

## ABSTRACT

BROWN, MATTHEW ISAAC. Evaluating Termination Methods of Four Winter Annual Leguminous Cover Crops for Optimizing Nitrogen Synchrony. (Under the direction of Dr. Julie Grossman).

In agroecosystems, synchronizing availability of legume nitrogen (N) to cash crop need is essential to efficiently utilize cover crops as a fertility source. Winter annual legume cover crop termination can be achieved through both tillage and non-tillage methods. However, the chosen approach may affect legume decomposition rate and consequent N mineralization by impacting availability to decomposer soil microorganisms. We predict that termination method in combination with biomass nitrogen content will govern rates of mineralization. Our objective is to evaluate various legume species and termination methods to determine nitrogen release rates in corn following winter annual legumes. In this study, four leguminous winter cover crop species, Austrian winter pea, hairy vetch, and balansa and crimson clovers, were terminated using a roller-crimper, flail mower, disk, or an herbicide. Bi-weekly inorganic soil tests and Plant Root Simulator ion resin probes were used to measure plant available  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Cover crop biomass, total carbon, and total nitrogen were measured for each species prior to termination. Mineralized nitrogen was most available from Austrian winter pea and hairy vetch across all termination methods at six to ten weeks after kill. Disked hairy vetch contributed the greatest plant available nitrogen amongst all 16 combinations. Biomass contributions of cover crops ranged from  $2.4 \text{ Mg ha}^{-1}$  to  $9.7 \text{ Mg ha}^{-1}$  in crimson clover and balansa clover, respectively. Crimson clover, Austrian winter pea, and hairy vetch averaged greater than  $7 \text{ Mg ha}^{-1}$  over three years. Balansa clover was the lowest contributor of both biomass and biomass nitrogen ( $2.4 \text{ Mg ha}^{-1}$  and  $40.3 \text{ kg ha}^{-1}$ , respectively). Hairy vetch had the greatest overall average biomass nitrogen of  $226.4 \text{ kg ha}^{-1}$ .

ha<sup>-1</sup>, while Austrian winter pea averaged 188.71 kg ha<sup>-1</sup> and crimson clover averaged 181.1 kg ha<sup>-1</sup>. Corn yield was not consistent with inorganic and PRS probe measurements of nitrogen. Results show though termination technique in combination with cover crop species does influence nitrogen contributions from winter annual leguminous cover crops.

Evaluating Termination Methods of Four Winter Annual Leguminous Cover  
Crops for Optimizing Nitrogen Synchrony

by  
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## **DEDICATION**

This acorn of knowledge I offer here is dedicated to my family and friends, who have supported me through the thick and thin. It took a village.

## **BIOGRAPHY**

Matthew Brown grew up in Larchmont, NY, going on to complete his undergraduate degree at Cornell University where he studied International Agriculture and Development. Following graduation, he spent several years producing music in New York City, NY and Miami, FL. In 2003, he joined the Central Park Conservancy where he developed curriculum to teach soil science to K-12 school children; managed a section of Central Park; and oversaw the successful development of a soil and water testing laboratory dedicated to monitoring the health of a natural resource. Seeking further learning opportunities and ultimately a career shift into agriculture, Matthew began work toward a Masters of Science degree at North Carolina State University in 2010. His hopes and dreams are to own a farm in the future.

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## CHAPTER 1

Review of the Literature: Evaluating nitrogen synchrony termination methods of leguminous cover crops for optimizing nitrogen synchrony.

### INTRODUCTION

Agriculture, organic or conventional, is an extractive process whereby crops use a portion of the nutrients in the soil for growth and reproduction. Harvesting typically removes plant portions richest in nitrogen, such as the seed or fruit, often leaving a net loss of nutrients with each cropping cycle. Maintaining adequate fertility in soils to generate optimum yields requires detailed nutrient management, with strong considerations given to tillage and cropping rotations. This becomes even more challenging when there are additional regulations in place such as those for farmers participating in the United States Department of Agriculture - National Organic Program (USDA-NOP), or when economics makes purchasing inputs unprofitable. Challenges exist for both organic and conventional growers. While certified organic farmers are limited by the NOP as to what they can use to fertilize their crops, conventional farmers are being confronted with rising synthetic nitrogen prices, increasing from \$212 per ton urea in 1990 to \$448 per ton in 2010, according to the United States Department of Agriculture - Economic Research Service (USDA-ERS). Sharp increases in the cost of synthetic fertilizers may however, open the door for mainstreaming legume cover crops. Despite research confirming leguminous species providing some or most of the required nitrogen in productive agroecosystems (Leigh, 2004, Smil, 2001), there still is a great deal unknown about the impacts of tillage, in particular tillage incurred through

spring termination of winter annual legume cover crops and the effects on plant available nitrogen. This literature review will outline the importance of biologically fixed nitrogen to organic and conventional agroecosystems, the current role of leguminous cover crops in organic systems, and the process of managed decomposition of legume cover crops to optimize nitrogen availability and subsequent uptake by organic crops. The current chapter provides background for this research into cover crops and how termination techniques influence the availability of nitrogen to corn, presented in chapter two.

### **Nitrogen in Agriculture**

Nitrogen is one of the most yield-limiting nutrients in agricultural systems worldwide and is applied annually to compensate for crop removal and natural losses (Brady, et al., 2010, Leigh, 2004). Statistics from 2009 show that synthetic nitrogen fertilizer accounted for approximately 105 Mg applied to agricultural land globally (FAO, 2012), the greatest percentage by weight amongst any other crop nutrient applied.

Since most plants are generally inefficient at taking up all available nitrate and ammonia, losses are ubiquitous in agroecosystems (Vitousek, et al., 2009). Ammonia is lost easily by returning to the atmosphere via surface-connected soil pores along with other gases such as nitric oxides, nitrous oxide, and  $N_2$ . When not bound in a stable organic form, nitrate is soluble the soil solution due to strong attraction to polar water molecules, readily moves downward as water percolates through the soil (Crews, et al., 2004, Di, et al., 2002). To minimize these losses, nutrient supply must be optimized to meet crop demand. Synthetic fertilizer application has evolved to include the use of coatings, stickers, and time release

technology with the intention to control solubility. State extension services have recommended split applications of synthetics to better meet crop needs, while federal programs promote the use of riparian buffers and artificial wetlands to biologically scrub runoff. Despite the growing awareness and action, levels of nitrate found in rivers like the Mississippi have not decreased as evidenced by the annual appearance of dead zones in the Gulf of Mexico. These events show that large N losses are still occurring (David, et al., 2010, Sprague, et al., 2009).

One management approach that requires a rethinking of nitrogen application is that of organic agriculture. Most certification programs, including the EU and USDA-NOP, prohibit organic producers from using any synthetic form of nitrogen (Canali, 2004). Available alternatives to synthetic nitrogen include legume cover crops, composted materials, and animal waste (Drinkwater, et al., 2007, Galloway, et al., 2004, Gaskell, et al., 2007, Smil, 2001). However the challenge of synchronizing nitrogen availability with the needs of cash crops in a timely manner is made often difficult when confronted with multiple options, none of which are perfectly controlled or predictable (Cassman, et al., 2002, Drinkwater, et al., 2007, Raun, et al., 1999).

### **Biological Nitrogen Fixation (BNF)**

Nitrogen (N) is a fundamental building block for all life on Earth, moving in a cyclical manner through abiotic and biotic systems. Along with hydrogen, oxygen, carbon and phosphorus, nitrogen is essential in forming deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It is also a major constituent of amino acids, protein, and a wide

range of other important biomolecules. Though gaseous nitrogen is found in abundance as 78.084% of Earth's atmosphere, this pool is largely inaccessible to most organisms because they lack a metabolic pathway for adsorption.

There are only a few distinct natural pathways through which nitrogen becomes biologically available, with the greatest proportion contributed globally by biological nitrogen fixation (BNF), accounting for as much as 170 Mt year<sup>-1</sup> (Smil, 2002). Rhizobia are soil-dwelling N-fixing bacteria capable of BNF through a symbiotic association with leguminous plants. Di-nitrogen gas is absorbed through soil pores into the anaerobic interior of root nodules housing the rhizobia, where it is converted to ammonium using the enzyme *nitrogenase*. Upon completion of the reaction, the ammonium is assimilated into organic molecules provided by the plant. Amides, amines, and ureides are all examples of these compounds (Postgate, 1998, Stacey, et al., 1992).

### **Cover Crops**

In general cover crops are a biological means to build better soils by adding organic matter; reducing nitrogen losses; preventing soil erosion through leaf interception; and suppressing weeds (Lenzi et al., 2009; Rhoton et al., 2002; Laloy and Biielders, 2010; Sarrantonio, 1994). Despite these benefits, lack of production knowledge, as well as increased labor costs, limit legume cover crop use in many crop rotations.

Choosing a cover crop to meet the needs of the subsequent crop in a rotation requires attention to tillage, soil chemical and edaphic characteristics, local climate, economics, and

water budgets (Wilke and Snapp, 2008). Various cover crop species are appropriate for different seasons and soil types, with least a dozen species for every climate in the U.S. Cover crop varieties have been successfully bred to work well within given climatic niches, producing sufficient growth without conflicting with cash crop planting or harvest windows (Clark, et al., 2007, Griffin, et al., 2000, Scharf, et al., 2002). For example, varieties of hairy vetch (*Vicia villosa*) ‘Auburn Early Cover’ and ‘Auburn Sunrise’, a crimson clover (*Trifolium incarnatum*), are examples that hold great potential for reaching peak nitrogen and biomass at a time fitting well within a southeastern and mid-Atlantic US typical cropping sequence (Bowen et al., 1988; Sarrantonio, 1991; Parr et al., 2011; Wagger et al., 1998; Ranells and Wagger, 1992). Termination should be timed to simultaneously yield the greatest N, but also arrest any potential weediness of a cover crop. In legume cover crops, biomass N has been shown to peak with seed filling, however it is during flowering that a plant becomes most vulnerable to termination (Ashford, et al., 2003).

Nitrogen contributions from legume cover crops are extremely variable. One meta-analysis found rates of plant available nitrogen supplied by a variety of legume cover crops to range from 40 kg ha<sup>-1</sup> to 231 kg ha<sup>-1</sup> across 33 studies carried out in temperate regions (Badgley, et al., 2007). In the Southeast, legume N contributions are equally as variable, ranging from 10 to 336 kg ha<sup>-1</sup> plant available nitrogen (Havlin, 2005, Parr, et al., 2011). Nitrogen fixation in particular is known to vary greatly depending on genetics, agronomics, climate, soil mineral N status, in addition to variations in rhizobia population size and effectiveness (Herridge, et al., 2008). Studies of nitrogen fixation in soybean have

demonstrated that even slight changes in water availability also affect biological N<sub>2</sub> transformations, with drought tolerance varying across genotypes (Goergen, et al., 2009, Sinclair, et al., 2000). As BNF is a costly process for legumes, soils with adequate soil mineral nitrogen present will stimulate legumes to conserve energy resources by not hosting rhizobia, where elevated levels of soil nitrates can negatively affect plant nodulation and nodule function (Day, et al., 1989, Herridge, et al., 1990).

