piedmont and Tidewater 2009, and Caswell in 2010, mean field $\delta^{15}N$ values were below zero for most legumes, and ranged from -0.62, to -0.31 for hairy vetch varieties, -0.74 to - 0.31 for common vetch, -0.53 to -0.13 for Austrian winter pea, -0.61 to -0.13 for crimson clover varieties, and -0.73 to -0.59, -0.38 to 1.12 and -0.68 to 0.19 for berseem clover, subterranean clover and lupin, respectively. Caswell $\delta^{15}N$ values for bicultures (composite sample of legume and rye) ranged from -0.31 for MXM and -0.05 for MXP. Piedmont 2010 $\delta^{15}N$ values were all greater than 0, ranging from 0.55 to 1.71 for hairy vetch varieties, 0.83 and 0.96-0.98 for common vetch and Austrian winter pea, respectively, 0.54 to 1.22 for crimson clover varieties, 0.14, 3.24 and 3.18 for berseem clover, subterranean clover and lupin respectively. Bicultures in Piedmont ranged from 1.29 for MXM to 1.58 for MXP. Higher $\delta^{15}N$ values suggest either lower BNF activity or greater natural ^{15}N enrichment of the soil in which the plants were grown.

In three of the four fields where both rye and weed non-fixing reference species were evaluated in 2009 and 2010, we found reference species $\delta^{15}N$ values to be lower than published values for agronomic soils, often ranging between 1.3 and 9.0, with a mean value of 5.5 (Peoples, et al. 2009; Shearer and Kohl. 1986). Mean rye and weed $\delta^{15}N$ values were found to be 1.50 and 1.90 at Piedmont and 1.71, 1.39 at Tidewater (2009), and 1.76 and 3.38 at Caswell and 7.76 and 9.17 at Piedmont (2010), respectively. All sites had been in long-term crop rotations that included soybean. Van Kessel showed that long term recycling of fixed N can reduce soil $\delta^{15}N$ values over time (Kessel et al., 1994), which may account for the low values at these three sites. Unlike the others, the Piedmont 2010 site received regular applications of composted chicken litter. Previous research comparing nitrogen isotope contents of various fertility sources has shown that carrots and tomatoes grown with pelleted chicken manure had higher $\delta^{15}N$ values than those fertilized with ammonium nitrate fertilizer (Bateman et al., 2005). Higher natural $\delta^{15}N$ values allow for greater precision and accuracy in determining Ndfa, thus the Piedmont 2010 values of %Ndfa may better reflect the BNF potential of these legumes.

B-Values by plant species and rhizobia occupants:

Initial calculations of %Ndfa using inoculants-derived B-values resulted in biologically impossible fixation values of higher than 100%. Mean commercial inoculant-derived B-values grown in N-free media were -0.09, -0.34 and -0.07 for hairy vetch, common vetch and Austrian winter pea respectively, -0.60 to -0.44 for crimson clover varieties, and -0.46, -0.40 and -0.62 for berseem clover, subterranean clover and lupin respectively. Corrected B-values are are -0.68 and -0.53 for hairy vetch, -1.38 and -0.92 for common vetch and -1.23 and -1.01 for Austrian winter pea at Piedmont and Tidewater respectively, Inoculant-derived B-values for hairy vetch, common vetch, Austrian winter pea and berseem clover were greater than the values of all field-grown plants in 2009 and those from Caswell in 2010, (Tables 2-8), yielding biologically-impossible Ndfa results indicating that more than 100% of the plant N was from fixation. There are several reasons why this could have occurred. The non-fixing control (all N from soil pools) and B-value (100% of N from fixation) set the limits of the range of values that are possible for a field value. Peoples et al. (1997) suggested that this inversion of values could occur in legume species that are especially promiscuous and nodulate readily with a variety of rhizobia. In a review analyzing B-values of many legume species, Unkovitch et al. (2008) found that soybean B-values from laboratories using different rhizobia partners range from -0.90 to -4.54. Grossman et al. (unpublished data, 2009) demonstrated that at the field sites used in this study, rather than the inoculant strains applied at planting, native strains of rhizobia dominated hairy vetch nodule occupants. We conclude that although some inoculant-derived B-values fell within the range of published literature values, -0.47 to -1.17 for *Vicia sp*. (Rochester and Peoples, 2005; Unkovich et al., 2008) and -0.72 for *Pisum sp.* (Hauggaard-Nielsen et al., 2003), corrected B-values from each field site, ranging in this study from -1.23 to -0.51, are likely to be more accurate as they result in realistic estimates of %Ndfa (Table 2-9).

These results were unexpected and may present complications for those using the ^{15}N Natural Abundance method to calculate BNF, as most studies assume that B-values are dependent on legume species, rather than nodule rhizobia occupant. For legume species that easily nodulate with a wide range of rhizoba partners, such as those used in this study, these results suggest that a new B-value derived at the beginning of an experiment to reflect the rhizobial population of the field may provide the most accurate

BNF estimations. It also suggests that reference plants found to have a ^{15}N signature abnormally similar to the legume in question may be a result of reference plant cultivation in agronomic soils with a history of legume cultivation, indicating that recycling of fixed-N may be contributing to the unreasonable estimations of Ndfa. Such a scenario reduces the accuracy as well as the precision of the ^{15}N Natural Abundance method, as both $\delta^{15}N$ of reference species and B-value determine the Ndfa values (Unkovich et al., 1994). For example, by using corrected B-values for hairy vetch, common vetch and Austrian winter pea, in Piedmont in 2009, we increased our denominator ($\delta^{15}N_{ref} - B$) by 27.6%, from 1.99 to 2.54 for hairy vetch varieties.

%Ndfa and total BNF of legumes:

Across all site years, %Ndfa calculated using corrected B-values, where available, and those derived from commercial inoculants when not available, accounted for between 34% and 100% of N in the legume biomass with the majority of the data falling between 70% and 100% for all treatments (Table 2-9). Within-species comparisons show that within hairy vetch in 2009, PRO relied less on BNF than the other varieties, however this trend did not continue in 2010. This finding suggests that PRO may be slightly more sensitive than other varieties to an environmental BNF constraint that was present in 2009. In 2010, EAR had a lower %Ndfa than AUE in Caswell or PRO in Piedmont ($p<0.05$). Varieties of crimson clover did not differ in their reliance on BNF in any site year, nor did varieties of Austrian winter pea. Within the rye bicultures, MXP was significantly lower than MXE ($p<0.05$).

While there were differences in the total mass of fixed N, this was most often a function of biomass production and not BNF efficiency. Overall, hairy vetch cover crops were able to add between 100 and 150 kg N ha^{-1} of fixed N to the system, crimson clover between 50 and 130 kg N ha^{-1}, Austrian winter pea between 62 to 130 kg ha^{-1}, berseem clover between 43 and 151 kg ha^{-1}, 4 and 68 kg ha^{-1} for subterranean clover and between 21 and 58 for lupine (Table 2-9). This is within the range of previously published values of Ndfa for these and related species from other parts of the world (Hauggaard-Nielsen et al., 2010; Ovalle et al., 2006; Rochester and Peoples, 2005).

Performance of roll-killed mulches in corn crop

Grain Yield N response

Grain yields for all cover crop and planting date treatments are reported in Table 2-10. Total yields from all sites ranged from 0.5 to 11.7 Mg ha^{-1} grain. With three exceptions, yield response to fertilizer nitrogen peaked at 112 kg ha^{-1} fertilizer N with grain yields between 7.3 and 11.7 Mg ha^{-1}, and declined at higher N rate of 168 kg ha^{-1} (Figure 2.2). Grain yield response to applied N peaked at 168 kg ha^{-1} N in the "early" termination date for Tidewater, the "mid" termination date for Caswell and the "late" termination date for Piedmont 2010, with 7.8, 3.0 and 7.6 Mg ha^{-1} grain, respectively. Highest overall yields were observed in Tidewater with a maximum yield of 11.7 Mg ha^{-1}grain in the 112N control treatment. Lowest overall grain yields were seen at Caswell in 2010 with a maximum yield of 3.0 g ha^{-1} in the 168N control treatment. This was possibly a result of low total biomass and biomass N in cover crops followed by a summer of low rainfall in a sandy soil (Figure 2.3). Symptoms of drought stress were observed throughout the season, including chlorosis, stunted growth and widespread incidents of *Ustilago maydis* (smut) in ears. However, treatment differences were still observable as evidenced in Table 2-10.

Cover crop effects on grain yields:

Cover crop treatment, termination date and the cover crop × termination date interaction had significant effects on corn yield for all site years. Highest overall yields among cover crop treatments were seen in 2009 by hairy vetch varieties, with AUE yielding 7.3 and 8.5 Mg ha^{-1} in the “Mid” and “Late” termination dates and between 7.5 and 7.9 Mg ha^{-1} in Piedmont. In 2010, the highest grain resulted from bicultures, with MXE yielding between 4.3 and 5.6 Mg ha^{-1} in Piedmont. The lowest yield was seen in Piedmont 2009 in the “early” termination date of berseem clover, with a least squares mean of -0.6 Mg ha^{-1} grain. Crimson clover produced yields between 0.5 Mg ha^{-1} (Caswell) and 5.9 Mg ha^{-1} (Tidewater). Austrian winter pea varieties produced similar yields as crimson clover, falling between 0.7 Mg ha^{-1} (Caswell) and 5.8 Mg ha^{-1} (Tidewater) .

Between-species contrasts indicate that hairy vetch treatments produced more grain than crimson clover for all site years except Tidewater ($p < 0.05$), with a significant interaction with termination date for all site years except Piedmont in 2010 (Table 2-10). Hairy vetch varieties yielded more grain that VET in 2009 ($p < 0.01$), but the difference in grain yield under the two vetch species was non-significant in 2010. MXE and MXM had higher grain yields in 2010 at both Caswell and Piedmont than the hairy vetch monocultures AUE, AUM, EAR and PRO ($p<0.05$). Within-species comparisons of hairy vetch signify that yields from the AUE treatment were greater than other all other varieties in Tidewater ($p<0.05$), and that the variety × termination date interaction was significant in Piedmont in 2009 ($p<0,05$), indicating that AUE treatments had greater yields at earlier termination dates. In 2010, AUE grain yields were not significantly greater than other hairy vetch varieties, although AUM had greater yields in Caswell at the "late" termination date. Within species comparisons of Austrian winter pea and crimson clover were non-significant.

It was expected that cover crop treatments with high amounts of biomass and N, would be predictive of high grain yields for all cover crop treatments. This was not the case. Treatments that resulted in poor corn yields were divided into two categories: (i) treatments where the roller-crimper failed to kill the cover crop placing corn in competition with an actively growing crop, and (ii) treatments where high biomass N was not correlated with increases in crop yield.

The roller crimper failed to kill all hairy vetch varieties, common vetch, berseem clover and Austrian winter pea at the "Early" roll-date in 2009, reflected in observations of growing cover crops competing with corn, and grain yields that are lower ($p<0.05$) than the 0N control. Figure 2.2 shows that as termination dates progress later in the season, corn yields resulting from hairy vetch treatments increase, and yields from corn planted in all hairy vetch varieties (AUE, AUM and EAR) at the "Late" planting date in 2009 were comparable to the 112N and 168N controls, averaging 6.7 to 8.5 Mg ha^{-1}. This trend was repeated in the berseem clover and the common vetch plots, where in Early

2009 termination date yields were significantly lower than all other treatment species (0.5 and 1.5 Mg ha^{-1} for VET, and 0.8 and -0.6 Mg ha^{-1} for BIG in Tidewater and Piedmont, respectively), yet improved dramatically at later planting dates, to 5.7 and 6.6 Mg $ha^{-1}$for VET and 2.3 and 4.3 kg ha^{-1} for BIG in Tidewater and Piedmont, respectively. Yields appeared to be affected by the interaction between cover crop treatment and corn planting time. This interaction was expected based on previous research showing that re-growth of hairy vetch after termination by a roller-crimper can be as much as 2.7 Mg ha^{-1} when the crop is at 50% flowering or less (Mischler et al., 2010). In 2010, this effect was not observed. Elimination of the "Early" termination date and low overall biomass yields at the Caswell site, are possible reasons for this.