### **Legume Decomposition**

Decomposition is the breakdown of complex organic materials into smaller biomolecule components, beginning upon death of an organism. Decomposition is driven by complex and interactive biologic systems, physical settings, and chemical reactions. The majority of decomposition occurs in the upper eight centimeters in the soil profile due to this being the location of greatest abundance of both dead material and the proximity of soil microorganisms. These microbes consume the myriad compounds complexed in fresh and decomposing substrates, deassembling once synthesized components of a plant or any living things into biologically obtainable nutrients (Gaillard, et al., 1999). The full suite of microorganisms and their associated biogeochemical processes are governed principally by the physical environment; the quality and quantity of the substrate; and properties of the present decomposer population characteristics (Honeycutt, 1999, Paul, 2007, Swift, et al., 1979).

Leguminous cover crops undergoing decomposition have been shown repeatedly to be capable of supplying adequate plant available nitrogen to the subsequent crop depending

on management and soil conditions (Cook, et al., 2010, Crews, et al., 2005, Smukler, et al., 2008, Thorup-Kristensen, et al., 2010). Environmental conditions such as changes in soil moisture, temperature, mineralogy, pH, and/or anaerobiosis will exert influence on decomposition kinetics (Hillel, 1998) and microbial communities (Andersson, et al., 2001, Linn, et al., 1984, Stotzky, 1986). Decomposition has been observed to be strongly influenced by soil moisture, where after five days without moisture and residue incorporation to a depth of 5 cm, decomposer activity can cease after 11 days without additional moisture (Coppens, et al., 2007). Further supporting the important role of moisture in decomposition are observations such as two-fold reductions in decomposition rate in biomass bags placed on the soil surface of soils at field capacity when compared to those placed on dry soil (permanent wilting point) (Cadisch, et al., 1997). Along with soil moisture, soil pH between 8-9 can also accelerate decomposition processes as seen with liming, while in low pH soils, decomposition processes are slower to initiate (Oades, 1988).

Substrate quality and size also influence processes driving decomposition (Holland, et al., 1987). Large debris laying on the soil surface insulates moisture and can more directly mitigate temperature oscillations in the soil profile by altering volumetric heat capacity, thermal diffusivity and conductivity (de Vries, et al., 1975, Teasdale, et al., 1993). Increased stability of favorable temperature and moisture conditions ultimately mean greater rates of decomposition as soil moisture and presence of detritus can positively affect enzyme activities and soil respiration measured by CO<sub>2</sub> flux (Fekete, et al., 2012). Plant tissues vary widely in quality, with plant constituents decomposing at varying rates. Cell walls

decompose more slowly than cytoplasmic materials in part due to a higher concentration of lignin (Kassim, et al., 1981). Lignin is a complex organic biopolymer with unique hydrophobic properties that make it extremely resistant to decomposition across a range of ecosystems (Fox, 1990; Marinari et al., 2010; Vahdat et al., 2011; Wagger et al., 1998; Kleber and Johnson, 2010). Lignin can comprise between 10-30% of biomass carbon, while more easily degraded carbohydrates like cellulose, hemicellulose, sugar and starch account for between 30-75% (Coyne, 1999). Other compounds in plant biomass that may negatively impact decomposition rates include pectins, tannins, waxes, etc. (Coyne, 1999).

Carbon can range from 30-75% of total legume biomass while nitrogen accounts for 2-6%. The ratio of carbon to nitrogen, in general, will predict some aspects of residue decay by directly affecting decomposition dynamics. Soil microbes, in the event of low nitrogen availability from fresh organic material (i.e., high C:N ratio), will draw from soil nitrogen pools and out-compete plant root absorption for N. Reserve nitrogen pools can be found as organic N previously applied as organic residues, as well as ammonium fixed in some mineral clays, however measured pool sizes do not necessarily predict turnover rates (Burger, et al., 2003, Nieder, et al., 2011). Biological N immobilization within the soil matrix is only temporary. As microbial populations increase with resource availability, rapid microorganism turnover releases the immobilized organic nitrogen for uptake by plants or leaching (Baggs, et al., 2000, Brady, et al., 2010, Mitchell, et al., 2000). Plants with higher polyphenolic content have been found to immobilize nitrogen through carbon complexation, limiting its decomposition and subsequent biological availability in the tropics (Palm and

Sanchez, 1990). Taylor et al. found the same phenomenon in temperate climates, where the C:N ratio accounted for more than 86% of the variation in the decay rates of different types of leaf litter with lignin being the immobilizing carbon compound (Taylor, et al., 1989).

Soil nitrogen mineralization potential can be measured using a variety of methods. These include anaerobic and aerobic incubations (Shelton, et al., 1997), hot and cold extractable inorganic N, Kjeldahl N, and ‘super-sinks’ such as resin probes and calcium hypochlorite oxidation (Schomberg, et al., 2009). Supply of mineralized N is never in a steady state along various biogeochemical pathways so it inherently needs to be quantified over a period of time (Hu, et al., 1997). This can be achieved using extractable inorganic soil nitrate-nitrogen and ammonia-nitrogen measurements recorded over a sequence of dates (Cahill, et al., 2010, Keeney, 1982, McSwiney, et al., 2010). Another option for measurement of N availability is the use of resin probes, which measure the flux of nitrate and ammonia ions over a known area (Bair, et al., 2008, Parr, et al., 2011). This method reflects the mass flow of cations and anions moving through the soil matrix. Resin probes simulate roots, by creating a down-gradient of nitrate and ammonia content so that any available source in the soil will contribute to driving the gradient toward equilibrium. When all sources are exhausted, or the ionic potential of the probe is saturated due to limited surface area, the probe holds a snapshot of N species made available from mass flow and diffusion.

## **Legume Cover Crop Termination**

Current methods for cover crop termination in both organic and conventional systems include tillage- and conservation tillage-based techniques. Common tillage implements such as a disk or plough are used to simultaneously cultivate soil while incorporating a cover crop, insuring the cover crop dies prior to planting the cash crop. Conservation tillage methods include no till and strip tilling, where only a small strip is cultivated for seed bed preparation, leaving the remaining soil undisturbed. In no till systems, herbicides, flail mowing, and an implement called the roller crimper have all been shown to successfully terminate a standing cover crop (Ashford, et al., 2003, Lawley, et al., 2011, Parr, et al., 2011, Snapp, et al., 2005). No till techniques are adopted in part to save money on fuel as well as take advantage of a wide array of benefits related to improvements in overall soil condition (Mulvaney, et al., 2010, Teasdale, et al., 2012, Triplett, et al., 2008). Some of these benefits include mitigating large soil moisture oscillations throughout the year (Schwartz, et al., 2010), increasing soil carbon and nitrogen internal cycling (Franzluebbers, et al., 1995, Rhoton, et al., 2002), and decreasing weed pressure (Derksen, et al., 2002, Teasdale, et al., 2012).

Synchrony is in essence a ‘Goldilocks’ phenomenon. In order to achieve optimal synchrony between decomposition of a legume cover crop and cash crop uptake, the decomposing plant material needs to harmonize mineralization with subsequent crop demand increases. If decomposition can be manipulated by increasing tillage then it would validate further investigation of this termination approach, along with evaluating decomposition rates of specific cover crop species (Balesdent, et al., 2000, Gaillard, et al., 1999, Hatfield, et al.,

1994, Malhi, et al., 2007, Mulvaney, et al., 2010). Tillage introduces air and surface debris to the soil profile by breaking apart aggregates that previously protected labile organic matter and by introducing a flush of new biomass and oxygen to support microbial activity. Decomposing microorganisms will then mobilize newly available nitrogen through enzymatic activity preferentially and only if the needs of the burgeoning microbial population do not outstrip the supply coming from the additional substrate (Findeling, et al., 2007). For these reasons it is critical that cover crop termination approach is considered in order to avoid losses through potential immobilization, leaching, or denitrification processes (Drinkwater, et al., 2007). Certified organic farmers are required by the NOP to improve the physical, chemical, and biological condition of soil and to minimize soil erosion (7 § 205.203 NOP, USDA). These goals may be met by carefully selecting tillage and cultivation practices that maintain or manage nutrients and soil fertility.

### **Agronomy of Organic Corn**

Among commodity crops, corn remains a perennial challenge for organic farmers due to high N requirements, weed pressure, and pest issues (Cavigelli, et al., 2008). To manage all of these, there are a wide range of products to which conventional farmers have access and organic farmers do not, including synthetic fertilizers and herbicide resistant crops. Organic growers, on the other hand, rely on animal manures, leguminous cover crops and NOP approved mineral fertilizers such as rock phosphate to provide fertility while using predominantly mechanical means for weed control (Lockeretz, et al., 1981, Pimentel, et al., 2005). To use legume cover crop biomass and manures effectively (and according to the

law), refining the synchrony of N availability with N uptake, it is critical to integrate growth demands of corn over time (Scharf, et al., 2002). By doing so, the maximum benefits of legume cover crops planted prior to cash crops are recovered, and N losses decreased (Andraski, et al., 2000, Kumar, et al., 2002).

Corn growth can be measured using the Leaf Collar Method, which is divided into vegetative stages starting with V1-V20, followed by vegetative tassel (VT). Within the first six stages of vegetative growth (V1-V6), N uptake increases relatively slowly, attributed to the accumulation of biomass and juvenile root development. By V6, the primary ear shoot tissue develops and the initiation of potential kernels commences (Stevens, et al., 1986). From V6 through VT, rows of potential kernels grow to produce a non-pollinated ear while N uptake and accumulation increases exponentially. When the corn plant reaches maximum biomass accumulation and the tassel has emerged completely for anthesis, reproductive development stages R1-R6 commence and are measured using established indicators of kernel development (Ritchie, et al., 1986). The potential size of an ear is measured by the number of kernel rows around the ear and number of kernels per row. The final number of kernels per row is strongly correlated with genetics in combination with soil moisture and nutrient conditions.

On the coastal plain of North Carolina, the NC State Agricultural Extension Service suggests applying  $147 \text{ kg ha}^{-1}$  to achieve optimum yield (Workgroup, 2003), which can be achieved using a productive legume cover crop such as hairy vetch (Cook, et al., 2010, Parr, et al., 2011). However, neither cited study produced equal or greater yields than their

conventionally fertilized counterparts, suggesting that synchrony between plant uptake and available N may be a strong limiting factor. As soil microbes are able to compete with crop plants for available N mineralized from decomposing residues, microbes may out-compete corn and thus suppress growth and yields. Long term planning, crop rotations, and tillage modifications will all help to increase soil mineral nitrogen and have a positive effect on synchrony, internal nutrient cycling, and potentially avoid immobilization (Cavigelli, et al., 2008, Rhoton, et al., 2002, Schomberg, et al., 2011).

## **CONCLUSION**

The aim of this research is to better understand how different legume cover crop termination approaches influence N availability for organic corn production. Our specific objectives were to 1) assess legume biomass and nitrogen at legume termination, 2) determine N release rates from decomposing legume residue, and 3) determine effect of cover crop species and termination approach on organic corn production. We hypothesize that the cover crop with the lowest C:N ratio in combination with the greatest biomass N, when coupled with the termination technique that increases surface area and soil-to-biomass contact, will deliver the greatest plant available nitrogen over the first several weeks after termination.

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## CHAPTER 2

### Evaluating Termination Methods of Leguminous Cover Crops for Optimizing Corn Nitrogen Synchrony.

#### INTRODUCTION

Plant available nitrogen is often a key limiting factor in agroecosystems, affecting crop growth and ultimately yield. Farmers counteract losses incurred through harvest, denitrification, and leaching with careful management of tillage and timing of fertility inputs. In particular, certified organic farmers face federal limitations regarding available fertility options. Synthetic fertilizers are restricted by the USDA National Organic Program (USDA-NOP), while animal manure and by-products such as blood, feather and bone meals are acceptable sources of fertility. Repeated applications of animal manure may present cumulative challenges, however, particularly related to over-application of phosphorus, as well as copper and zinc accumulation in some soils, which in large enough plant-available pools can limit plant growth (Mantovi, et al., 2003, Novak, et al., 2004). Raw manure is subject to narrow application time frames set by the USDA-NOP (USDA, 2012), creating further challenges. Winter annual leguminous cover crops provide an alternative to manure, providing some or most of the required nitrogen in productive agroecosystems (Smil 2001; Leigh 2004).

Under optimal conditions legume cover crops can provide sufficient total N to support subsequent crop growth. Work evaluating eight different winter annual cover crop legume species and several cultivars highlighted several high performing crops with

potentially adequate N contributions for corn growth in North Carolina (Parr, et al., 2011). In this study, Austrian winter pea (*Pisum sativum* subsp. *arvense*), hairy vetch (*Vicia villosa*, var. 'AU Early Cover'), and crimson clover (*Trifolium incarnatum*, var. 'Dixie') were found to contribute 208 kg ha<sup>-1</sup>, 204 kg ha<sup>-1</sup>, and 163 kg ha<sup>-1</sup>, respectively, agreeing with earlier research performed in Maryland (Holderbaum, et al., 1990). These three cover crops can be incorporated into a cash crop rotation in the Piedmont and Coastal Plain of North Carolina without major disruption to traditional cash crop planting times.

Biologically and physically mediated residue decomposition and mineralization is critical for organic cropping systems because it is the major mechanism through which nutrients are returned to crops. Synchronizing availability of legume nitrogen (N) to cash crop need is essential to efficiently utilize cover crops as a fertility source. Winter annual legume cover crop termination can be achieved through a variety of means, including tillage and non-tillage methods. However, the approach may affect legume decomposition rate and consequently N mineralization by placing residue at various points in the soil profile and increasing or decreasing particle size, thus impacting availability to decomposing soil microorganisms. Intensive tillage approaches can increase the reactive surface area available to microbial populations and lead to greater access and faster decomposition (Gaillard, et al., 1999, Hatfield, et al., 1994, Holland, et al., 1987), where in no-till systems, such as with an herbicide or a roller crimper, a senescing standing plant or surface mulch may reduce microbial access and retard decomposition (Dungait, et al., 2012, Swift, et al., 1979).

In tillage systems, chisel and moldboard plows as well as discs are commonly used to incorporate a cover crop terminated by a mower, where no-tillage systems utilize herbicides or terminate cover crops mechanically with a roller crimper, or flail mower with no incorporation. Organic farmers are required by the National Organic Program standards to “select and implement tillage and cultivation practices that maintain or improve the physical, chemical, and biological condition of soil and minimize soil erosion” as stated in 7 § 205.203 (USDA, 2012), which effectively encourages low-tillage or no-till systems even if not a mainstream practice yet. No-till termination methods for organic producers present management challenges with regards to weed control, as tillage is the primary weed management tool in such systems. Tillage is therefore more frequently used in organic systems than in conventional systems for this purpose (Rasmussen, et al., 1995). The tractor mounted roller crimper, which rolls down the cover crop and crimps the shoots while leaving the roots undisturbed, may be an effective no-till tool for both nutrient and weed management through creation of a thick surface mulch that limits weed germination, provided adequate N is made available to the subsequent crop (Davis, 2010).

The overall goal of this study is to measure the effects of cover crop termination strategies on nitrogen mineralization of four leguminous cover crop species nitrogen in an organic corn system. Our objectives were to 1) assess legume biomass and nitrogen at legume termination, 2) determine N release rates from decomposing legume residue under different termination approaches, and 3) determine effects of cover crop species and termination approach on organic corn production. We predict that termination method in

combination with biomass nitrogen content will govern mineralization, therefore termination treatments that place residue deeper in the soil profile, increase the rate of N availability relative to those that leave a surface residue.

## **MATERIALS AND METHODS**

### **Site Descriptions**

This study was conducted in 2011 and 2012 at the Caswell Research Station (KIN) in Kinston, North Carolina and 2012 at the Center for Environmental Farming Systems (CEFS) in Goldsboro, North Carolina. Kinston is located at 36°16'N, 77°37'W, 14 meters above sea level, on the central coastal plain; CEFS is 40 km west of KIN, at 35°38'N, 78°04'W, 24 meters above sea level. The KIN 2011 field was a Johns Sandy Loam (fine loamy over sandy or skeletal, siliceous, thermic Aquic Hapludults) and KIN 2012 a Portsmouth Loam (fine loamy fluviomarine deposits over sandy skeletal fluviomarine, mixed, thermic Typic Umbraquult). CEFS 2012 was a Wickham Loamy Sand (fine loamy, mixed, thermic Typic Hapludult). Annual precipitation averages 124 cm in the region. Temperatures range from 11° C to 23° C.

### **Cultural Practices**

#### **Cover Crop Planting**

On October 12, 2011 and October 17, 2012, crimson clover (CC) (*Trifolium incarnatum*, var. 'Dixie'), hairy vetch (HV) (*Vicia villosa*, var. 'AU Early Cover'), Austrian winter pea (AP) (*Pisum sativum* subsp. *arvense*), and balansa clover (BC) (*Trifolium balansae*) were planted at KIN. On October 3, 2012, CC, HV, and AP were planted at

CEFS, but not BC. A 1000 Series Drill Planter (Hege Equipment, Colwich, KN) was used at both sites. Individual plots measured 3 m by 15 m at KIN 2011, 6.1 m by 15.2 m at KIN 2012, and 6.1 m by 13.7 m at CEFS 2012.

Both a positive and negative control were implemented at KIN, while only a negative control was used at CEFS due to restrictions on conventional fertilizer usage in the certified organic research plots. The positive control at KIN received an application of 30% urea ammonium nitrate (UAN) while the negative control received zero nitrogen inputs. All controls were not seeded and were maintained vegetation-free until corn planting. At CEFS, BurnOut II (St Gabriel Organics, Orange, VA), an organic herbicide was utilized instead of glyphosate (Round Up Weather Max, Monsanto, St. Louis, MO) used at KIN for plot maintenance.

Planting was performed at densities chosen by averaging seed distributor recommended rates, comprehensive literature review, and informal regional farmer conversations. At all three site years, crimson clover was planted at 4 kg ha<sup>-1</sup>, hairy vetch at 4.6 kg ha<sup>-1</sup>, Austrian winter pea at 16.6 kg ha<sup>-1</sup>, and balansa clover at 4 kg ha<sup>-1</sup>. Seed that had not been pre-inoculated was done so with commercially available peat-based inoculants. For Austrian winter pea and hairy vetch, N-Dure inoculant (INTX Microbials, Kentland, IN) was used. R/WR (Nitragin, Milwaukee, WI) was used for crimson clover, while the balansa clover arrived pre-coated.

Prior to planting the first site year winter cover crop at KIN 2011, the field was amended with lime at 1121 kg ha<sup>-1</sup> in conjunction with an application of 112 kg ha<sup>-1</sup>

potassium on September 15<sup>th</sup>, 2010. On January 4<sup>th</sup>, 2011, manganese sulfate formulated for a 0.28 kg ha<sup>-1</sup> foliar application was applied across all the winter cover crop treatments due to a deficiency observed by station management. Prior to site year KIN 2012, lime was applied at a rate of 2,242 kg ha<sup>-1</sup> on October 4, 2011. All applications were based on recommendations made by the NCDA Soil Analysis Lab with the intended crop to be field corn.

### **Cover Crop Termination and Corn Planting**

The KIN cover crops were terminated on April 26<sup>th</sup>, 2011 and April 19<sup>th</sup>, 2012. The four termination treatments were disk, flail, roll, and spray. Disk included flail-mowing of the cover crop using a 4.6 meter John Deer Model 163 mower (Moline IL), followed by several passes with a 4.3 meter disk (John Deere Model 215, Moline, IL) to incorporate residues to approximately 15 cm. The flail treatment utilized the 4.6 meter flail mower (John Deer Model 163, Moline IL) with no incorporation, and the roll treatment a 3.1 meter rear-mount roller-crimper (I &J Manufacturing, Gap, PA). The spray treatment terminated cover crops through herbicide application of 48.8% Round Up Weather Max, 2.33 L ha<sup>-1</sup> spray rate with an 80/20 non-ionic surfactant added at .95 L per 378.6 L of tank solution (Monsanto, St. Louis, MO).

In 2012 CEFS cover crops were terminated on May 11. For no-tillage treatments the 1.8 meter flail mower (John Deer Model 370, Moline IL); and a 3.1 meter rear-mount roller-crimper (I &J Manufacturing, Gap, PA) were used. The spray treatment was the herbicide BurnOut II, with a 4.4% final concentration of clove oil, applied at 187.08 liters ha<sup>-1</sup> (St.

Gabriel Organics, Orange, VA). For tillage a 3.65 meter disc (John Deere Model 225, Moline, IL) was passed several times through each plot to an average depth of 15 cm.

Field corn ('N631', Doeblers Pennsylvania Hybrids, Jersey Shore, PA) was planted across all three site years, in eight rows, at 76 cm spacing, with population densities of 82,000 seeds ha<sup>-1</sup>, on May 12<sup>th</sup> 2011 (KIN 2011), May 1, 2012 (KIN 2012), and May 18<sup>th</sup> and 25<sup>th</sup>, 2012 (CEFS 2012) with a John Deere 7200 MaxEmerge 2 Drawn Four Row Conservation Planter (John Deere, Moline, IL). Planting on all fields was performed in the same direction so as to minimize hair-pinning of seeds and optimize seed to soil contact among both till and no-till kill treatments. Planter settings for debris clearing, downward seed pressure, and closure completeness were set to optimally plant across tillage regimes.

At KIN positive control plots were sprayed with liquid UAN calculated to deliver 168 kg ha<sup>-1</sup> on April 10<sup>th</sup>, 2011 and April 25<sup>th</sup>, 2012, prior to corn planting. The negative control plots in all three site years received zero nitrogen inputs and were maintained vegetation free with herbicide from the time of cover crop planting until two weeks prior to corn planting at all sites.

## **SAMPLING AND ANALYSIS**

### **Biomass Sampling**

Quadrats measuring 0.25 x 0.25 m<sup>2</sup> were used to take representative biomass samples from all cover crops on the same day as termination. Non-cover crop plants (i.e. early spring weeds) did not present themselves to be enough of a significant contributor to require samples of them to be removed from the field. Samples were dried at 70° C in a drying oven

(Mid-Pines Small Grains Lab, NCSU, Raleigh, NC) until a constant weight was achieved and ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2 mm sieve. Ground samples were then submitted to the Environmental and Agricultural Testing Services Lab (NCSU, Raleigh, NC) for analysis of total elemental carbon and nitrogen using a Perkin Elmer 2400 CHNS/O Elemental Analyzer (Waltham, MA).