The second category consisted of treatments where high biomass N and adequate termination failed to induce a response in grain yield. Cover crops in the second category included crimson clover, balansa clover, and subterranean clover, which yielded 0.7 to 5.9 Mg ha^{-1}, 0.7 to 1.8 Mg ha^{-1} and 0.7 to 5.5 Mg ha^{-1}, respectively. While the crimson clover residues contained >100 kg N ha^{-1} in 3 of 4 site years, the corn crop planted in these treatments did not perform significantly better than a 0N control plot, or as well as other cover crop treatments that contained equivalent biomass N. At the "Mid" termination date in Piedmont 2010, balansa clover contained 124 kg ha^{-1} N, but the resulting corn crop only yielded 1.8 Mg ha^{-1} grain, not significantly different from the 0N control of 2.7 Mg ha^{-1}. While the reasons for this remain unclear, there are several possible contributing factors. First, following roll-kill there was noticeable reseeding and germination of crimson clover, likely to effectively compete with the corn crop.

Secondly, nitrogen contained in organic residues, such as these cover crops, must be mineralized by soil microbes in order for that N to be made available to the succeeding crop. Lack of a yield response to the biomass N of the crimson clover treatments suggest that mineralization either did not occur, or was not sufficient to meet the needs of the corn crop. Previous research on crimson clover provide mixed results as to the net N mineralization value of crimson clover residues. Wagger and Ranells (Ranells and Wagger, 1996; Wagger, 1989a) repeatedly show that crimson clover residues release nitrogen following kill, in both tilled and no-tilled systems, however earlier research shows that crimson clover that has been allowed to senesce and re-seed offered no benefit to a sorghum grain crop without additional applications of nitrogen. (Touchton et al., 1982). Snapp et al. (2005) found that herbicide treated residues decompose more quickly than un-treated residues. Possible reasons for the poor grain yields in rolled crimson clover could be due to plant tissue chemistry. Third, mineralization rates are largely dependent on residue quality, including N concentration, C:N ratios, lignin and phenolic content (Palm and Sanchez, 1990; Swift et al., 1979). The C:N ratios of the crimson clover residue was significantly higher than that of the hairy vetch, however it was not higher than the rye-vetch mixes MXE or MXM, which in Piedmont in 2010 saw corn grain yields comparable to the 168N rate. Neither crimson nor balansa clover phenolic content was available for this experiment; however previous research shows cellulose and hemicelluloses content of crimson clover to be 20% and 70% greater than hairy vetch, respectively (Wagger et al., 1998).

In 2010, biculture mulches had higher grain yields than the individual legume stands alone, 4.3 to 5.6 for MXE and MXM vs. 3.0 to 4.1 for hairy vetch varieties in Piedmont. At Piedmont MXM and MXE had the greatest yields of cover crop treatments ($p<0.05$). Despite the significantly higher C:N ratios compared to hairy vetch, previous research has shown that rye/vetch bicultures release N at a rate equal to or faster than crimson clover, although not as fast as hairy vetch (Ranells and Wagger, 1996). An additional reason for the yield increase could relate to the mulch weed suppressive ability. These treatments produced more than 8 Mg ha^{-1} of biomass, which likely provided a substantial barrier against weeds. Teasdale and Mohler have shown that increased mulch mass for a variety of mulches results in a decrease in weed emergence (Teasdale and Mohler, 2000). Research on rolled rye cover crop in soybean production has shown reduced weed populations under rolled rye leading to an increase in soybean yields (Davis, 2010).

Peak N content corresponds to optimum roll-kill/planting date

General trends in biomass N content of the roll killed legumes (Figure 2.1) show that earlier maturing legumes, including crimson clover, balansa clover and subterranean clover reached their peak biomass N content in mid to late April. After this point, biomass and N content declined hairy vetch varieties; PEA, WHI and BIG reached their peak N content in at the “Mid” and “Late” termination dates. In both cases, the peak N content period corresponded to the initial successful roll kill. Corn planted into hairy vetch, Austrian winter pea and berseem clover at the time of peak biomass N had higher yields than when planted at other times ($p<0.05$). This is likely a result of both the N content, lack of competition from living cover crop in the case of the later maturing vetches, and lack of competition from re-seeded cover crops in the case of late killed clovers.

Conclusions

The roller crimper is a popular new tool being evaluated for cover crop termination in organic corn production. This study sought to identify cover crop species compatible with the roller-crimper system in North Carolina using defined criteria of high biomass and N production, susceptibility to termination by the roller crimper in mid April, and high grain yield from corn planted into a roll-killed mulch. Tested species included four varieties of hairy vetch and crimson clover, two varieties of Austrian winter pea, a common vetch, a berseem, subterranean and balansa clover, and lupin. Attempted termination took place at “Early,” “Mid” and “Late” planting dates corresponding with mid-April, early-May and late-May, respectively. None of the cover crops we tested fulfilled all criteria: earlier flowering crimson clover failed to produce adequate yields, hairy and common vetch did not mature until May, however resulted in high grain yields, despite the later maturity dates. As evidenced by increasing yields with later termination dates in hairy and common vetch and berseem clover treatments, cover crop termination is crucial to the succeeding corn crop. Adequate cover crop biomass is also important to for N contribution as evidenced by the low yields in Caswell in 2010. All species derived a majority of their tissue N from BNF, with most having more than 70% Ndfa. Research on hairy vetch has shown that peak N fixation period as measured using acetylene reduction occurs between late April and early May (Anugroho et al., 2009). By waiting until this peak N period, producers are likely to successfully terminate legume cover crops using a roller-crimper and take advantage of greater fixed N for corn production in North Carolina. Conversely, the data relating to grain yields from corn planted into crimson clover mulch suggest that N mineralization rates are different in a roll-kill system than in one managed through tillage or herbicide treatment. Additionally, research is needed to investigate potential disease risks producers would face by moving planting dates later in the season and how these risks compare to those in a conventionally managed system.

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FIGURE CAPTIONS, TABLES AND FIGURES

Table 2-1: Cover crop treatments

Species	Common Name	Variety	Seed rate (kg ha^{-1})	Abbreviation
Vicia villosa	Hairy Vetch	AU Merit	28	AUM
		AU Early Cover	28	AUE
		Early Cover – Winter Hardy	28	EAR
		Purple Prosperity	28	PRO
Vicia sativa	Common Vetch	Variety Unstated	28	VET
Pisum sativum subsp. Arvense	Austrian Winter Pea	Whistler	67	WHI
		Variety Unstated	67	PEA
Trifolium incarnatum	Crimson Clover	Tibbee	22	TIB
		Dixie	22	DIX
		AU Sunrise	22	AUS
		AU Robin	22	AUR
Trifolium alexandrinum	Berseem Clover	Bigbee	22	BIG
Trifolium michelianum *	Balansa Clover	Frontier	6	FRO
Trifolium subteraneum	Subterannean Clover	Denmark	22	DEN
Lupinus angustifolius	Blue Lupin	TifBlue78	135	TIF
Secale cereale	Cereal Rye	Wrens Abruzzi	118	WRE
Bicultures:				
Vicia villosa + *Secale cereale* *		“AU Early Cover” + Wrens Abruzzi	28+56	MXE
Vicia villosa + *Secale cereale* *		“AU Merit” + Wrens Abruzzi	28+56	MXM
Pisum sativum + *Secale cereale* *		“Variety Unstated “ Wrens Abruzzi	50+56	MXP

* Planted during the 2009/2010 season only

Table 2-2: Field operation

	2008/2009						2009/2010			
	Tidewater			Piedmont			Caswell		Piedmont	
	Early	Mid	Late	Early	Mid	Late	Mid	Late	Mid	Late
Cover Crop Planting	25 Sept 2008			2 Oct 2008			28 Oct 2009		8 Oct 2009	
Cover Crop Termination/ Corn Planting	16 April	29 April	13 May	23 April	8 May	21 May	23 April	6 May	30 April	28 May
Fertilizer N Split Application	13 May	28 May	10 June	21 May	2 June	16 June	24 May	9 June	28 May	24 June
Corn Harvest	11 Sept 2010			4 Sept 2010			1 Sept 2010		21 Sept 2010	

Table 2-3: Cover crop biomass, nitrogen and C:N; Piedmont 2009

		Early Roll-Killed			Mid Roll-Killed			Late Roll Killed		
	Variety	Biomass Mg ha^{-1}	C:N	Total N Kg ha^{-1}	Biomass Mg ha^{-1}	C:N	Total N Kg ha^{-1}	Biomass Mg ha^{-1}	C:N	Total N Kg ha^{-1}
Hairy Vetch	AU Early	4.1 bcd	10.4 d	168 a	5.2 abc	13.4 cde	163 ab	4.9 bcd	12.7 f	168 abc
	AU Merit	4.2 bcd	10.7 d	166 a	6.0 a	12.1 de	197 a	5.5 abcd	12.5 f	190 ab
	Purple Prosperity	3.8 cde	12.3 cd	122 a	6.0 a	13.8 cde	151 ab	6.4 ab	12.7 f	217 a
Common Vetch	Common Vetch	3.6 def	11.8 cd	122 a	4.4 bcd	11.4 e	153 ab	5.0 bcd	11.9 f	179 ab
Crimson Clover	AU Robin	5.5 ab	18.1 b	129 a	6.1 a	17.6 bc	144 b	4.6 cd	19.9 bcd	99 de
	AU Sunrise	5.8 a	16.4 bc	152 a	6.6 a	17.7 bc	156 ab	6.0 abc	17.4 cde	152 bc
	Dixie	5.0 abc	15.8 bc	134 a	5.6 ab	17.3 bc	136 bc	5.0 bcd	23.8 b	87 ef
Berseem Clover	BigBee	2.6 efg	13.0 cd	73 b	4.3 bcd	14.4 bcde	122 bc	5.2 bcd	15.6 def	139 bcd
Subterannean Clover	Denmark	2.0 g	15.7 bc	38 c	1.6 e	18.8 b	27 de	1.7 g	21.8 bc	28 h
Austrian Winter Pea	Variety unstated	2.4 fg	14.9 bcd	67 b	3.9 cd	16.1 bcd	99 c	4.2 de	14.6 ef	126 cde
	Whistler	1.7 g	12.0 cd	60 bc	3.4 d	14.1 cde	95 c	3.2 ef	13.6 ef	99 de
Narrow Leaf Lupin	TifBlue 78	0.5 h	13.1 cd	15 d	0.3 f	11.8 de	11 e	2.1 fg	15.5 def	56 fg
Rye	Wrens Abruzzi	5.0 abc	42.5 a	52 bc	6.4 a	63.8 a	45 d	7.1 a	77.5 a	42 gh

Contrasts	Biomass		C:N		Biomass N	
	CC	CC*Rolltime	CC	CC*Rolltime	CC	CC*Rolltime
Hairy vetch vs. crimson clover	ns	***	***	ns	***	***
Hairy vetch vs common vetch	*	ns	ns	ns	**	ns
Hairy vetch: AUE vs others	ns	ns	ns	ns	ns	ns
Crimson clover: AUS vs others	ns	ns	ns	ns	*	**

Treatments with the same letter signify no significant difference at α=0.05

Table 2-4: Cover crop biomass, nitrogen and C:N; Tidewater 2009

		Early Roll-Killed			Mid Roll-Killed			Late Roll Killed		
	Variety	Biomass Mg ha^{-1}	C:N	Total N Kg ha^{-1}	Biomass Mg ha^{-1}	C:N	Total N Kg ha^{-1}	Biomass Mg ha^{-1}	C:N	Total N Kg ha^{-1}
Tidewater										
Hairy Vetch	AU Early	4.0 abc	10.3 f	153 ab	5.2 abc	11.9 de	182 ab	6.4 ab	12.4 ef	203 a
	AU Merit	4.1 abc	10.2 f	157 a	4.6 bc	11.4 e	166 abcd	5.6 bc	12.7 def	179 abc
	Winter Hardy Purple	4.5 ab	10.3 f	168 a	5.4 abc	12.4 de	177 abc	5.8 bc	11.1 f	203 a
	Prosperity	3.9 abc	10.8 ef	148 ab	5.3 abc	11.8 de	191 a	6.1 abc	13.3 def	195 a
Common Vetch	Common Vetch	2.6 cd	10.8 ef	103 bc	4.6 abc	12.0 de	159 abcd	4.3 cd	12.7 def	134 bcd
Crimson Clover	AU Robin	3.0 bcd	17.8 bc	69 c	6.3 ab	20.4 bc	126 cde	4.6 bcd	20.5 c	97 defg
	AU Sunrise	3.5 abcd	16.5 bcd	88 c	5.4 abc	18.2 bc	124 cde	5.2 bcd	18.3 cd	117 cde
	Dixie	3.5 abcd	16.9 bcd	82 c	4.8 abc	17.1 bcd	117 de	3.6 de	21.3 bc	73 fgh
	Tibbee	3.2 bcd	16.0 bcde	81 c	4.6 bc	18.5 bc	105 e	5.5 bc	20.8 bc	114 cdef
Berseem Clover	BigBee	0.8 e	13.3 cdef	26 de	1.8 d	15.5 cde	48 fg	3.4 de	17.3 cde	74 efgh
Subterannean Clover	Denmark	2.1 d	21.2 b	29 de	1.5 d	22.3 b	26 f	0.9 f	26.5 b	16 i
Austrian Winter Pea	Whistler	2.1 d	10.5 ef	79 c	3.6 c	11.5 de	133 bcde	4.6 bcd	12.4 ef	146 abc
Narrow Leaf Lupin	TifBlue 78	0.3 ef	12.7 cdef	10 ef	0.9 d	16.2 cde	23 g	2.3 e	14.2 def	60 gh
Rye	Wrens Abruzzi	5.4 a	33.4 a	54 cd	6.7 a	56.2 a	56 f	8.3 a	72.6 a	53 h