### **Inorganic Nitrogen**

For all plots, a composite sample was created by removing surface residue and compositing twelve soil cores (AMS, American Falls, IN) taken from 0-15 cm deep. Samples were taken across 10 time points in 2011 at KIN, starting with a baseline taken March 31<sup>st</sup>, then seven days after terminating the cover crop on May 2, then every 14 days through Aug 4<sup>th</sup>. The baseline samples from KIN 2011 were found to have abnormally high ammonia values, possibly due to contamination, and were excluded from this study.

There were nine sampling points at KIN and CEFS in 2012 using the same baseline, seven day, 14 day scheduling routine as in 2011. The KIN 2012 baseline samples were taken April 5<sup>th</sup>, followed by the first sampling April 26<sup>th</sup> and ending July 26<sup>th</sup>. At CEFS 2012, baseline samples were taken April 5<sup>th</sup>, sampling commenced May 17, ending August 16<sup>th</sup>.

Soils were removed from the field and dried on the same day at 45° C in an oven for 24 hours or until a steady mass was achieved. Oven dry soils were ground immediately to pass through a 2 mm sieve at the Small Grains Research Unit (NCSU, Raleigh, NC). Dry ground soil samples were stored at room temperature until available N was extracted using 8 g soil to 40 mL of 1 mol/L KCl and shaken for one hour. Samples were then filtered through

Whatman #42 paper (Kent, UK) into scintillation vials and frozen until N analysis could be performed. Soil KCl extracts were analyzed for ammonia using the sodium salicylate-based method and the cadmium reduction method for nitrate using a Flow Injection Analysis Autoanalyzer (Lachat Omnion 8000, Hach Company, Loveland, CO).

### **Ion Resin Probes**

To measure plant available soil nitrogen, ion resin exchange Plant Root Simulator probes (Western Ag, Saskatoon, Canada) were exchanged at two week intervals over the course of two site years at KIN for a total of seven adsorption intervals starting on May 12, 2011 and May 5<sup>th</sup>, 2012. The third sampling interval during KIN 2012 was pushed back 14 days due to probe shortage. Four anion and four cation probes were buried in all of the experimental plots containing Austrian winter pea and hairy vetch, as well as the positive and negative controls, in flailed and disked treatments to represent a till and no-till system. When integrated with soil water content, ion resin probes are designed to provide a measurement of ammonia and nitrate flux for the period deployed in the soil on a known surface area (Qian, et al., 1992).

### **Corn Plant Total N Analysis**

Two separate analyses were conducted to monitor total corn plant nitrogen status. For the first, three corn plants were selected from the center two rows, at least two meters in from the perimeter, and cut 8-10 cm from the soil surface during vegetative stage six (V6) on June 2, 2011 at KIN, again on May 31, 2012. At CEFS V6 occurred for the majority on May 31, 2012. The second analysis was carried out at was vegetative tasseling stage (VT). At KIN,

this took place on July 7, 2011 and July 12, 2012, and at CEFS on July 7<sup>th</sup>, 2012. Three ear leaves were removed from plants in the center two data rows at a minimum distance of two meters from the plot perimeter. All corn plant biomass was dried, ground, and analyzed in a manner identical to the cover crop biomass.

### **SPAD Meter**

During the VT stage, corn leaf chlorophyll readings were taken at all sites with a Minolta Single Photon Avalanche Diode (SPAD) 502 meter (Konica Minolta, Ramsey, NJ). Sampling was conducted over two hours for two days on July 7<sup>th</sup> and 8<sup>th</sup>, 2011, and July 12<sup>th</sup> and 13<sup>th</sup>, 2012 at Kinston from 10:00 am to 12:00 pm to standardize light variation. At CEFS SPAD was measured on July 5<sup>th</sup>, 2012 from 10:00 am to 12:30 pm. Five randomly chosen plants bearing ears from the two center rows had three readings apiece taken midway up the ear leaf avoiding venation. The fifteen readings were averaged for each experimental plot.

### **Corn Yields**

Corn was harvested on September 22<sup>nd</sup>, 2011 and August 23<sup>rd</sup>, 2012 at KIN. At CEFS it was harvested September 13<sup>th</sup>, 2012. Prior to harvest, in KIN 2011, stand count was measured by combining two, 3.05 meter long sections from the center two rows 1 m from perimeter. The same method was carried out for KIN and CEFS 2012, however the stand count was doubled in length to a total of 12.2 meters. From sections that had stands counted, corn cobs were hand harvested into cotton sacks and labeled. Cobs were placed the same day on all three harvest days into a drying oven at 70° C (Mid-Pines Small Grains Lab, NCSU, Raleigh, NC). Targeted moisture for shelling was monitored using a handheld grain moisture

monitor (Farmex Moisture Master Grain Analyzer, Farmex Electronics, Streetsboro, OH). Once kernels achieved <15% moisture content by weight, cobs were shelled and yield data measured for each plot. Yields were adjusted in the final analysis to 15.5% moisture content.

## **TREATMENT STRUCTURE AND EXPERIMENTAL DESIGN**

Experimental design was a split plot randomized complete block design with the main unit factor being termination, and the sub-unit factor cover crop species. Four termination levels were applied across four cover crop treatments (Till, Flail, Roll and Spray) and two controls among four repetitions. Three cover crops and one control were used at CEFS.

### **Statistical Analysis**

Statistical analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC). Cover crop, total N and C:N of the biomass, PRS Probe N, inorganic N, and grain yield data were analyzed using the mixed procedure for analysis of variance. Termination, Cover Crop and their interaction were considered fixed-effects. Analysis of Repeated Measures in the Mixed Procedure were performed on all inorganic N and PRS probe N data where time was considered a repeated measure, utilized due to its best fit for covariance structures. Nesting was implemented for PRS probe N data due to imbalanced observations. Square root transformations prior to analysis were performed in inorganic N and PRS probe N, corn biomass N at V6 and VT, and total corn grain yield. Stand counts from 12.2 meter sections in KIN 2011 and 24.4 meter sections in KIN and CEFS 2012 were used to normalize stand density across all experimental plots.

$$Yield (kg ha^{-1}/Stand count) * Total Stand count = Total Grain Yield$$

A 1<sup>st</sup> Order Taylor Expansion was used to derive standard error for least squares mean in the original scale. (Jørgensen, et al., 1998).

A partial correlation between SPAD and yield was calculated by running a MANOVA with SPAD and yield as dependent variables in the Mixed Procedure to evaluate SPAD values as a predictor of yield, after controlling for the experimental factors (block, termination method, and cover crop effects). This more conservative approach takes into consideration the wide variances within the data.

## **RESULTS**

### **Cover Crop Biomass, Nitrogen Content, and C:N Ratio**

Biomass production across all three site years, with the exception of balansa clover, contributed more than 5.8 Mg ha<sup>-1</sup>, with amounts as great as 9.7 Mg ha<sup>-1</sup> accumulated by crimson clover (Table 2.1). Total biomass N was found to be as low as 40.4 kg ha<sup>-1</sup> in balansa clover to all site-year high of 288.5 kg ha<sup>-1</sup> in crimson clover (Table 2.1). Excluding balansa clover's rather limited N production, hairy vetch, crimson clover, and Austrian winter pea produced a mean of 198.7 kg ha<sup>-1</sup> N for all three site years ( $p < 0.05$ ). Within site years, crimson clover at KIN 2011 (7.5 Mg ha<sup>-1</sup>) produced significantly more biomass than Austrian winter pea (5.8 Mg ha<sup>-1</sup>) and hairy vetch (6.6 Mg ha<sup>-1</sup>), with hairy vetch and Austrian winter pea producing similar amounts of biomass N at 216 kg ha<sup>-1</sup> and 191 kg ha<sup>-1</sup>, respectively ( $p < 0.05$ ). The same pattern for biomass N held among the three cover crops in KIN 2012 where hairy vetch and Austrian winter pea accumulated similar amounts of N, 174.7 kg ha<sup>-1</sup> and 170.4 kg ha<sup>-1</sup>, respectively. However while crimson clover's biomass

production in this year ( $6.4 \text{ Mg ha}^{-1}$ ) did not outperform hairy vetch ( $6.5 \text{ Mg ha}^{-1}$ ), or Austrian winter pea ( $6.1 \text{ Mg ha}^{-1}$ ), it accumulated significantly less biomass N ( $110.25 \text{ kg ha}^{-1}$ ) than the other two cover crops ( $p < 0.05$ ). At CEFS 2012, a different trend emerges for biomass and biomass N production. Instead of a disparity from those two factors as was seen in crimson clover in both KIN 2011 and KIN 2012, crimson clover and hairy vetch accumulated similarly substantial amounts of biomass of  $9.7 \text{ Mg ha}^{-1}$  and  $8.8 \text{ Mg ha}^{-1}$  respectively, while their biomass N ( $263.6 \text{ kg ha}^{-1}$  and  $288.4 \text{ kg ha}^{-1}$ , respectively) remained the greatest out of the three cover crops ( $p < 0.05$ ). This trend indicates the possibility of higher N-fixation rates or greater N accumulation per unit of biomass in hairy vetch and Austrian winter pea.

Ratios of C:N for cover crop species remained fairly consistent within species among the site years (Table 2.1). Hairy vetch and Austrian winter pea C:N ranged from 12.9:1 – 15.8:1, while crimson clover and balansa clover C:N ratios were higher, ranging from 16.1:1 – 24.9:1. Total lignin varied among the site years and no clear trend was observed among species, with hairy vetch ranging from 12.4% to 20.2%; crimson clover ranging from 8.7% to 13.9%; Austrian winter pea ranging from 10.7% to 15.5%; and balansa clover with values ranging from 9.2% to 14.6%. Lignin was not statistically analyzed.

### **PRS Probe Nitrogen**

A two-way interaction was observed between *cover crop\*time (termination)* ( $p < 0.001$ ) at both sites, demonstrating the interactions of cover crop species and termination over time had a significant effect on the availability of nitrogen in the form of PRS

adsorbable N, defined here as N flux, during KIN 2011 and 2012 site years (Table 2.2). Among all legume species, nitrogen flux was greatest from hairy vetch residues for both site years at 14 ( $927.3 \mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$  in KIN 2011) and 42 ( $894.4 \mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$  in KIN 2012) days after termination (Figures 2.1C and 2.2B).

### **KIN 2011**

Among the two cover crops, hairy vetch was more affected by termination approach than was Austrian winter pea, as indicated by separation of termination approaches at each measured time point (Figure 2.1 and 2.2). Termination method only made a significant impact on differences of N flux from Austrian winter pea at 28 days after termination, when N flux from disked cover crops was greater than all other treatments (Figure 2.1A) ( $p < 0.05$ ). At 56d after cover crop termination, N flux declined sharply in both legume species, then increased slightly at 70 days, but not to levels observed at 42 days. For both legume species, disking of cover crops contributed more to N flux than other termination methods for the first 28 days following termination, and then again at 70 days after termination. However, during the interim two sample dates, 42 and 56 days after termination, rolled surpassed the N contributed from disked treatments, with values of  $927.3 \mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$  and  $390.8 \mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$ , respectively.