Contrasts	Biomass		C:N		Biomass N	
	CC	CC*Rolltime	CC	CC*Rolltime	CC	CC*Rolltime
Hairy vetch vs. crimson clover	*	**	***	ns	***	**
Hairy vetch vs common vetch	ns	ns	ns	ns	**	ns
Hairy vetch: AUE vs others	ns	ns	ns	ns	ns	ns
Crimson clover: AUS vs others	ns	ns	ns	ns	ns	ns

Table 2-5: Cover crop biomass, nitrogen and C:N; Caswell 2010

		"Middle" Roll						"Late" Roll					
Species	**Variety**	Biomass Mg ha^{-1}		C:N		Total N Kg ha^{-1}		Biomass Mg ha^{-1}		C:N		Total N Kg ha^{-1}	
Caswell													
Hairy Vetch	AU Early	3.1	bcdef	15.2	gh	79	abc	2.6	defghi	17.3	ef	64	cdef
	AU Merit	4.3	a	13.6	h	107	a	5.1	ab	15.6	f	121	ab
	Winter Hardy	2.7	defgh	15.5	gh	72	bcdef	2.9	cdefgh	15.9	f	77	cde
	Purple	2.9	cdefg	15.8	gh	73	bcdef	2.2	fghi	16.9	ef	55	defgh
Common Vetch	Unstated	2.5	efgh	13.3	h	71	bcdef	2.3	fghi	15.6	f	60	def
Crimson Clover	AU Robin	3.1	bcdef	22.3	cd	51	efg	2.1	ghi	26.0	bc	35	ghij
	AU Sunrise	3.5	abcde	20.6	cde	58	cdefg	2.0	hi	26.0	bc	32	ij
	Dixie	3.6	abcd	19.9	cdef	62	cdefg	2.4	fghi	27.6	b	34	hij
	Tibbee	3.8	abc	22.1	cd	64	cdefg	2.6	defghi	21.0	d	49	fghi
Balansa Clover	Frontier	3.2	abcdef	21.4	cd	50	fg	2.4	efghi	20.1	de	50	fghi
Berseem Clover	BigBee	2.4	fgh	17.1	fg	42	g	1.8	i	16.5	f	43	fghi
Sub. Clover	Denmark	3.2	abcdef	19.4	def	53	defg	3.4	cde	21.4	d	52	efgh
Austrian Winter Pea	Pea	3.2	abcdef	17.4	efg	77	bcd	3.6	cd	15.7	f	91	bc
	Whistler	2.8	cdefg	15.6	gh	70	bcdef	3.3	cdef	16.3	f	81	cd
Lupin	TifBlue 78	2.1	gh	14.3	h	57	cdefg	2.3	fghi	16.7	f	57	defg
Rye/H. Vetch	Mix Early	2.7	cdefg	21.9	cd	49	fg	3.0	cdefg	23.2	bcd	56	defg
	Mix Merit	4.1	ab	17.8	efg	92	ab	6.2	a	16.4	f	152	a
Rye/Pea	Mix Pea	4.2	ab	23.1	c	75	bcde	4.0	bc	22.5	cd	75	cde
Rye	Wrens Abruzzi	1.8	h	66.7	a	12	h	2.6	defghi	52.6	a	21	j

Contrasts	**Biomass**		**C:N**		**Biomass N**	
	CC	CC*Rolltime	CC	CC*Rolltime	CC	CC*Rolltime
Hairy vetch vs. crimson clover	ns	***	***	ns	***	**
Hairy vetch vs common vetch	**	ns	ns	ns	*	ns
Hairy vetch: AUE vs others	ns	ns	ns	ns	ns	ns
Crimson clover: AUS vs others	ns	ns	ns	ns	ns	ns
Rye+Hairy vetch vs hairy vetch	***	ns	***	ns	ns	***
MXE,MXM&MXP vs hairy vetch and pea	***	ns	***	ns	ns	ns

Treatments with the same letter signify no significant difference at α=0.05, for values within the same site and termination date.

Table 2-6: Cover crop biomass, nitrogen and C:N; Piedmont 2010

Species	Variety	"Middle" Roll Biomass Mg ha^{-1}		C:N		Total N Kg ha^{-1}		"Late" Roll Biomass Mg ha^{-1}		C:N		Total N Kg ha^{-1}	
Piedmont													
Hairy Vetch	AU Early	6.2	cdef	12.1	e	204	ab	4.7	defg	15.3	fg	136	bc
	AU Merit	6.3	cdef	11.7	e	202	ab	5.1	defg	14.6	g	141	b
	Winter Hardy	5.8	def	11.3	e	204	ab	5.4	cdef	13.9	g	162	ab
	Purple Prosperity	6.6	cdef	11.5	e	213	a	5.4	cdefg	14.0	g	163	ab
Common Vetch	Unstated	5.4	f	11.7	e	188	abcd	4.9	defg	15.8	efg	133	bc
Crimson Clover	AU Robin	7.8	bcde	19.5	cd	163	abcde	3.4	gh	30.6	b	47	efg
	AU Sunrise	7.0	cdef	18.2	d	155	abcde	3.6	fgh	26.9	bc	56	defg
	Dixie	5.9	def	17.8	d	138	def	5.7	cde	20.3	d	90	cd
	Tibbee	6.7	cdef	19.0	d	141	cdef	3.9	efg	25.5	bc	68	def
Balansa Clover	Frontier	5.8	ef	18.8	d	124	ef	3.5	gh	20.3	d	71	de
Berseem Clover	BigBee	6.3	cdef	16.3	d	155	abcde	7.7	abc	19.0	def	169	ab
Sub. Clover	Denmark	7.6	bcdef	18.8	d	102	fg	2.1	hi	24.0	cd	33	g
Austrian Winter Pea	Pea	6.2	cdef	11.6	e	208	a	4.7	defg	14.2	g	128	bc
	Whistler	6.3	cdef	12.6	e	199	abc	4.4	defg	14.1	g	130	bc
Lupin	TifBlue 78	1.0	g	17.9	d	23	h	0.8	ij	27.7	bcd	14	gh
Rye/H. Vetch	Mix Early	8.2	bcd	18.4	d	190	abcd	6.6	bcd	22.6	cd	130	bc
	Mix Merit	8.4	bc	16.4	d	212	a	9.7	a	19.7	de	217	a
Rye/Pea	Mix Pea	9.6	b	27.6	b	149	bcdef	8.1	ab	23.5	cd	152	b
Rye	Wrens Abruzzi	12.6	a	79.0	a	69	g	8.3	ab	99.4	a	37	fg

Contrasts	**Biomass** CC	**Biomass** CC*Rolltime	**C:N** CC	**C:N** CC*Rolltime	**Biomass N** CC	**Biomass N** CC*Rolltime
Hairy vetch vs. crimson clover	ns	***	***	ns	***	***
Hairy vetch vs common vetch	ns	ns	ns	ns	ns	ns
Hairy vetch: AUE vs others	ns	ns	ns	ns	ns	ns
Crimson clover: AUS vs others	ns	ns	ns	ns	ns	ns
Rye+Hairy vetch vs hairy vetch	***	ns	***	ns	ns	ns
MXE,MXM&MXP vs hairy vetch and pea	***	ns	***	ns	ns	**

Treatments with the same letter signify no significant difference at α=0.05, for values within the same site and termination date.

Table 2-7: $\delta^{15}N$ Natural Abundance of field grown cover crops

Species	Variety	2009		2010	
		Piedmont	Tidewater	Caswell	Piedmont
Vicia villosa L.	AU Early Cover	-0.47	-0.06	-0.51	1.14
	AU Merit	-0.62	-0.20	-0.43	0.99
	Winter Hardy	nd	-0.31	-0.21	1.71
	Purple Prosperity	-0.03	0.31	-0.27	0.55
Vicia sativa L.	Common Vetch	-0.74	-0.31	-0.48	0.83
Pisum sativum subsp. Arvense	Variety Unstated	-0.35	nd	-0.44	0.98
	Whistler	-0.44	-0.37	-0.53	0.96
Trifolium incarnatum	AU Sunrise	-0.35	0.08	-0.37	0.79
	AU Robin	-0.19	-0.01	-0.46	1.22
	Dixie	-0.13	0.13	-0.48	0.54
	Tibbee	nd	-0.37	-0.61	0.74
Trofolium alexandrium	Bigbee	-0.69	-0.59	-0.73	0.14
Trifolium subteraneam	Denmark	-0.38	1.12	-0.35	3.24
Lupinus	Tifblue 78	-0.42	0.19	-0.68	3.18
V. villosa + S. cereale	Mix Early Cover	nd	nd	-0.20	1.33
	Mix Merit	nd	nd	-0.05	1.29
P. sativum + S. cereale	Mix Pea	nd	nd	-0.31	1.58
Non-Fixing References:	Rye	1.50	1.71	1.76	7.76
	Weeds	1.90	1.39	3.38	9.17

Table 2-8: ^{15}N isotope discrimination B-values by rhizobia origin

Species	Variety	Inoculant $\delta^{15}N$	Piedmont $\delta^{15}N$	Tidewater $\delta^{15}N$
Vicia villosa L.	AU Early Cover	-0.09	-0.64	-0.51
	AU Merit	-0.14	-0.64	-0.60
	Winter Hardy	-0.24	-0.72	-0.54
	Purple Prosperity	0.09	-0.73	-0.50
Vicia sativa L.	Variety Unstated	-0.34	-1.38	-0.92
Pisum sativum subsp. Arvense	Whistler	-0.07	-1.23	-1.01
	Variety Unstated	-0.07		
Trifolium incarnatum	AU Sunrise	-0.44		
	AU Robin	-0.50		
	Dixie	-0.60		
	Tibbee	-0.46		
Trofolium alexandrium	Bigbee	-0.46		
Trifolium subteraneam	Denmark	-0.40		
Lupinus	TifBlue78	-0.62		

Corrected B-values used for Ndfa calculations

Species	Inoculant $\delta^{15}N$	Piedmont $\delta^{15}N$	Tidewater $\delta^{15}N$
Vicia villosa L.	-0.09	-0.68	-0.53
Vicia sativa L.	-0.34	-1.38	-0.92
Pisum sativum subsp. Arvense	-0.07	-1.23	-1.01

Table 2-9: Nitrogen derived from the atmosphere

Species		Piedmont2009		Tidewater2009		Caswell 2010		Piedmont 2010	
		Ndfa (%)	TotalN (kg ha^{-1})	Ndfa (%)	TotalN (kg ha^{-1})	Ndfa (%)	TotalN (kg ha^{-1})	Ndfa (%)	TotalN (kg ha^{-1})
Vicia villosa L.	AUE	99 ±10	148 ±25	68 ±10	131 ±14	99 ±3	77 ±9	82 ±3	136 ±13
	AUM	100* ±10	174 ±25	87 ±10	152 ±14	97 ±3	109 ±9	83 ±3	135 ±14
	EAR			87 ±10	164 ±14	92 ±3	69 ±9	76 ±3	143 ±13
	PRO	76 ±10	135 ±25	59 ±10	109 ±14	93 ±3	63 ±10	87 ±4	150 ±15
Vicia sativa L.	VET	82 ±10	123 ±25	74 ±10	106 ±14	90 ±3	57 ±9	79 ±3	125 ±13
Pisum sativum subsp. Arvense	PEA	71 ±10	69 ±25			87 ±3	80 ±9	79 ±3	130 ±13
	WHI	75 ±10	62 ±25	73 ±10	101 ±14	89 ±3	71 ±10	79 ±3	122 ±13
Trifolium incarnatum	AUS	100 ±13	127 ±30	66 ±15	67 ±20	97 ±4	47 ±13	87 ±5	140 ±21
	AUR	95 ±13	136 ±30	71 ±15	77 ±20	100 ±4	58 ±13	83 ±5	133 ±21
	DIX	85 ±13	101 ±30	71 ±15	73 ±20	97 ±4	62 ±13	88 ±5	119 ±21
	TIB			89 ±15	91 ±20	100 ±4	64 ±13	87 ±5	115 ±21
Trofolium alexandrium	BIG	100 ±13	139 ±30	96 ±15	73 ±20	100 ±3	42 ±10	94 ±3	151 ±13
Trifolium subterraneam	DEN	100 ±13	16 ±30	34 ±15	4 ±20	99 ±4	53 ±13	62 ±5	68 ±21
Lupinus agustifolus	TIF	98 ±13	43 ±30	73 ±15	37 ±20	100 ±4	57 ±13	61 ±5	21 ±21
V. villosa + *S. cereale*	MXE					92 ±3	56 ±10	80 ±3	136 ±13
	MXM					88 ±3	117 ±9	80 ±3	174 ±13
P. sativum + *S. cereale*	MXP					84 ±3	65 ±9	73 ±3	120 ±13

Estimates are in the form of Least Squares Mean±SE. Data was calculated using Proc Mixed.

Table 2-10: Grain Yields (Mg ha-1)

	Tidewater 2009			Piedmont 2009			Caswell 2010		Piedmont 2010	
Treatment	Early	Mid	Late	Early	Mid	Late	Mid	Late	Mid	Late
0 N	5.6 abcd	6.2 cde	4.8 cde	5.0 cde	5.7 bcde	5.1 cde	1.1 cd	1.4 bcd	2.7 cdefgi	4.4 bcde
56 N	6.1 abc	8.0 bc	7.4 ab	6.4 bc	5.5 bcde	5.4 bcde	1.7 b	1.3 bcde	4.4 abc	5.2 bcd
112 N	7.2 ab	11.7 a	8.8 a	9.6 a	9.3 a	7.3 ab	2.9 a	2.3 a	5.5 a	6.4 ab
168 N	7.8 a	10.3 ab	8.3 a	8.4 ab	5.8 bcd	5.6 bcd	3.0 a	1.6 bc	5.4 ab	7.6 a
AU Early	2.6 fghi	7.3 cd	8.5 a	7.6 ab	7.5 ab	7.9 a	1.0 cd	0.8 defg	3.8 abcdef	3.4 cdefg
AU Merit	1.0 hi	3.6 fgh	7.8 ab	5.3 cd	7.0 bc	8.4 a	0.9 cd	1.9 ab	3.4 abcdefg	3.1 defgh
Winter Hardy	2.2 ghi	4.7 efg	6.7 abc				1.3 bcd	0.8 defg	4.1 abcde	3.4 cdefg
Purple Prosperity							1.3 bc	1.1 cdef	3.2 bcdef	3.0 defgh
Common Vetch	0.5 i	1.7 hi	5.7 bcd	1.5 gh	5.5 bcde	6.6 abc	0.7 d	0.9 cdefg	4.2 abcde	4.0 cdef
AU Robin	4.5 cdefg	5.9 cdef	4.3 cdef	4.0 def	5.7 bcd	3.6 def	0.7 cd	0.7 efg	2.6 cdefg	2.0 fgh
AU Sunrise	4.0 cdefg	4.2 efg	3.1 ef	5.4 cd	5.6 bcde	4.7 cdef	0.9 cd	0.5 fg	3.4 abcdefg	2.5 efgh
Dixie	4.8 bcdef	4.2 efg	5.5 bcde	3.8 def	5.0 cde	3.5 ef	1.1 bcd	0.6 defg	2.1 efg	1.8 fgh
Tibbee	3.2 defgh	4.2 efg	4.3 cdef				0.9 cd	0.7 efg	2.5 cdefg	1.6 gh
Frontier							0.7 cd	0.5 fg	1.8 fgh	0.9 h
BigBee	0.8 hi	0.7 i	2.3 f	0 h	1.0 f	4.3 def	1.0 cd	0.7 efg	2.2 defg	5.0 bcd
Denmark	5.0 bcde	5.5 def	4.4 cdef	2.5 fg	3.6 e	2.9 fg	0.8 cd	1.1 cdef	1.1 g	0.7 h
Pea				3.1 efg	5.3 cde	5.0 cde	0.8 cd	0.9 defg	3.1 cdefg	2.0 fgh
Whistler	4.4 cdefg	5.5 def	5.8 bcd	3.5 defg	4.3 de	4.2 def	0.7 cd	0.8 defg	3.9 abcdef	3.0 defgh
Mix Early							1.1 bcd	0.7 efg	4.3 abcd	5.6 abc
Mix Merit							1.0 cd	1.0 cdefg	5.4 ab	5.1 bcd
Mix Pea							1.0 cd	1.1 cdef	3.2 cdefg	4.4 bcde

Contrasts	**Tidewater 09**		**Piedmont 09**		**Caswell 10**		**Piedmont 10**	
	CC	CC*Rolltime	CC	CC*Rolltime	CC	CC*Rolltime	CC	CC*Rolltime
Hairy vetch vs. crimson clover	ns	***	***	***	***	**	*	ns
Hairy vetch vs common vetch	***	ns	***	ns	ns	ns	ns	ns
Hairy vetch: AUE vs others	**	ns	ns	**	ns	ns	ns	ns
Crimson clover: AUS vs others	ns	ns	ns	ns	ns	ns	ns	ns
Rye+Hairy vetch vs hairy vetch					**	ns	***	ns
MXE,MXM&MXP vs hairy vetch and pea					ns	ns	***	***

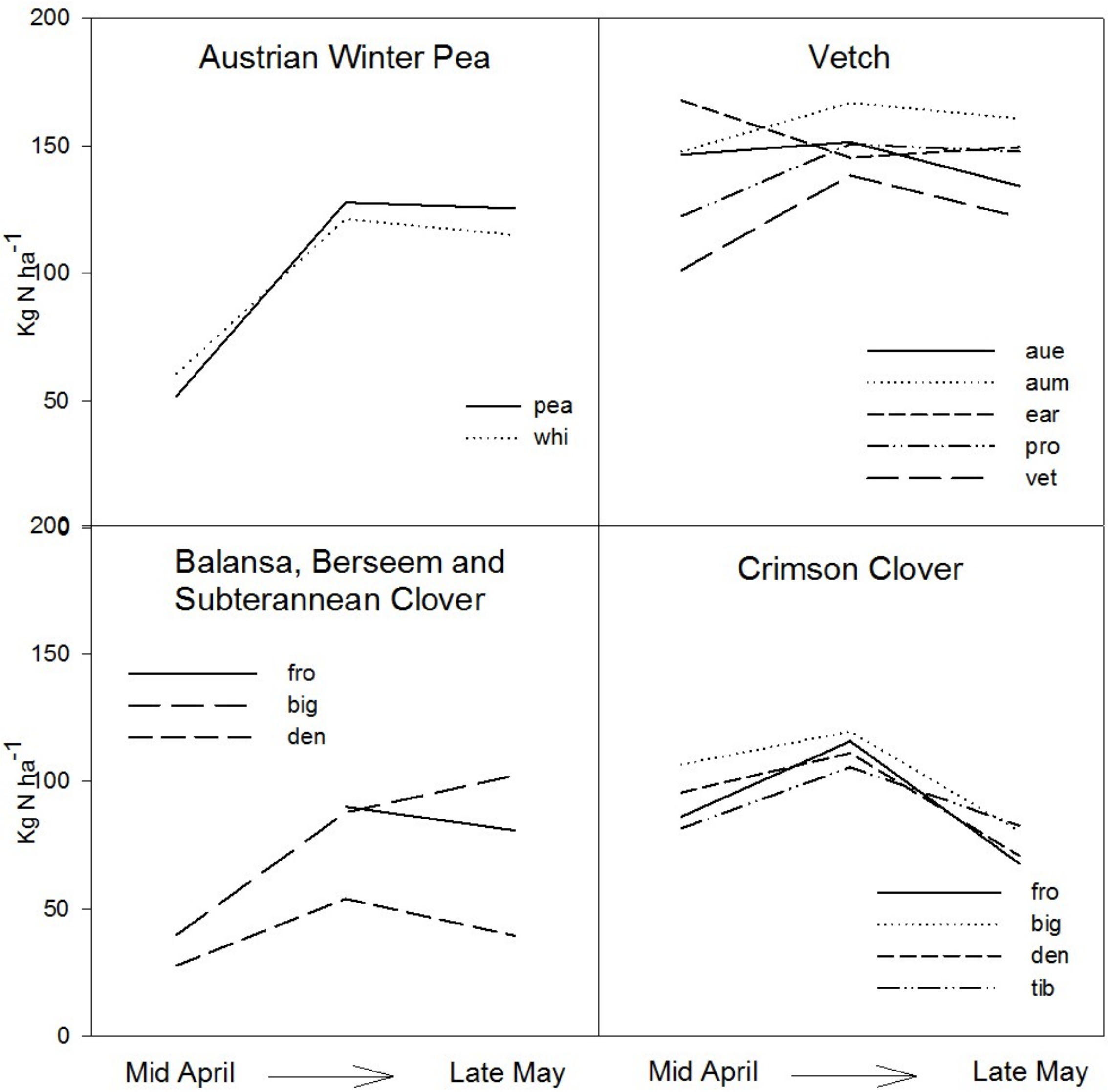

Figure 2-1: Change in biomass N accumulation for winter annual cover crops in North Carolina.

Figures are based on combined least squares means for biomass N accumulation in 2009 and 2010. Crimson, balansa and subterranean clover peak in total biomass N in mid to late April, Hairy and common vetch, Austrian winter pea and berseem clover maintain and increase biomass N through late May.

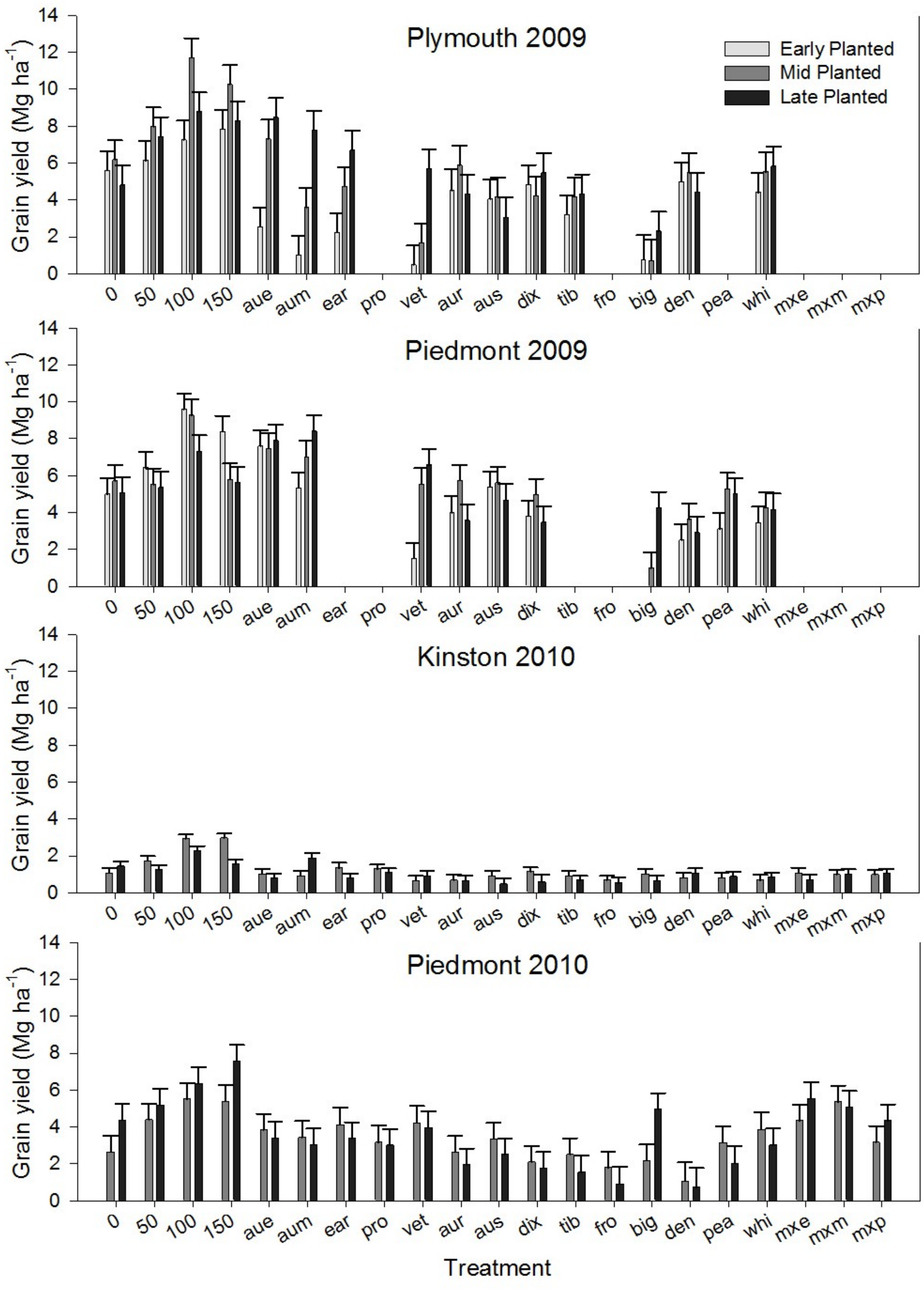

Figure 2-2: Corn Grain Yields 2009 and 2010

Chapter 3: **Nitrogen (N) cycling under roll-killed winter annual cover crops in North Carolina organic corn production**

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INTRODUCTION

Over the past decades, a variety of cultural practices have been developed to reduce the impact of agriculture on the environment. Seeking to integrate the benefits of organic farming, cover crop use and no-till agriculture, producers have recently shown increased interest in the roller-crimper, a tractor-mounted implement with a water-filled rolling drum that mechanically terminates a cover crop by crimping the stems while leaving the root system and soil undisturbed, as a tool with potential to resolve the often conflicting limitations of these three systems. This new "no-till organic system" presents questions regarding how roll-killed residue acts in terms of nutrient cycling, nitrogen (N) mineralization, and plant nutrient availability.