### **KIN 2012**

In 2012, though the significant three way interaction is the same, a different pattern emerged over time for both hairy vetch and Austrian winter pea, with all termination methods peaking within 14-28 days following kill followed by a steady decline until 98 days

after termination. Disking and flail mowing of both legume species produced the greatest N flux at 14 days after termination, with values ranging from 711 to 750.2  $\mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$  in Austrian winter pea and 798.3 to 894.4  $\mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$  in hairy vetch. After this period, flux in the disked treatment decreased after 14d, while flail-mowed Austrian winter pea had the greatest N flux until 28 days after termination (688.0  $\mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$ ) ( $p < 0.05$ ). In hairy vetch, by Day 28, the N flux under disk and flail termination approaches were indistinguishable from other termination methods (636.0  $\mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$  and 486.2  $\mu\text{g N } 10 \text{ cm}^{-2} 14 \text{ days}^{-1}$ , respectively).

### **Inorganic Soil Nitrogen**

KIN 2011 was the only site year to have a significant three way interaction between *termination\*cover crop\*time* (Table 2.3). The two-way interaction of *Cover crop\*time* were significant at KIN and CEFS 2012 ( $p < 0.001$ ). Additionally, *termination\*time* was only significant at CEFS 2012 ( $p < 0.001$ ), providing evidence that cover crops, termination, and time across all three site years had a measured impact on soil inorganic N, defined here as soil N.

#### **KIN 2011**

Soil N data at this site under Austrian winter pea residue in 2011 is presented in Figure 2.1B. Over the first 14 days after Austrian winter pea termination, the disk, flail, and spray treatments maintained similar soil N levels. Soil N under Austrian winter pea for all termination methods increased until 28 days after kill, at which point it temporarily decreased in disked treatments when measured at 42 days after termination. Soil N values under

Austrian winter pea increased over the greatest number of time points under flail and roll from the first observation until peaking 56 days after termination (24.9 mg kg<sup>-1</sup> and 17.3 mg kg<sup>-1</sup>, respectively). When Austrian winter pea was terminated by disking, soil N also peaked at 56 days after termination, with values similar to that of flailed pea, 23.4 mg kg<sup>-1</sup>.

Hairy vetch soil N data for Kinston 2011 is presented in Figure 2.1D. Seven days after hairy vetch termination, flail mowed treatments showed greater available soil N than all other termination methods. At 28 days after termination, flail mowed treatments reached their maximum soil N of 17.0 mg kg<sup>-1</sup>, statistically equivalent to that of soil N under disked treatments at that same time-point, 20.9 mg kg<sup>-1</sup>. At 42 days after termination, available N had decreased for disk, flail, and spray. Available N in soil under rolled treatments, however, continued to increase up until this point, with a maximum of 14.1 mg kg<sup>-1</sup> reached. Disking peaked two weeks later at 56 days after termination (24.2 mg kg<sup>-1</sup>), a significantly greater soil N than all other termination methods but achieving the same temporal peak when compared to Austrian winter pea.

## **KIN 2012**

Baseline pre-termination values of soil N varied for each cover crop species with Austrian winter pea > hairy vetch > balansa clover/crimson clover (Figure 2.2C) ( $p < 0.05$ ). Effects of termination approach were observed 7d after kill, with Austrian winter pea and hairy vetch contributing the greatest amount of available soil N. Under balansa clover and crimson clover, soil N decreased slightly from baseline values. Fourteen days after termination Austrian winter pea and hairy vetch begin a temporary negative trend, while

balansa and crimson clover increase soil N, resulting in soil N values that were statistically indistinguishable among cover crop species for the remainder of the experiment. Overall Austrian winter pea and hairy vetch contributed greater available N to soil than observed in balansa clover and crimson clover. Peak N occurred under hairy vetch and Austrian winter pea seven days after termination with  $6.7 \text{ mg kg}^{-1}$  and  $7.2 \text{ mg kg}^{-1}$ , again, higher than other cover crop treatments across the entire site year.

### **CEFS 2012**

Among the four termination methods at CEFS, disking contributed the greatest amount of soil N over the entire experiment period (Figure 2.3A). Prior to termination, the disked treatment baseline had  $1.7 \text{ mg kg}^{-1}$  less total soil N than the sprayed and rolled treatment, enough to be significant ( $p < 0.05$ ). Soil N peaked across all termination approaches 42 days after termination, with  $28.0 \text{ mg kg}^{-1}$  observed in disked plots, one and half times greater than all others. The flail, roll, and till methods peaked at 16.2, 15.0, and  $15.0 \text{ mg kg}^{-1}$  respectively and did not vary significantly from each other during the monitoring period.

Baseline pre-termination measurements showed similar amounts of soil N under Austrian winter pea, crimson clover, and hairy vetch treatments (Figure 2.3B). Following termination, hairy vetch provided the greatest soil N for 14 days, peaking at 42 days after termination with  $26.7 \text{ mg kg}^{-1}$  soil N. Over the duration of time though, Austrian winter pea and hairy vetch are the greatest contributors of soil N between 28 and 42 days after termination. After this time point both Austrian winter pea and hairy vetch intersect with

crimson clover. Crimson clover soil N increases until day 42, peaking at 16.5 mg kg<sup>-1</sup>, significantly lower than the other two species. At 56 days of decomposition, soil N in all three cover crop treatments returns similar to baseline levels, and sometimes below, becoming indistinguishable from each other.

## **Agronomy**

### **Grain Yields**

All site years demonstrated a significant two-way interaction between cover crop \* termination ( $p < 0.001$ ), with the exception of KIN 2011 (Table 2.4). Termination method in Kinston 2011 was a greater driver in predicting corn yield than cover crop species (Figure 2.4). Among the termination methods, spray and flail produced the greatest corn yields of 5.95 Mg ha<sup>-1</sup> and 5.71 Mg ha<sup>-1</sup> that year, respectively, while disked treatments yielded somewhat less but not that much greater than the roll treatment. ( $p > 0.05$ ; Figure 2.4).

Within each cover crop species of Austrian winter pea, crimson clover, and hairy vetch in 2012 at Kinston termination approach was not shown to significantly affect grain yields (Table 2.5 & Figure 2.5). This did not hold true for balansa clover, or the N0 and N168 controls, where higher yields resulted from disking and spraying when compared to flail. At this site year, excluding controls, no yield differences were observed among any combination of species and termination method, except for corn yields under balansa clover when killed with a herbicide, where yields were significantly lower than all other combinations at 0.52 Mg ha<sup>-1</sup> ( $p < 0.05$ ). However, there is a trend towards peak yield among cover crop species across all termination methods being achieved by Austrian winter

pea and hairy vetch when terminated with a disk (5.44 and 5.61 Mg ha<sup>-1</sup>, respectively). Austrian winter pea terminated via spraying also returned nominally greater yields at a mean of 5.45 Mg ha<sup>-1</sup>.

Yields at CEFS 2012 were exceptionally low for this experiment with a mean of 0.93 Mg ha<sup>-1</sup>, across all species by treatment combinations excluding the control, falling well below the North Carolina average 9 Mg ha<sup>-1</sup> (NCSU, 2012). Differences in yield among all cover crop species and termination method combinations were statistically indistinguishable, with the exception of disked hairy vetch, which had lower yields than sprayed and rolled vetch. When no cover crop biomass N was applied (N0), disked treatments produced greater yields (1.69 Mg ha<sup>-1</sup>), than flailed rolled or sprayed treatments (Table 2.5;  $p < 0.05$ ). Peak yields of 2.2 Mg ha<sup>-1</sup> were observed under hairy vetch terminated by the organic herbicide, but fell as low as 0.3 Mg ha<sup>-1</sup> in Austrian winter pea terminated by the flail mower (Table 2.5 & Figure 2.5). In Austrian winter pea and crimson clover, no differences were observed across all termination treatments. The opposite is true within hairy vetch, where spray and flail methods had a positive effect on yields over yields observed in treatments terminated with disk and roll.

In general, corn biomass N at V6 growth stages did not vary greatly among treatments (Figure 2.6). Year over year comparisons were not performed. At KIN 2011, corn grown in cover crop treatments measured comparable amounts of biomass N at this stage. Results from KIN 2012 show the greatest biomass N found in the 168 kg ha<sup>-1</sup> nitrogen control, varying significantly from crimson clover, hairy vetch, and Austrian winter pea. The

negative control and balansa clover yielded the least. At CEFS 2012, where there is no positive control, corn growing in soils that had previously been cover cropped had significantly greater biomass N than the negative control. Austrian W Based on the outcomes of regression analysis performed on SPAD values and grain yields, SPAD values were within those found in the North Carolina Coastal plain (Table 2.6) ( R. Heiniger, personal conversation, 2012)

## **DISCUSSION**

This study showed that amongst termination treatments, disking of biomass most rapidly increases plant available nitrogen found in the soil, above all other termination treatments. Flail mowing also demonstrated a rapid increase in N availability but only slightly different overall from the roller crimper and herbicide termination treatments. Unlike the other three methods, the disking treatment both chopped and incorporated residue to a minimum of 15 cm into the soil profile. Earlier work by Holland and Coleman as well as Ambus and Jensen found similarly increased rates of decomposition when incorporating plant residues as compared to leaving residues on the surface (Ambus, et al., 1997, Holland, et al., 1987).

Cover crop species also influenced nitrogen availability as evidenced by the high rates of N flux and inorganic soil N contributions for all three site years in hairy vetch and Austrian winter pea plots. At both Kinston 2011 and CEFS 2012, all soil tests showed peak N availability to occur between 42 and 56 days after termination. Soil N dynamics at Kinston 2012 differed with peak N occurring a week after termination, agreeing more with

the results of previous decomposition studies that have shown the greatest rates of decomposition to occur within ten days to four weeks after termination (Cherr, et al., 2006, Wagger, 1989, Wilson, et al., 1986).

Total cover crop biomass, biomass N, and C:N ratios varied by species and within species across site years. Driven by genetic differences, variations may be explained by inconsistent periods of growth occurring between planting and termination. Growing and/or accumulated degree days have been repeatedly shown to exert an influence on biomass and total N accumulated (Cook, et al., 2010, Ranells, et al., 1992) , but species appear to be differentially affected. For example, crimson clover at CEFS 2012 accumulated 9.7 Mg ha<sup>-1</sup> of biomass and 263.6 kg ha<sup>-1</sup> of biomass N, after 221 days of growth. Compared to Kinston 2011 and 2012, cover crops grew for 196 days and 185 days, respectively, resulting in 22-34% less biomass and 36-58% less biomass N. Austrian winter pea and hairy vetch were less affected by changes to the growing period, providing generally consistent additions of biomass N. The same conclusion was reached by Parr et al., who found no interaction between termination date, total biomass, and biomass N (Parr, et al., 2011). These findings are in contrast to Drinkwater et al, who found that a 3.5 week delay in terminating hairy vetch resulted in greater biomass but not greater N (Drinkwater, et al., 2000). Cook et al observed a strong relationship between cumulative growing degree days and biomass production in hairy vetch, but biomass N remained inconsistent between two site years (2010).