Legume cover crops are a major source of N in organic production systems, with estimates of added N from winter annual cover crops ranging between 100 and 200 kg N ha^{-1} $year^{-1}$ (Drinkwater et al., 2008; Fageria et al., 2005; Ott and Hargrove, 1989). In addition to fixing atmospheric N, cover crops provide numerous benefits including reduced soil erosion, increased water infiltration, increased soil organic matter and soil nutrient scavenging (Torbert et al., 1996; Vieira et al., 2009).

Reducing tillage in agriculture has been shown to have numerous environmental benefits including conservation of soil carbon through decreased respiration, improved infiltration and decreased bulk density (Blevins et al., 1983; Golabi et al., 1995). There are distinct differences between reduced / no-till and organic production systems, with the former relying on herbicides for weed and cover crop management, and the later on tillage. Cover crop management often requires multiple tillage operations in order to terminate the cover as well as provide an adequate seed bed for the succeeding crop, which can result in a loss of soil organic matter through increased soil respiration (Yaduvanshi and Sharma, 2008) as well as a destruction of soil aggregates and increased soil erosion (Jasa and Dickey, 1991). Further, incorporating residues increases decomposition and N release leading to a lack of crop-N synchrony, or coincidental timing of soil N availability and crop N needs (Gaskell and Smith, 2007), where cover crop N is mineralized before the subsequent crop has the root structure to access it.

Success of the roller-crimper technology as an alternative cover crop management tool may pave the way for widespread adoption of a reduced-till organic system. This system would differ dramatically from either a tilled organic system or a no-till non-organic system in nutrient availability, timing and weed control. First, organic producers are limited in their use of synthetic N applications (Rosen and Allan, 2007), so the N supplied by the rolled cover would need to be adequate to support the subsequent crop. Second, the roller-crimper leaves rolled mulch on the soil surface. Since the amount of soil contact is known to affect decomposition (Swift et al., 1979), nutrient release patterns from rolled residues may be significantly different than incorporated cover crop residues. Third, research with small grain cover crops has shown that the roller-crimper is most effective after anthesis (Ashford and Reeves, 2003). In legumes, this stage is early pod set, which, in the Northeastern US has been shown to occur in May, later than desired corn planting time in North Carolina (Mischler et al., 2010; Teasdale et al., 2004, Heiniger et al., 2000).

Hairy vetch (*Vicia villosa* Roth) and crimson clover (*Trifolium incarnatum* L.) are two commonly used legume cover crops in organic production in North Carolina. Hairy vetch is reported to fix more than 100 kg N ha^{-1} and up to 211 kg N ha^{-1}, and crimson clover up to 150 kg N ha^{-1} (Fageria et al., 2005; Mueller and Thorup-Kristensen, 2001; Sainju and Singh, 2008). Early flowering, southern adapted varieties, "AU Early Cover" hairy vetch and "AU Sunrise" crimson clover were developed at Auburn University. AU Early Cover has been known to reach flowering 15 days earlier than common vetch and AU Sunrise has been shown to flower 12 to 28 days earlier than older cultivars of crimson clover, making them potentially ideal candidates for use with the roller-crimper (Mosjidis and Owsley, 2002; Teasdale et al., 2004).

Estimated N release and availability from cover crop residues can vary based on measurement approach. Plant available N is a dynamic pool within the soil, regulated by both microbial decomposition of residues, plant uptake and losses to the environment, such as leaching and volitalization (Drinkwater and Snapp, 2007). Point measurements based on one of these pools, such as N remaining in residues, soil mineral N, or crop uptake, may result in an incomplete understanding of nutrient movements. For example, a study in which ^{15}N labeled cover crop residues were applied to a corn crop found that 90% of the N in the residues was released, but the corn N recovery values were less than 40% (Wagger et al., 1985). However, litter bag study on similar residues found that 70 to 90% of all biomass N was released from hairy vetch and crimson clover residues in the first 10 weeks after kill, much of it earlier than the period of highest N demand by corn (Ranells and Wagger, 1996b). Various studies reviewed by Crews and Peoples (2005) show that legume residues are often subject to 'pool substitution' where the flush of N released from decomposing residues is immobilized in the soil and resident pools of microbial N are mineralized (Crews and Peoples, 2005).

If microbial mineralization approximates plant uptake, the inorganic pool may be small despite the total N flux being large (Drinkwater et al., 2008). Traditional soil extractions measure inorganic N at a specific point in time, but this snapshot of a single pool may fail to account for the flux of nutrients between pools. Ion-exchange membranes have been shown to measure nitrogen-supply power of soils by acting as an ion sink, collecting nutrients over time (Qian and Schoenau, 2002). Similarly to a plant root, ion exchange membranes continuously remove accumulated NO_3^--N and NH_4^+-N and can account for ion diffusion over distances greater than what is in immediate contact with the probe, as well as fluctuating rates of microbial mineralization, making it a reliable measure of time-integrated plant available N (Bair et al., 2008; Qian and Schoenau, 2007).

We hypothesized that N from the biomass of roll-crimped crimson clover and hairy vetch residues would be made available to the corn crop through microbial mineralization in synchronous timing to the N needs of a corn crop. We tested this by: (i) measuring soil N availability by chemical extraction and in-situ ion exchange resin methods under hairy vetch and crimson clover mulches, (ii) comparing these measurements to split applied inorganic N fertilizer and (iii) determining how N availability affected grain yield.

MATERIALS AND METHODS

Site Descriptions

The experiment was conducted over two years. In 2008/2009 it the experiment at the Tidewater Research Station in Plymouth, NC on a Portsmouth (Fine-loamy over sandy or sandy-skeletal, mixed, semiactive, thermic Typic Umbraquults) soil and at the Piedmont Research Station in Salisbury, NC on a Lloyd soil (Fine, kaolinitic, thermic Rhodic Kanhapludults), with previous crop at both sites corn, and the experiment was repeated on a new field each year. In 2009/2010, the experiment was planted at the Caswell Research Farm in Kinston, NC on a Pocalla loamy sand (Loamy, siliceous, subactive, thermic Arenic Plinthic Paleudults) and again at the Piedmont Research Station in Salisbury NC on a Mecklenburg Loam (Fine, mixed, active, thermic Ultic Hapludalfs).

Cultural Practices

Hairy vetch, cv. “AU Early Cover” (HV) and crimson clover cv. “AU Sunrise” (CC) were planted in the fall of 2008 and 2009. The HV treatment was planted at a seeding rate of 28 kg ha^{-1} and CC at a seeding rate of 22.4 kg ha^{-1}. In 2009, a rye (*Secale cereale)* and hairy vetch biculture (RYE+HV) was added with a seeding rate of 56 and 28 kg ha^{-1} respectively. Cover crops were roll-killed on dates that corresponded well with early pod set in hairy vetch and corn was no-till planted directly into the residue immediately following roll-kill (Table 3-1). For the Tidewater site, CC was roll-killed on April 29, 2009 and HV on May 13, 2009. At Piedmont, both cover crops were roll-killed May 8, 2009 and May 28 2010, and the Caswell site on May 6, 2010. Bare ground control plots were treated with 0 kg ha^{-1} N and 168 kg ha^{-1} N (39.2 kg ha^{-1} N applied as urea-ammonium nitrate at planting and the remainder applied at 4 weeks after planting were used as negative (0N) and positive (168N) controls, respectively. Bare ground 0N control plots were used to measure existing organic matter mineralization rates. Control plots were treated with 0.84 kg glyphosate to control weeds, and a pre-emergent herbicide in the form of 1.41 kg a.i. ha^{-1} metolachlor and 1.82 kg a.i. ha^{-1} atrazine; corn within control plots was planted on the same dates as the CC plots for all sites.

Sampling and Analysis

Cover Crop Sampling

At roll-kill, a 0.5m^2 quadrat of cover crop biomass was removed from each plot, dried at 65°C, weighed and ground using a Wiley mill to pass a 2mm sieve. The sample was then homogenized and a subsample was collected and analyzed for total N and carbon(C) at the NCSU Analytical Services Lab using a Perkin Elmer 2400 CHNS/O Elemental Analyzer.

Soil NO_3^- and NH_4^+:

Soil samples were taken at roll-kill and then at approximately 2 week intervals for a total of 12 weeks (Table 3-1). Nine samples per plot were collected with a 2 cm diameter soil probe from 0 – 20 cm, bulked, and sub-sampled. Due to dry conditions and difficulty sampling, four 10 cm samples were collected per plot at Piedmont on June 30, 2009, and in 2010, all soil samples in Piedmont were collected with a 5cm auger to a depth of 20 cm. Samples were transported to NCSU where soils were air dried at 40°C for 2 days, ground and sieved to pass a 2mm screen. Samples were extracted using 1M KCl and shaken for 1 hour. The samples were allowed to settle for 20 minutes before filtration through pre-rinsed #42 Watman filter paper. Extracts were frozen until analysis for NH_4^+ and NO_3^- on a QuikChem 2000 flow injection autoanalyzer (Lachat Instruments, Loveland CO).

Root Simulator Probes:

Mineral NO_3^--N and NH_4^+-N fluxes were measured using ion-exchange resin strips (Plant Root Simulator (PRS) Probes; Western Ag Innovations, Saskatoon SK Canada). At the time of roll-kill, 4 pairs of PRS probes, including one anion and one cation, were inserted vertically into the soil beneath the roll-killed legume mulch to a depth of 10 cm. In order to minimize root competition for ions adsorbed to the membrane, probe pairs were inserted into root exclusion devices consisting of PVC cylinders 15 cm in length and 10 cm in diameter that had been driven vertically into the soil at four points of the center inter-row space, allowing consistent monitoring of the same area of soil. Probes were exchanged at approximately 2-week intervals on the dates of soil sampling. Upon removal, probes were scrubbed clean with de-ionized H_2O and shipped to Western Ag Innovations for extraction and analysis.

Corn Grain Yield

In 2009, six meters of corn were hand-harvested from each plot. Total ear weight was taken in the field. Ears were transported back to NCSU and shelled using a single ear electric sheller. Total ears, ear weight and kernal weights were recorded. Moisture content of grain was measured by drying sub-samples at 70°C for 48 hours. In 2010, plots were trimmed to 6 m in length and outer edge rows were removed, grain was harvested from the center two rows using an Allis Chalmers K2 Gleaner combine. Final plot dimensions and grain weights were taken in the field; moisture content was determined using a Dickey John miniGAC Plus Moisture Tester, and re-adjusted to reflect 15.5% moisture.

Data Analysis

All statistical analysis was conducted using Statistical Analysis Software (SAS, Cary NC). Separate analysis was conducted for each location and year. Cover crop attributes, including biomass, %N, C:N ratios and total N were analyzed using the Proc mixed command for mixed models; block and block×treatment were included as random factors. Total biomass and biomass N were square root transformed for analysis and least squared means were back transformed for reporting. Means separations were performed using protected LSD at $\alpha = 0.05$.

Potassium chloride extractable soil N and PRS probe data were analyzed using the Proc GLM procedure for repeated measures, with block, block×treatment and block×time included as random factors. Means separations were performed for each timepoint of both the KCl extractable soil N and PRS probe N using a protected LSD.

RESULTS

Cover crop mulch attributes

Cover crops were well established both years. In 2009, HV produced 203 and 163 kg N ha^{-1} and CC, 124 and 156 kg N ha^{-1} in Tidewater and Piedmont, respectively. Across sites, mean N concentrations were 3.1% and 2.3% for HV and CC respectively. In 2010, total N production for CC was only 56 and 32 kg N ha^{-1} and for HV was 163 and 64 kg N ha^{-1} for Piedmont and Caswell, respectively. Total tissue N concentrations were also lower than in 2009 at 2.5% for HV and 1.5% for CC. Mean biomass in 2009 was approximately two to three times greater than Caswell 2010 and total N levels were three to four times greater (Table 3-2). This can be attributed to low winter temperatures in 2010 and a drier spring. In three of the four site years, HV produced more total N than CC, with the exception being Piedmont in 2009, where there was no significant difference in biomass N between the two species. In 2010, RYE+HV produced significantly more biomass than the HV cover crop but due to lower %N in the mixed residue, total N for the two treatments remained the same. Ratios of C:N are often used to predict whether residue N will mineralize or be immobilized by soil micro-organisms. HV, CC and

RYE+HV C:N ratios were below the theoretical "mineralization threshold" of 30:1(11-17, 17-27 and 22-23 respectively), suggesting that N from these residues will be available for crop uptake (Figure 3.1).

N release patterns differ for cover crop treatments

Nitrogen release in the form of soil extractable N as well as PRS adsorbable N, defined as N flux, was found to be significantly affected by treatment, time and time×treatment interaction. In all site years, 168N had the greatest values of extractable N and N flux when compared to both cover crop treatments and the 0N control ($p<0.0001$). Among cover crop treatments, in 2009 HV had greater extractable N and N flux than CC ($p<0.05$). In 2010, both HV and HV+RYE had greater N than CC in Piedmont but no cover crop treatments were significantly different than the 0N control at the Caswell site.

Soil inorganic N under hairy vetch

In 2009, peak extractable N under HV occurred at 4 weeks after roll-kill, and declined in subsequent sampling dates from 20.9 mg kg^{-1} at wk4 to 4.4 mg kg^{-1} at wk12 in Piedmont and 36.2 mg kg^{-1} at wk4 to 8.2 mg kg^{-1} at wk12 in Tidewater (Figures 3.2 and 3.3). The greatest N flux was measured during the 2-6 weeks after roll-kill for both the Plymouth and Piedmont locations (734 and 876 µg $10cm^{-1}$ $2weeks^{-1}$, respectively). This pattern was similar in Caswell in 2010, with extractable soil N peaking at 4 weeks and soil N flux peaking between 4 and 6 weeks under all cover crop treatments. This trend was not observed in Piedmont 2010. While extractable N declined over the course of the growing season from 12.4 mg kg^{-1} to 3.2 mg kg^{-1}, N adsorbed by PRS probes declined for the first 6 weeks after roll-kill, and then peaked between 6 and 8 weeks for HV (500 µg $10cm^{-1}$ $2weeks^{-1}$) and between 8 and 10 weeks for RYE+HV (521 µg $10cm^{-1}$ $2weeks^{-1}$). It was notable that extractable soil N was equal to or lower than the 0N treatment throughout the growing season at this site. N flux also remained lower than the 0N treatment until 10 to 12 weeks after roll-kill it began to increase, suggesting net N immobilization. However, grain yields only reflected low soil N in the HV treatment, which was not significantly different than 0N. However RYE+HV grain yield was not significantly different from the 168N control (Figure 3.6).

<u>Soil N under crimson clover</u>

Soil N flux data, as measured with the PRS probes, suggested net soil N mineralization of CC residues was inadequate to compensate for N uptake of the cover crop while it was growing, resulting in net immobilization in the CC treatment plots for much of the corn season. Under CC, Tidewater extractable N at wk0 was significantly lower ($p<0.05$) than the 0N treatment (19.5 mg kg^{-1} vs 8.5 mg kg^{-1} for 0N and CC, respectively), suggesting soil N uptake by the CC cover crop. CC extractable N peaked at 20.1 Mg kg^{-1} and declined to 8.9 mg kg^{-1} at wk12 in Tidewater. The 0N treatment, however, increased from 19.5 at wk 0 to 31.2 mg kg^{-1}at wk4, and CC extractable N increased from 8.5mg kg^{-1} at wk0, to 20 mg kg^{-1} at wk4. In Piedmont, extractable N under CC closely followed soil N under 0N, with an initial peak at wk4 of 19.4 mg N kg^{-1}, followed by a decline at wk6, and a subsequent increase to 15.2 mg kg^{-1} at wk8. Following roll-kill, extractable N under CC was significantly lower than 0N from week 0 to week 4 in Tidewater 2009, and from 0-6 weeks in Piedmont 2010 ($p<0.05$). In Piedmont 2009 and Caswell 2010, there was no significant difference between extractable N of CC and 0N. Nitrogen adsorbed by PRS probes in CC remained less than N flux in the 0N treatment until 6 weeks after roll kill in Plymouth 2009, and 8 weeks in Piedmont 2009 (Figures 3.2 and 3.3). This was also reflected in the lack of response by corn grain yields CC residues (Figure 3.5) In 2010, CC N flux in Piedmont was lower than 0N during the 6 to 8 week period ($p<0.05$), but was not significantly different during the rest of the season. At Caswell, there was no significant difference in N flux between CC and 0N the entire season (Figures 3.4 and 3.5), however corn grain yields from the CC treatment were significantly less those from 0N.

DISCUSSION

Mineralization of rolled mulches

Trends showing crimson clover differing in N release compared to hairy vetch are not well documented in the literature. In a tilled study exploring N contributions of HV and sweet clover to a Concord grape crop, Bair et al. found that the two legumes did not differ in their N release rates following incorporation (Bair et al., 2008), however previous work with crimson clover in no-till settings show mixed crop response to surface residues. Touchton et al. (1982) failed to observe a sorghum crop yield response to CC residues in instances where one was observed to applied N fertilizer. Previous work on the use of legume cover crop residues in no-till systems has shown that both hairy vetch and crimson clover release N from their residues, and can result in significant yield increases (Ranells and Wagger, 1996a; Wagger, 1989; Wagger et al., 1998). This previous work on no-till cover crop residues relied on chemical dessication to terminate crops, which has been shown to increase N mineralization compared to untreated residues (Snapp and Borden, 2005). A combination of termination methods and quality seems to have a significant impact on N mineralization potential, which could explain why net immobilization was observed in CC and more frequently than in HV. Ratios of C:N in the CC residues were higher than HV, but equal to those of the RYE+HV mixture (Table 3-2 and Figure 3-1), and while soil N was low under the biculture, it was much less pronounced and did not result in yield reductions compared to 0N. Crimson clover is known to have higher cellulose and hemicelluloses content than HV (Wagger et al., 1998), and while phenolic content of CC is not documented in the literature, it is also know to affect N mineralization potential (Palm and Sanchez, 1990).

Soil core experiments have compared decomposition and N mineralization potential of immature rye residues (C:N=16, lignin=1.4%) with those of mature oilseed rape, *Brassica napus* L. (C:N=29, lignin=6.7%) in both incorporated and surface applied situations. Decomposition was affected by both residue quality and treatment: after 3 and 9 weeks, only 33% and 31% of applied rye C remained, whereas 69% and 45% rape residue C remained in incorporated treatments, and 66% or rye C and 82% of rape C remained in surface applied treatments after 9 weeks.

Mineral N concentrations were also greater for both surface applied and incorporated rye residues than for rape residues (Coppens et al., 2007). This could potentially be compared to this study, with the low C:N rye residues comparing to HV and higher C:N ratio oilseed rape residue comparing to CC.

Soil N and plant available N affected by moisture

In this study, we used two methods for measuring plant available N, traditional soil extractions and ion resin probes. Extracting inorganic N from soil samples with KCl is an established protocol for measuring inorganic N in agricultural soils, but does not quantify mineralizable N or that contained in microbial pools (Burger and Jackson, 2003). Because traditional extractions are conducted with a solution, they do not change based on moisture content of the soil. PRS probes on the other hand adsorb N ions in situ, over time, theoretically similar to a plant root, and are therefore subject to soil physical properties that would inhibit transport and diffusion of N ions in the bulk solution (Qian and Schoenau, 2002).

The difference in measurements between KCl extractions and ion exchange membranes was clearly demonstrated in this experiment, particularly in Piedmont, and Caswell 2010. Under consistently moist conditions as observed in Plymouth in 2009, these differences were minimal. However, Piedmont in 2010, there was a three week period shortly after roll kill with nearly no rainfall. During this time, soil N measured using PRS probes fell sharply for all treatments, including 168N, which received a surface application of urea during this period, suggesting that N diffusion, required for PRS probes to adsorb nutrients, and theoretically for nutrients to move to roots, slowed considerably during this period. The fertilizer application appears in the KCl extractable data as a spike at 4 weeks after planting. The PRS probes, however do not detect this additional N until 2 to 4 weeks later, following a rain event. (See Figure 3.4) A similar pattern is visible in Piedmont 2009 and Caswell, where a period of little to no rainfall corresponds with a drop in PRS adsorbable N and a rise in KCl extractable N. This suggests that despite mineral N being present in the soil, less was available to the crop as a result of low soil moisture, and that the use of PRS probes give a more complete picture of soil N dynamics than extracted soil N alone. Coppens et al. (2007) found that decomposition of surface applied residues essentially ceased within 5 days following a simulated rain event as a result of moisture limitations, which could also explain reduction in N flux under residues during these periods.

Grain yields and N synchrony

Nitrogen synchrony is defined as matching soil N availability with crop needs, this requires reducing periods of excess-asynchrony as well as periods of N limitations (Crews and Peoples, 2005). To this end, roll-killed mulches show promise in that, despite low levels of soil N, corn planted into rolled mulches performed better than corn in what kind of? plots where soil N was higher for most of the season (See figure 3.6). In Piedmont 2009, HV had lower extractable N and smaller N fluxes than 168N, but grain yields were higher at 7.5 Mg ha^{-1} for HV vs. 5.8 Mg ha^{-1} for 168N.

This was also true of the RYE+HV biculture where in Piedmont 2010, soil N under this mulch was lower than for HV alone or the 0N treatment, yet the RYE+HV treatment had higher grain yield, 5.6Mg ha^{-1} vs 4.4 and 3.4 Mg ha^{-1} for RYE+HV, 0N and HV respectively. If the yield response between 0N and 168N suggests that this was an N limited system, other N pools could be responsible for tighter N cycling. Previous research comparing N pools in an organic system (N sources = animal manure and legume cover crops) with a conventional system (N sources = inorganic fertilizer) has shown that soils supplied with organic additions had significantly greater potentially mineralizable N and microbial biomass N pools, despite showing no difference in inorganic NH_4^+ or NO_3^- concentrations, and that NO_3^- immobilization by microbes was higher in the organic system (Burger and Jackson, 2003). Higher microbial activity as a result of residue inputs under mulch treatments in this experiment could account for lower soil extractable N pools and N fluxes seen in RYE+HV and HV treatments as compared to 168N. Greater yield responses to RYE+HV over HV in Piedmont 2010 suggest that the slower release rate may better synchronize with the N needs of a corn crop. This agrees with previous research that has found that N contribution of *Sesbania rostrata* to a rice crop coincided better with the N needs of the rice crop when mixed with rice straw (Becker and Ladha, 1997).

Conclusions

This research shows that in a roll-killed cover crop system, nitrogen release patterns differ dramatically by residue type, with HV having a faster release than CC and RYE+HV, and CC causing net soil N immobilization and yield reductions compared to HV and RYE+HV. Greater yield response to RYE+HV suggest that when cover crop biomass is high, the slower N release rate of a legume-grass biculture may synchronize better with corn N uptake. More research is needed to determine the source of the observed reduced N mineralization by CC residues when terminated by the roller-crimper, and how this might compare to other mechanical or chemical cover crop management methods relative to the N synchrony of a corn crop.

The PRS ion exchange resins complimented extracted total soil inorganic N data to demonstrate differences between total N, and plant available N related nutrient flux and mobility. Continued research investigating nitrogen pools and microbial response to roll-killed cover crops would further illuminate questions regarding cover crop N fate and lead to more efficient management of N in agroecosystems.