Increases in biomass production due to a longer growing season, when coupled with biomass N content, did not appear to have a strong relationship to C:N ratio. The highest ratio of 25:1 was measured in crimson clover growing at Kinston 2012, the site with the shortest growing period of all three site years. This runs counter to increases in C:N ratios that occur when plant growth is allowed to continue, as observed by both Parr and Wagger (Parr, et al., 2011, Wagger, et al., 1998). Ratios of C:N for other species fell below 20:1 for all site years, with no observation of net N immobilization. If greater than 25:1, net immobilization would have been a large possibility, slowing decomposition and subsequent N availability (Kuo, et al., 1997, Sall, et al., 2007). Trinsoutrot et al came to similar conclusions after decomposing 47 different types of plant residues in controlled environments (Trinsoutrot, et al., 2000).

Soil N evaluated through extraction and PRS probes at all three site years followed similar patterns of availability over time, however at one site (Kinston 2012) stark differences were observed between the two methods of soil N analysis. Kinston 2012 probe N flux started high then underwent a logarithmic decrease in both Austrian winter pea and hairy vetch, under all termination methods, in contrast to what was observed the previous year at this site where soil N began low and increased over the decomposition period. Remarkably, in the same plots, our extracted soil N values showed a distinctly different pattern, peaking twice over the same time period. When comparing PRS probe N to N values generated via extraction, Bair et al. show dissimilarities in the timing of N release from cover crop legumes between PRS probe N flux and inorganic soil N, but does not expand on these

differences (Bair, et al., 2008). In the findings of Parr et al (2013), probe N flux peak occurred two weeks after extracted soil N peak, whereas the opposite trend emerged in our study. At Kinston 2011, measurements of soil N through extraction among termination treatments generally showed an earlier response to disking in accumulation of nitrogen than probe N. Based on the kinetics and movement of nitrogen mineralized from an organic form into an adsorbable inorganic form, the methodologies will most likely be inconsistent, with Kinston 2012 providing an ideal example. As such, KIN 2011 began as a cool wet spring becoming extremely dry 56 days after termination, whereas KIN 2012 had more consistent precipitation throughout about 84 days after termination. This suggests that variation in soil type and climatic conditions such as those encountered in this experiment, may present inorganic nitrogen extractions to better reflect measurement of point-in-time measurement of soil N than PRS probes.

In this study corn yields did not appear to be limited by nitrogen as demonstrated by both a lack of yield response to variable available soil N values, which ranged from 8 kg ha<sup>-1</sup> to a high of 54 kg ha<sup>-1</sup> over the growing period. At the CEFS 2012 site for example, hairy vetch and Austrian winter pea both supplied greater than 20 kg ha<sup>-1</sup> of soil N for more than 70 days after termination. The range of soil N is consistent with other studies of leguminous cover crops including the work of Decker et al (1994) , Teasdale et al (2012), as well as findings by Snapp et al (2005). Over all three site years, plant available N was greater than the negative controls, and relatively successfully supplied N during the critical growth points in corn under all termination treatments. Additional evidence supporting a positive effect

from cover crops on plant available nitrogen in soil was measured in the corn tissue biomass N. Corn grown in the cover crop treatments had greater biomass N than the positive and negative control for two of the site years. In ideal agronomic conditions, nitrogen availability can explain 70-81% of resulting yield, but weeds are also a factor, explaining a considerable portion of around 25% of yield variability (Cavigelli, et al., 2008, Kuo, et al., 2000). On the N supply side, levels from 0.5 to 6 kg ha<sup>-1</sup> N day<sup>-1</sup> of fertigated nitrogen have produced competitive yields by essentially ‘spoon feeding’ nitrogen to sweet corn. This method also reduced potential nitrogen losses as summarized by Bar-Yosef (Bar-Yosef, 1999).

It may be that other factors not quantified in this study impacted yields. In Kinston, from June 15 until July 20, 2011 only four precipitation events of two centimeters each occurred. As a result, corn at this site appeared drought and heat stressed. Nitrogen uptake by corn would have likely been impacted, as would have grain filling (Dou, et al., 1995). Prior to harvest, on August 27, 2011, Hurricane Irene made landfall about 120 km east of Kinston, NC with wind speed averaged 140 km hr<sup>-1</sup>, impacting a number of the mature plants through lodging and shredding by high winds, which encouraged disease and ultimately impacted total grain yield. Yet soil inorganic N and N flux measurements in KIN 2011 were greater than in 2012, specifically during the period between V6 and VT, possibly offering a protective advantage that year over KIN 2012, when soil N and probe N was relatively lower, but precipitation more consistent.

Weed pressure was likely a strong driver of negative crop yield. At the Kinston site, sickle pod (*Senna obtusifolia*) and morning glory (*Convolvulus arvensis*), and Palmer

amaranth (*Amaranthus palmeri*) appeared to impose a great deal of competition to the corn in many plots. At CEFS, a slightly different population emerged, and where some plots were almost covered by Palmer amaranth (*Amaranthus palmeri*), rye grass (*Lolium perenne*), Johnson grass (*Sorghum halepense*), or curly dock (*Rumex crispus*). Curly dock in particular, with low-spreading leaves, presented a formidable challenge to stand establishment in mid-May. Possibly as a result, the average yield across all treatments was 0.8 Mg ha<sup>-1</sup> at CEFS, far below 7.2 Mg ha<sup>-1</sup>, the 2012 state average. There is growing evidence of weed control using cover crop mulches and residues as discussed by Reberg-Horton et al (2012). Given sufficient biomass production and resulting thick surface mulches, hairy vetch when evaluated alone and with different cover crops can successfully check weed growth (Cook, et al., 2010, Decker, et al., 1994, McVay, et al., 1989, Mitchell, et al., 1977). If sufficient moisture has accumulated in the root zone prior to termination, resulting weed control and moisture retention through use of surface mulches may produce improved yields.

Weediness also appeared to be a function of termination method as well. Flail and spray termination methods at Kinston during both site years consistently achieved the greatest yields among termination treatments. Similar to Kinston, yields at CEFS in flailed or sprayed hairy vetch showed slight increases in yield. Further, at this site, the Wickham loamy sand did not appear to hold moisture for long periods of time, and with a large fine sand fraction, made the soil extremely hard and slow to re-wet. Results suggest that no-till approaches may have led to greater soil moisture retention and therefore slightly higher

yields, however this result was not observed in the roller-crimper termination (Schwartz, et al., 2010).

## **CONCLUSION**

This research shows that biomass from all four winter annual legumes had a measurable impact on soil N after termination, increasing N that is available to corn for the first four to ten weeks after termination. Termination method also made a considerable difference in how cover crops affected soil N released over the observation period. When terminating cover crops with a disk, the observed peak soil nitrogen ranged from 42-56 days after cover crop termination. Measurements of soil inorganic N and N flux as measured through PRS probes were inconsistent with each other for both Kinston 2011 and 2012, however similar patterns emerged among termination treatments. Measuring nitrogen mineralizing from a decomposing cover crop presents many challenges due to the dynamic nature of nitrogen kinetics in the biological and chemical systems driven by biotic and abiotic factors (Schimel, et al., 2004, Schomberg, et al., 2009).

Increasing grain yield requires more than adequate available N. In particular, improved weed control in a no-till system are going to be required if it is to be widely adopted by organic farmers. While conventional growers have herbicides for control, organic farmers do not yet have inexpensive, acceptable alternative (Crews, et al., 2004). As a result organic farmers have to rely on a variety of approaches, especially tillage, for weed control. Alternatives have been researched such as the roller crimper which may be a viable alternative for weed control (Davis, 2010, Moyer, 2011). Despite the great importance

placed on decomposition and subsequent movements of nitrogen, water will be an increasingly limiting factor and also the greatest challenge to control. Hairy vetch and Austrian winter pea clearly provide a viable alternative source to synthetic nitrogen for cash crops.

Long term cover cropping may be the key to increasing soil N values over time by stemming N losses (Drinkwater, et al., 1998); fixing ammonium to the clay fraction (Nieder, et al., 2011); and increasing microbial populations which in turn will prime internal nutrient cycling and potentially promote immobilization and provide short-term capture of available soil N (Burger, et al., 2003, McSwiney, et al., 2010). All this will result in a more resilient soil supporting and maximizing the performance of cash crops.

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## **APPENDICES**

**Table 2.1: Cover crop attributes by site year**

		<b>Kinston</b>				<b>CEFS</b>		
		HV	CC	AWP	BC	HV	CC	AWP
2011	Biomass (Mg ha <sup>-1</sup> )	6.56 b	7.53 a	5.81 b	4.88 c	nd	nd	nd
	Total N (kg ha <sup>-1</sup> )	215.97 a	169.46 b	190.99 ab	107.59 c	nd	nd	nd
	C:N Ratio	13 b	18.5 a	12.9 b	19.7 a	nd	nd	nd
	Plant Lignin (%)	12.35	8.65	10.73	14.6	nd	nd	nd
2012	Biomass (Mg ha <sup>-1</sup> )	6.54 a	6.39 a	6.12 a	2.35 b	8.83 a	9.66 a	6.53 b
	Total N (kg ha <sup>-1</sup> )	174.69 a	110.25 b	170.37 a	40.36 c	288.44 a	263.58 a	204.76 b
	C:N Ratio	15.7 c	24.9 a	15.8 c	19.9 b	12.9 b	16.1 a	13.2 b
	Plant Lignin (%)	12.93	11.33	12.55	9.2	20.2	13.9	15.45

**Table 2.2 Analysis of variance for soil nitrogen as measured by PRS Probes.**

Source of Variation	KIN 2011	KIN 2012
	Significance	
Termination	NS	***
Cover Crop (Termination)	***	***
Time	***	***
Termination*Time	***	***
Cover Crop*Time (Termination)	***	***

\*\*\* Significant at 0.001

**Table 2.3: Analysis of variance for inorganic soil nitrogen.**

Source of Variation	KIN 2011	KIN 2012	CEFS 2012
	Significance		
Termination	***	NS	***
Cover Crop	***	***	***
Termination*Cover Crop	***	NS	NS
Time	***	***	***
Termination*Time	***	NS	***
Cover Crop*Time	***	***	***
Termination*Cover Crop*Time	***	NS	NS

\*\*\*Significance at 0.001

**Table 2.4 Analysis of variance for total corn grain yield.**

Source of Variation	KIN 2011	KIN 2012	CEFS 2012
	Significance		
Cover Crop	NS	***	***
Termination	***	***	NS
Cover Crop*Termination	NS	***	***