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FIGURE CAPTIONS, TABLES AND FIGURES

Table 3-1: Soil Sample and PRS Probe Measurements

	Tidewater Year 1		**Piedmont Year 1**	**Caswell Year 2**	**Piedmont Year 2**
	Hairy Vetch	CC N-rate controls	All Treatments	All Treatments	All Treatments
Cover crop planted	2 October 08		25 September 08	28 October 09	9 October 09
Cover crop killed / corn planted	13 May 09	29 April 09	8 May 09	6 May 10	28 May 210
Soil Sample 1 / PRS 1 inserted	13 May 09	29 April 09	8 May 09	6 May 10	28 May 10
Soil Sample 2 / PRS exchanged	28 May 09	13 May 09	21 May 09	20 May 10	11 June 10
Soil Sample 3 / PRS exchanged	11 June 09	28 May 09	2 June 09	3 June 10	24 June 10
Soil Sample 4 / PRS exchanged	24 June 09	11 June 09	16 June 09	17 June 10	8 July 10
Soil Sample 5 / PRS exchanged	8 July 09	24 June 09	30 June 09	1 July 10	22 July 10
Soil Sample 6 / PRS exchanged	22 July 09	8 July 09	14 July 09	15 July 10	5 August 10
Soil Sample 7 / PRS removed	5 August 09	22 July 09	28 July 09	29 July 10	19 August 10

Table 3-2: Cover Crop Attributes

		Tidewater			Piedmont				Caswell			
		CC	Hairy Vetch		CC	Hairy Vetch	Rye+ HV		CC	Hairy Vetch	Rye+ HV	
2009	Biomass (Mg ha^{-1})	5.4	6.4	ns	6.6	5.2	-	ns	-	-		
	%N	2.30 b	3.16 a	*	2.36 b	3.11 a	-	*	-	-		
	C:N	18.3 a	11.9 b	*	17.7 a	13.4 b	-	*	-	-		
	Total N (kg ha^{-1})	124 b	203 a	*	156	163	-	ns	-	-		
2010	Biomass (Mg ha^{-1})	-	-		3.6 b	4.7 ab	6.6 a	*	2.6	2.0	3.0	ns
	%N	-	-		1.57 b	2.89 a	1.96 b	*	1.58 b	2.46 a	1.90 b	*
	C:N	-	-		26.9 a	15.3 b	22.6 a	*	26.0 b	17.3 a	23.2 b	*
	Total N (kg ha^{-1})	-	-		56 b	163 a	130 a	*	32 b	64 a	56 a	*

Table 3-3: Age of Cover Crops at roll kill

Cover Crop	Year	Location	DAP	Julian Date	GDD(F)
Hairy Vetch	08/09	Tidewater	223	133	1591
		Salisbury	225	128	1245
	09/10	Caswell	190	126	1304
		Salisbury	231	148	1336
CC	08/09	Tidewater	209	119	1332
		Salisbury	225	128	1245
	09/10	Caswell	190	126	1304
		Salisbury	231	148	1336

GDD is calculated for a base temperature of 40F (4C)

Table 3-4: Grain Yelds (Mg ha^{-1})

Treatment	Plymouth 2009		Piedmont 2009		Caswell 2010		Piedmont 2010	
0N	6.2	c	5.7	b	1.4	ab	4.4	bc
168 N	10.3	ab	5.8	b	1.6	a	7.6	a
Hairy Vetch	8.5	b	7.6	a	0.8	b	3.4	bc
CC	3.1	d	5.4	b	0.5	b	2.5	c
Rye + HV					0.7	b	5.6	ab

Means with the same letter are not different from one another at α=0.05, determined by protected LSD

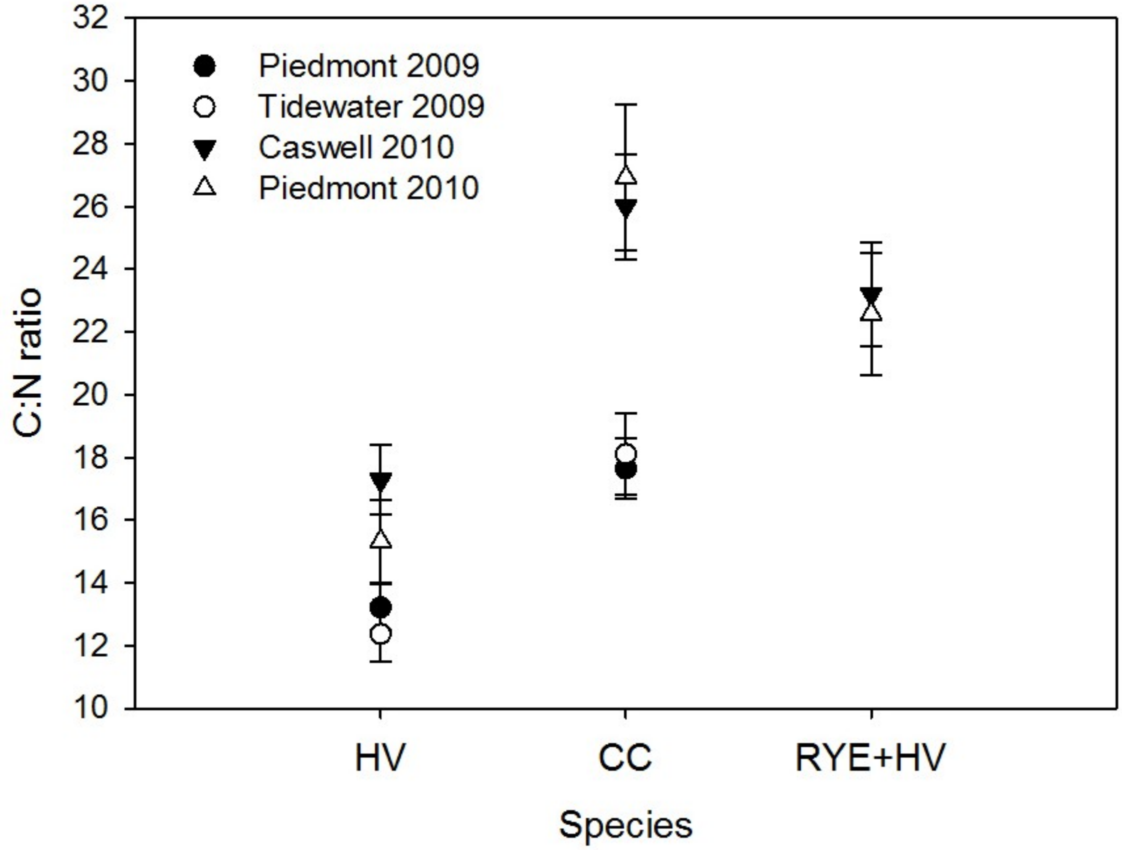

Figure 3-1: Ratios of C:N for cover crop treatments. 2010 C:N ratios (triangles) were significantly greater than in 2009 (circles) for both HV and CC.

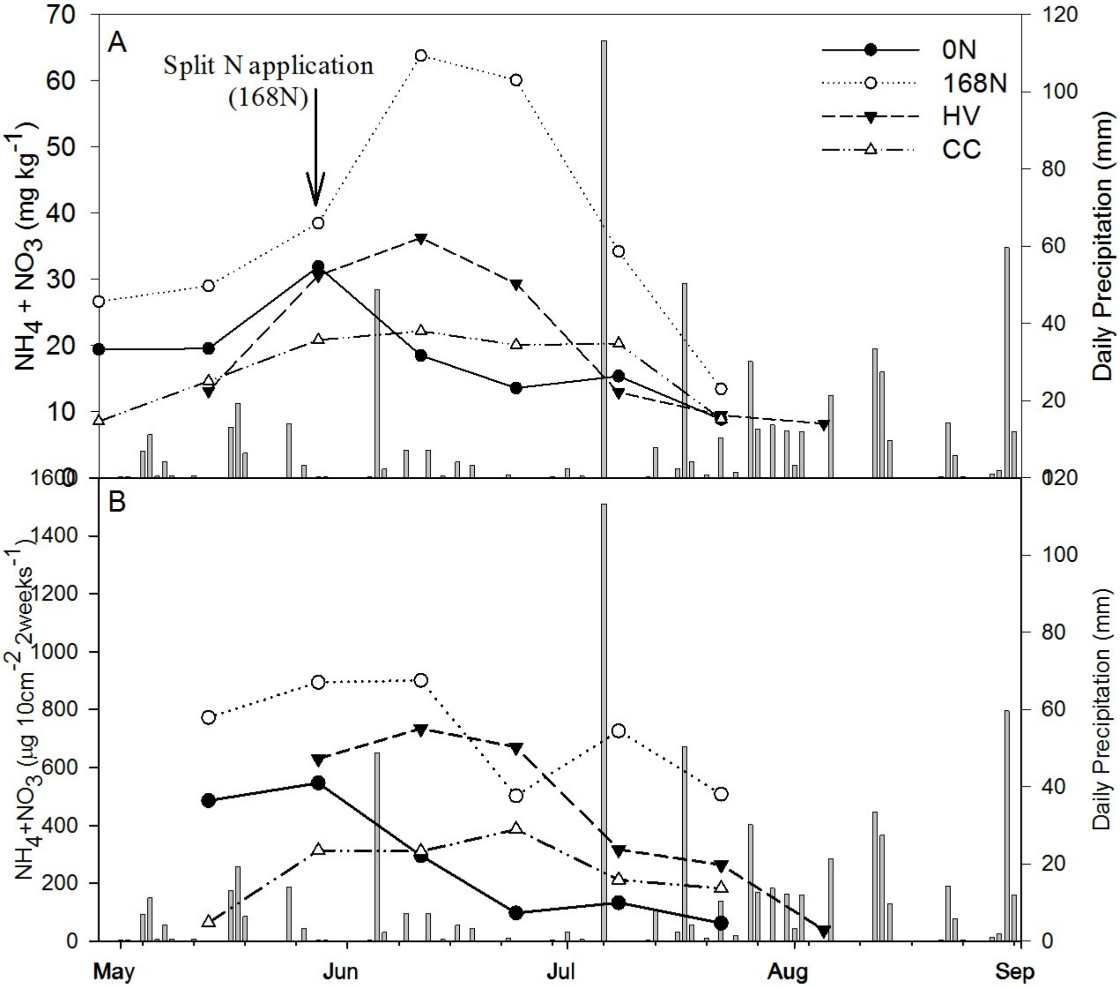

Figure 3-2: Plymouth 2009 Soil N as measured by (A) KCl extraction and (B) PRS adsorbable soil N flux. Hairy vetch (dashed line) was planted 2 weeks after CC and control treatments (0N and 168N)

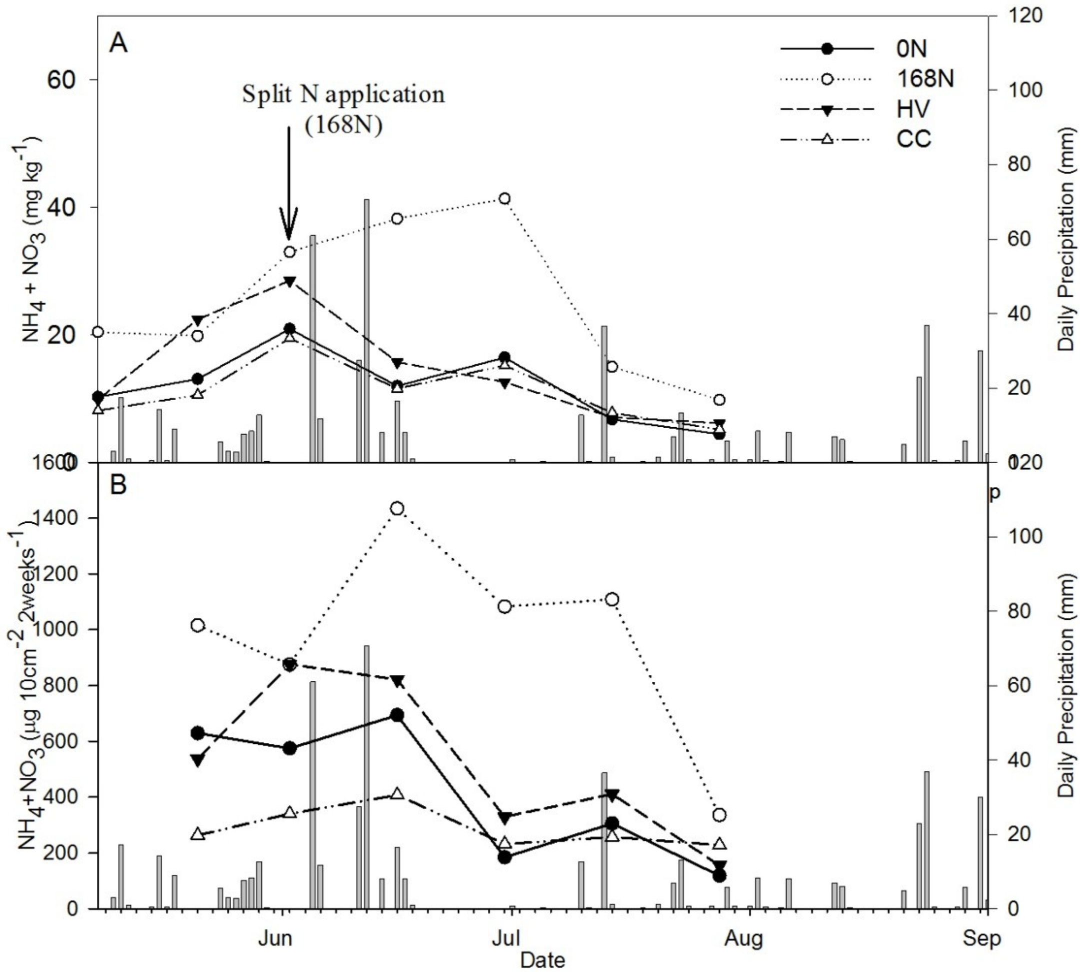

Figure 3-3: Piedmont 2009 Soil N as measured by (A) KCl extraction and (B) PRS adsorbable soil N flux.

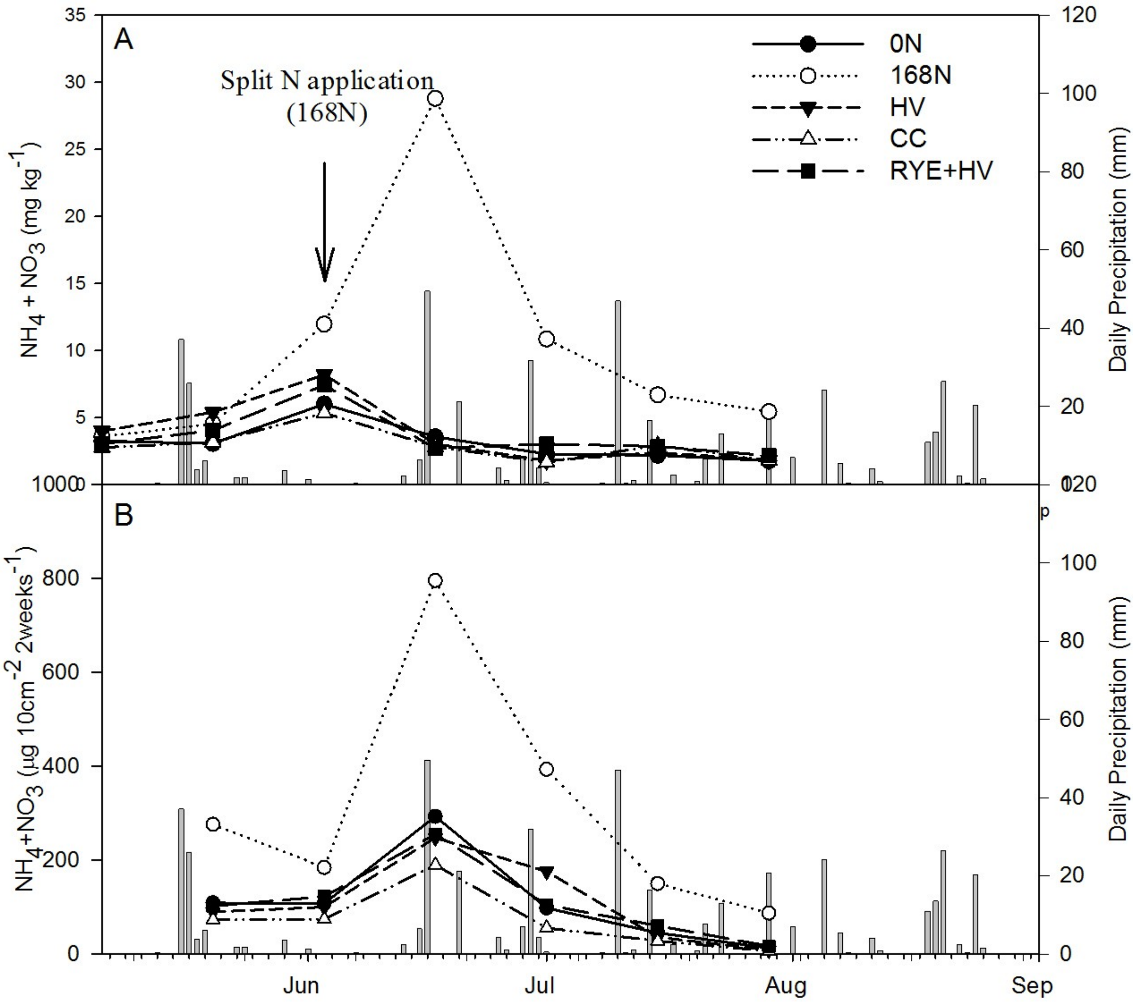

Figure 3-4: Caswell 2010 Soil N as measured by (A) KCl extraction and (B) PRS adsorbable soil N flux.

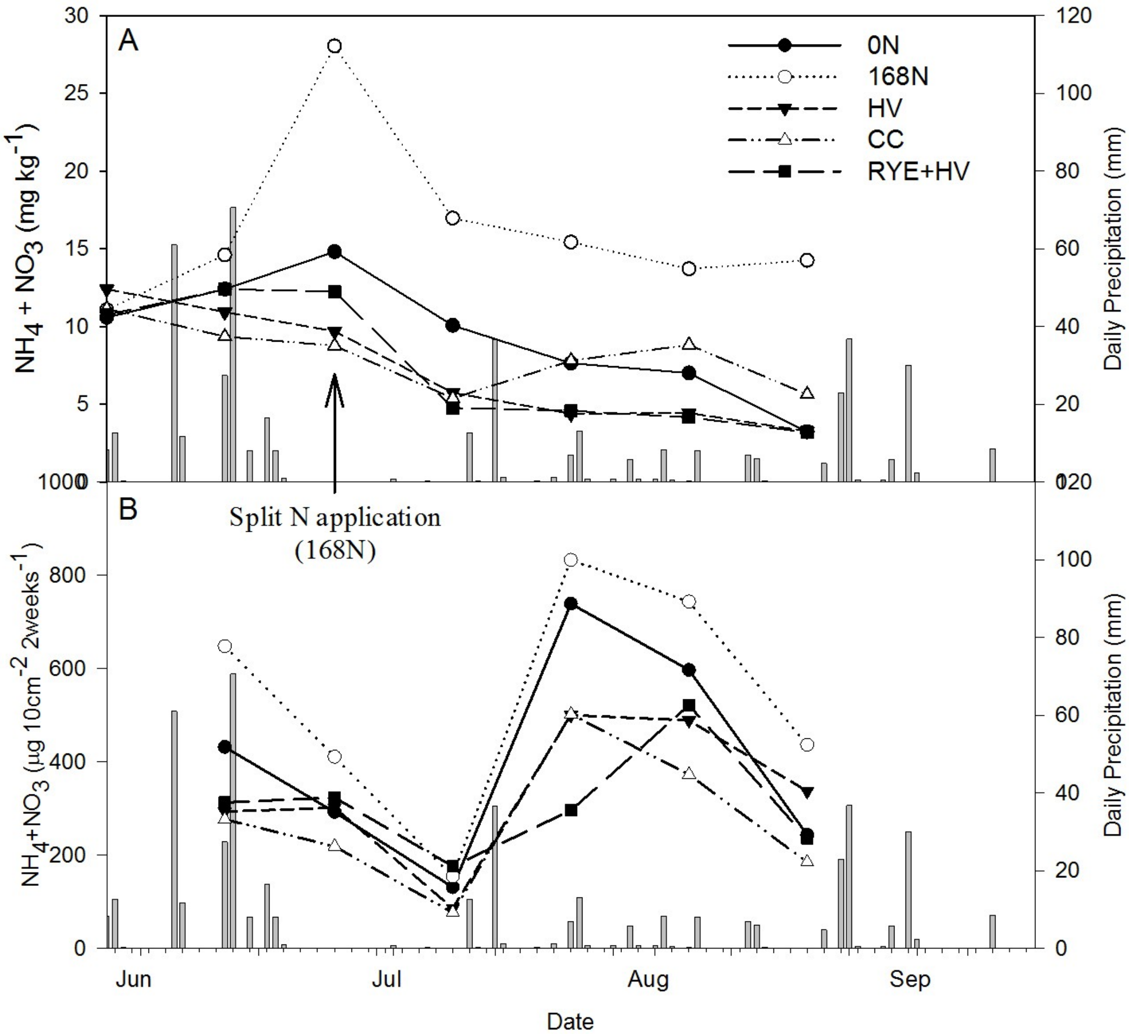

Figure 3-5: Piedmont 2010 Soil N as measured by (A) KCl extraction and (B) PRS adsorbable soil N flux.

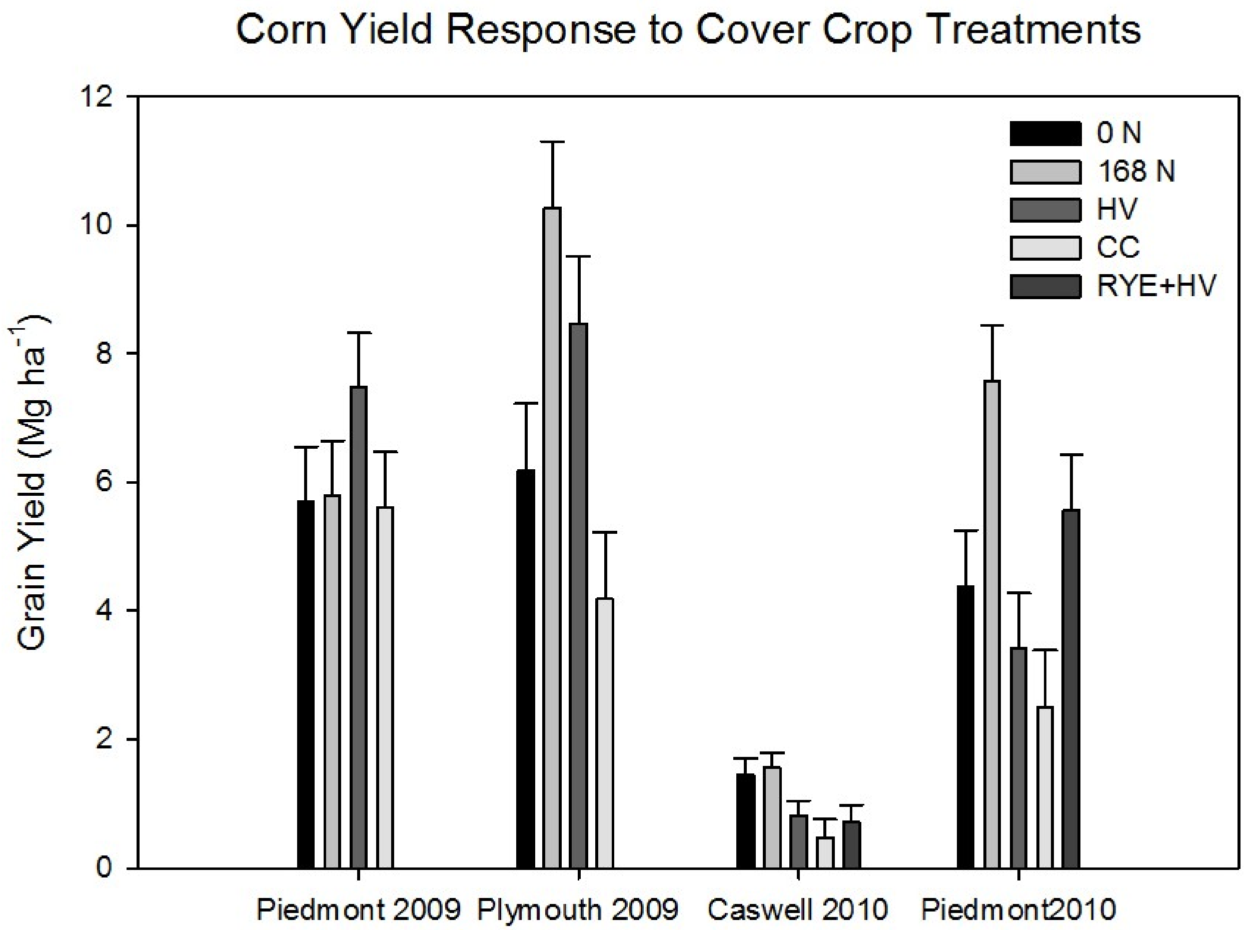

Figure 3-6: Grain yields by treatment