\*\*\* Significant at 0.001

**Table 2.5: Grain yields among cover crops by termination treatments (Mg ha-1)**

<i>Treatment</i>	KIN 2011								KIN 2012								CEFS 2012							
	DISK		FLAIL		ROLL		SPRAY		DISK		FLAIL		ROLL		SPRAY		DISK		FLAIL		ROLL		SPRAY	
Austrian Winter																								
Pea	4.28	ab	6.97	a	2.18	b	5.12	ab	5.44	a	4.95	a	4.06	a	5.45	a	0.73	a	0.28	a	1.26	a	1.02	a
Balansa Clover	5.01	ab	5.38	ab	1.56	b	6.03	a	2.11	a	0.52	b	1.77	ab	3.07	a	nd		nd		nd		nd	
Crimson Clover	2.69	ab	4.28	ab	1.92	b	6.51	a	4.60	a	4.39	a	4.06	a	2.93	a	0.34	a	0.92	a	0.93	a	0.36	a
Hairy Vetch	3.09	a	2.78	a	3.63	a	3.54	a	5.61	a	4.65	a	3.94	a	4.78	a	0.54	b	1.72	a	0.88	ab	2.15	a
N0	5.21	ab	6.81	ab	2.85	b	7.64	a	4.21	a	0.44	b	0.89	b	4.01	a	1.69	a	0.04	b	0.05	b	0.09	b
N168	4.62	ab	9.26	a	3.77	b	7.42	ab	7.29	a	3.70	b	3.18	b	7.82	a	nd		nd		nd		nd	

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

**Table 2.6: Correlation values for SPAD and Grain Yields**

<i>R</i> =	KIN 2011	KIN 2012	CEFS 2012
	0.4462	0.3936	0.4509

**Table 2.7: Schedule of field operations**

	KIN 2011	KIN 2012	CEFS 2012
Cover Crop Planting	12 October 10	17 October 11	3 October 11
Cover Crop Termination	26 April 11	19 April 12	11 May 12
Corn Planting	12 May 11	1 May 12	18, 25 May 12
V6	2 June 11	31 May 12	21 June 12
VT	7 July 11	12 July 12	5 July 12
Corn Harvest	22 September 11	23 August 12	13 September 12

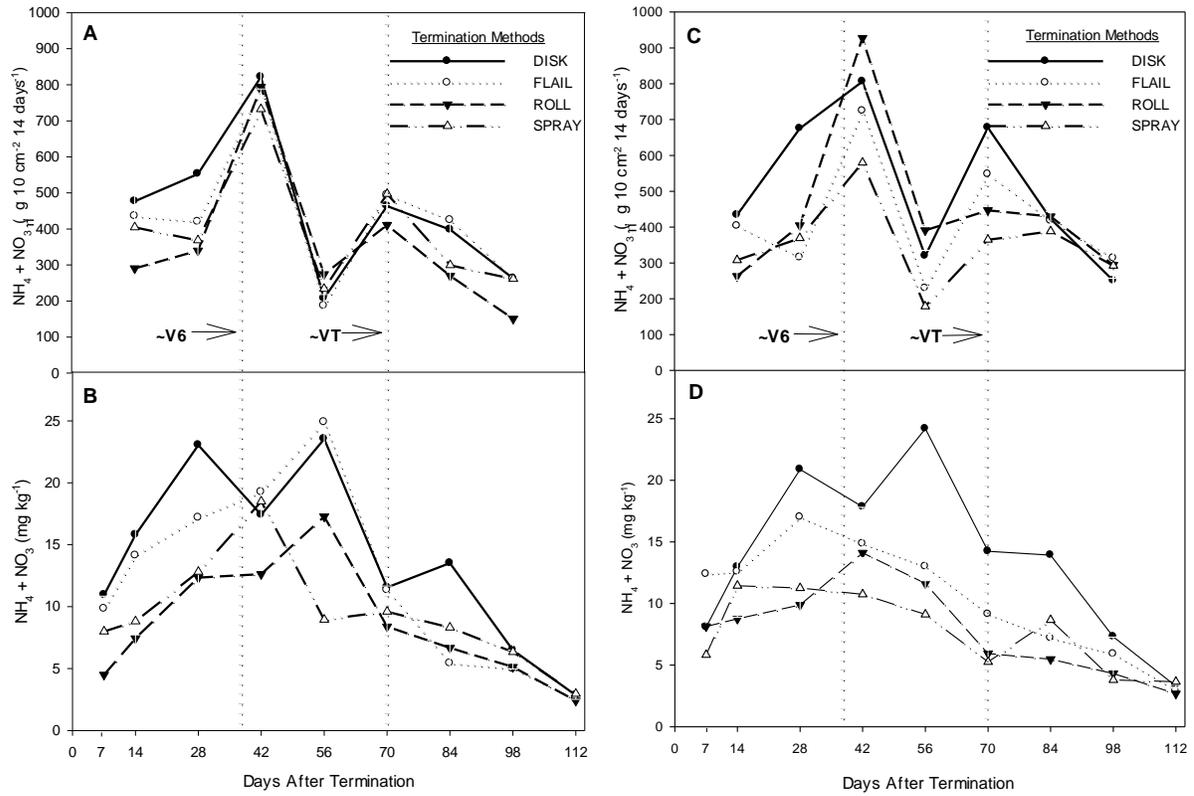
**Table 2.8: Schedule of Soil Sampling**

Sample No.	KIN	KIN	CEFS
1	30 March 12	05 April 12	05 April 12
2	02 May 11	26 April 12	17 May 12
3	12 May 11	04 May 12	24 May 12
4	26 May 11	17 May 12	07 June 12
5	09 June 11	31 May 12	21 June 12
6	23 June 11	14 June 12	05 July 12
7	07 July 11	28 June 12	19 July 12
8	21 July 11	12 July 12	02 August 12
9	04 August 11	26 July 12	16 August 12

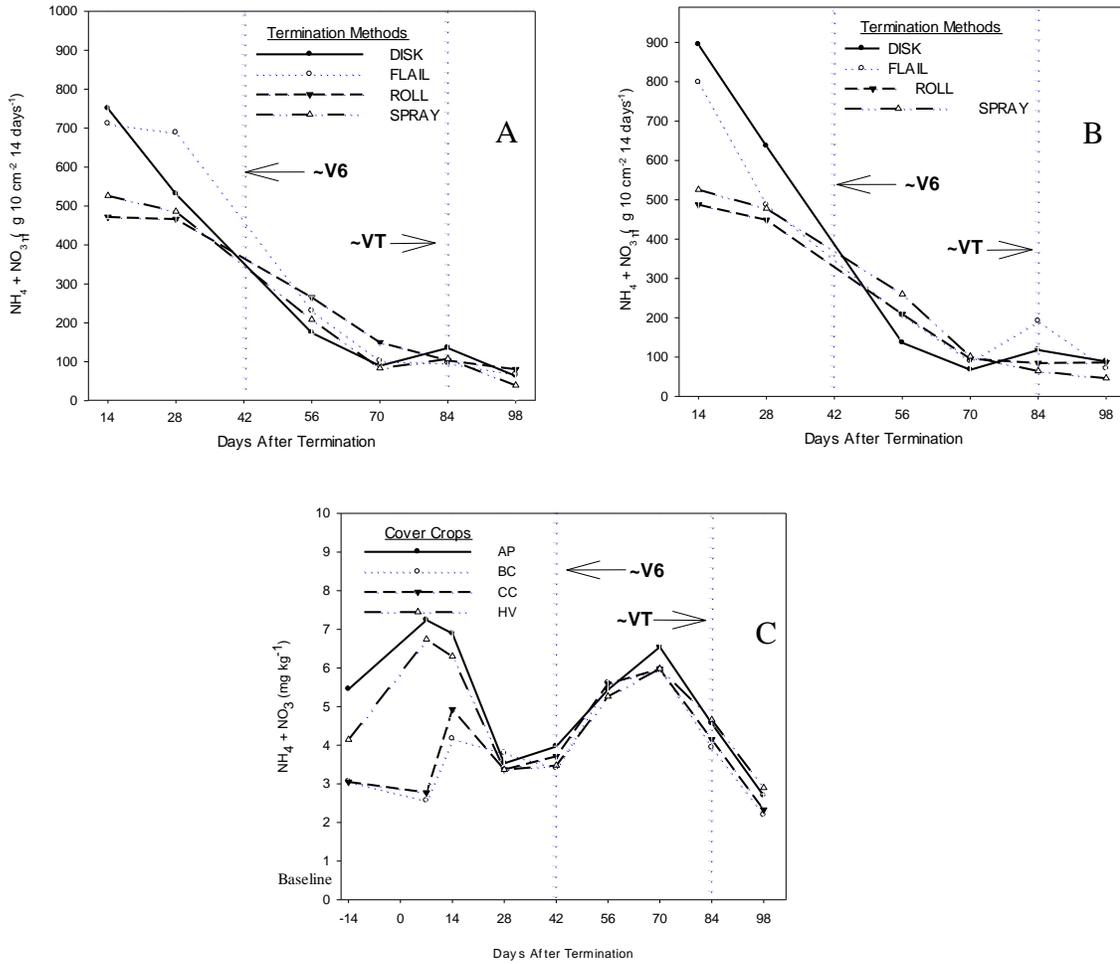
**Table 2.9: PRS Probe insertion and extraction calendar.**

	KIN 2011	KIN 2012
PRS ->1 inserted	12 May 11	5 May 12
PRS ->2 exchanged	26 May 11	17 May 12
PRS ->3 exchanged	9 June 11	31 May 12*
PRS ->4 exchanged	23 June 11	14 June 12
PRS ->5 exchanged	7 July 11	28 June 12
PRS ->6 exchanged	21 July 11	12 July 12
PRS ->7 exchanged	4 August 11	26 July 12
PRS 7-> removed	18 August 11	9 August 12

\* Only sixteen probes were available to replace the existing 48. This data point has not been included in the analysis.



**Figure 2.1: Kinston 2011 Soil N as measured by PRS probe adsorbable N flux in Austrian winter pea (A) and hairy vetch (C). Figure B & C is soil N as measure by KCl extraction in Austrian winter pea (B) and hairy vetch (D).**



**Figure 2.2: Kinston 2012 Soil N as measured by PRS probe adsorbable N flux in Austrian winter pea (A) and hairy vetch (B). Figure (C) is soil N as measure by KCl extraction.**

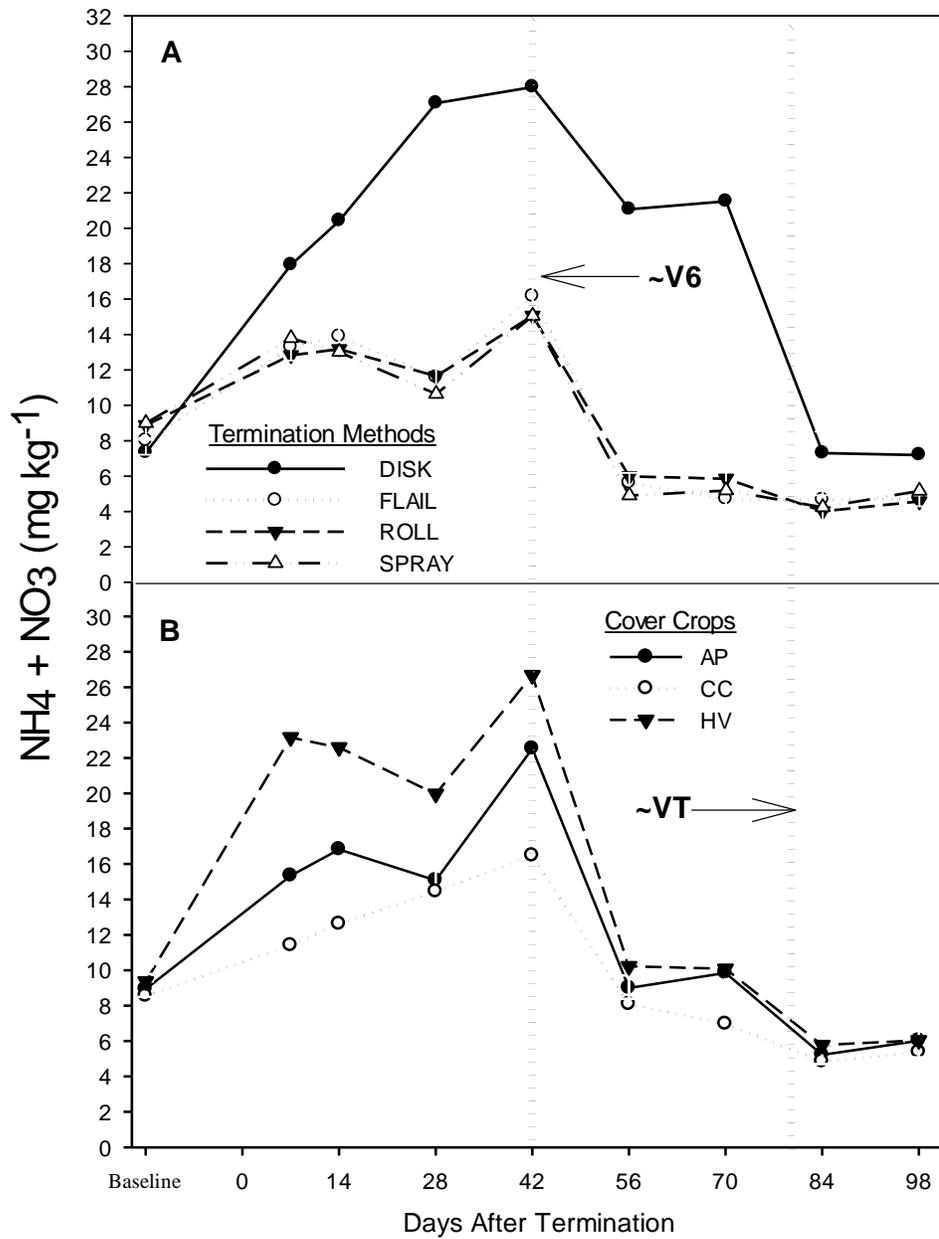
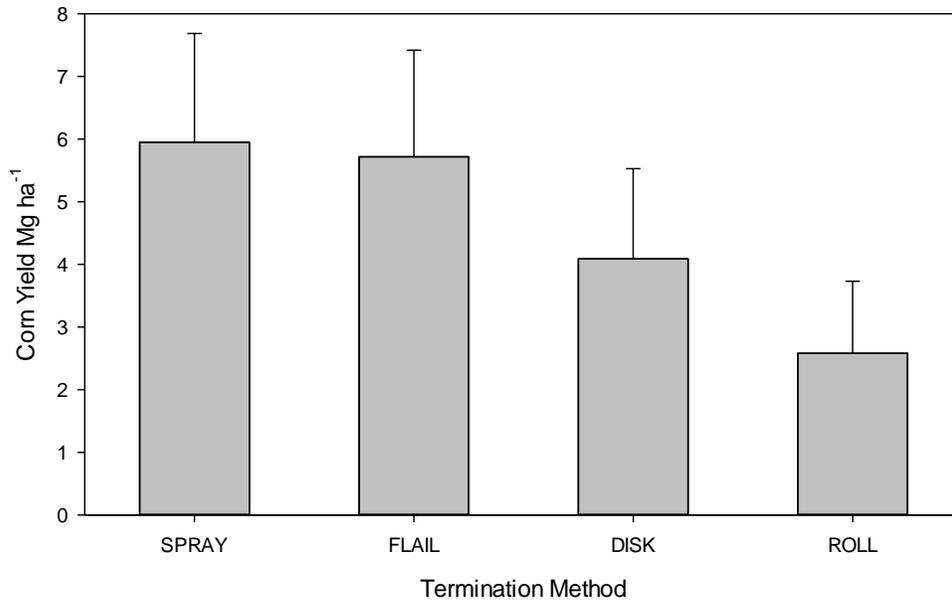
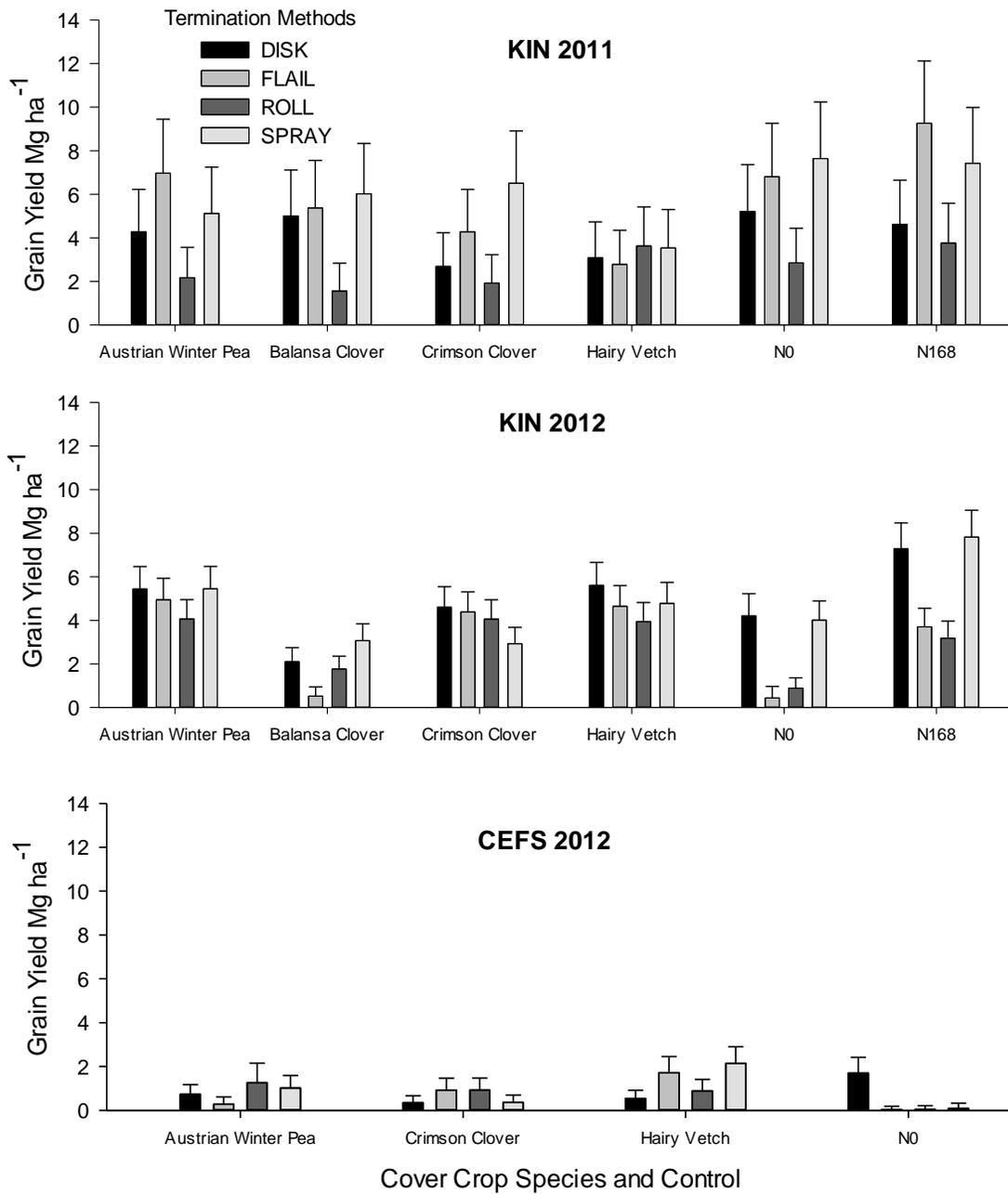


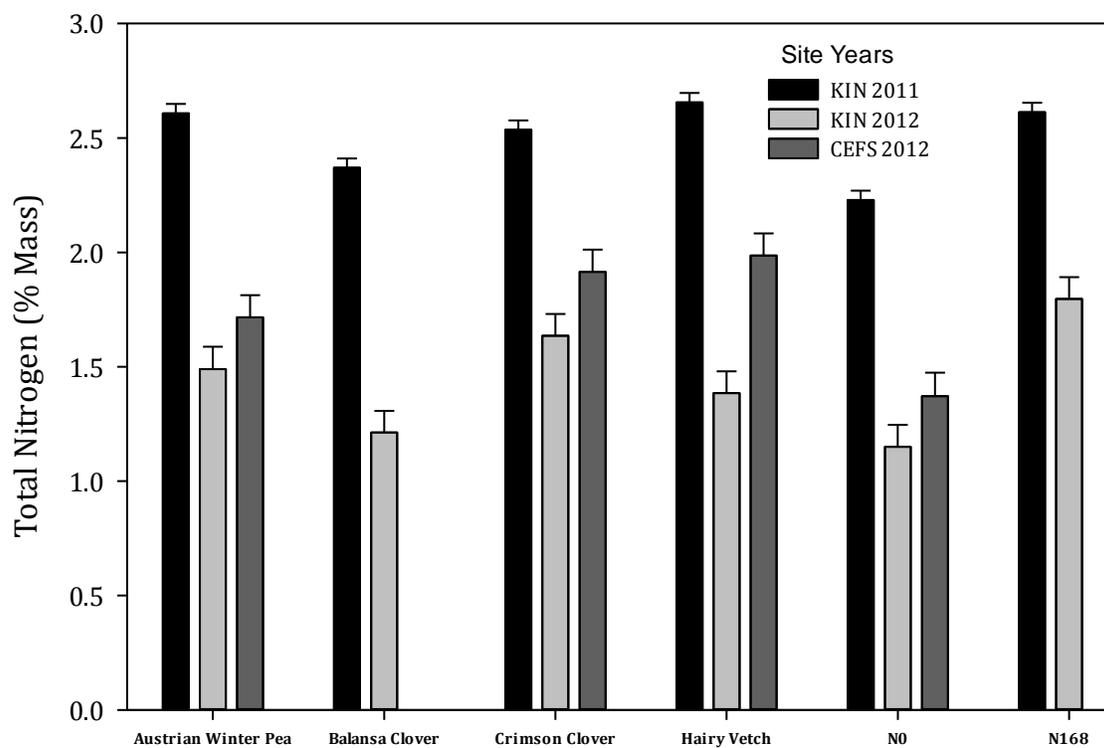
Figure 2.3: CEFS 2012 Soil N as measured by (A), (B) KCl extraction.



**Figure 2.4: Kinston 2011 Grain yields by termination method.**



**Figure 2.5: Grain yields ( $\text{Mg ha}^{-1}$ ).**



Cover Crops and Controls

**Figure 2.6: Corn biomass N at V6 growth stages (% mass).**