

ABSTRACT

JANI, ARUN DILIPKUMAR. Winter Annual Legume Cover Crop Root Decomposition as a Function of Spring Termination Approach and Root Morphology. (Under the direction of Dr. Julie Grossman).

Winter annual legume cover crops have traditionally not been viewed as a major contributor to long-term soil organic carbon (SOC) due to their rapid rate of shoot decomposition.

However, there is evidence that legume cover crop root residues decompose at a slower rate relative to shoots and estimates of legume cover crop-derived SOC based solely on shoot decomposition have led to an incomplete understanding of total legume cover crop contribution to long-term SOC. The objectives of this study were to (i) determine how traditional and novel legume cover crop termination approaches affect root decomposition rate (ii) gain greater understanding about the influential role of soil inorganic N in root decomposition (iii) characterize root morphological properties relevant to decomposition for Austrian winter pea (AWP) (*Pisum sativum*), crimson clover (CC) (*Trifolium incarnatum*) and hairy vetch (HV) (*Vicia villosa* Roth) and (iv) investigate decomposition rate of cover crop coarse and fine root fractions. To determine the effect of cover crop termination approach on root decomposition, two field studies were conducted in Goldsboro and Kinston, North Carolina. Root materials from AWP, CC, and HV were collected from living plants at each site and washed, air-dried, and placed in litterbags, which were buried in AWP, CC, HV, and bare control plots terminated by disking or roller-crimping. Initial root C, N and lignin percentage was determined. Litterbags were retrieved 2, 4, 6, 8, 12, and 16 weeks after burial to determine root mass loss. Soil inorganic N and percent root C and N were also determined at each collection. Termination method did not affect root decomposition rate. There also was no evidence for a relationship between soil inorganic N status and root

decomposition rate at either site; neither did percent root N, root lignin/N and root C/N ratio improve the prediction of decomposition rate for cover crop species. Root decomposition was best characterized at both sites by a double exponential, four parameter model. In Goldsboro, roots from all species decomposed at similar rates, while in Kinston CC decomposed faster than other species. To determine root morphological characteristics, two greenhouse studies were conducted in which plants were grown in PVC cylinders in a completely randomized design. Roots were harvested and root morphological characteristics were determined. Cover crop species did not differ in root morphological properties. However, in both studies, all cover crop species had greater than 70% fine roots (< 1-mm), which have been reported to have high turnover. A 12-week temperature, light, and humidity-controlled incubation study with soil from the Goldsboro field site was conducted to better understand decomposition dynamics of coarse and fine root fractions of CC and HV. Half of treatments received urea ($\text{CH}_4\text{N}_2\text{O}$) at a rate of $133 \mu\text{g}/\text{cm}^3$ (200 kg N/ha). Samples were collected at 1, 2, 3, 4, 8, and 12 weeks after root litterbag burial. Fine roots decomposed faster than coarse roots for both species and after 12 weeks greater HV root mass remained compared to CC when averaged across root size fractions and N treatments. Root C/N and lignin/N ratios served as valuable predictors for root decomposition as fine roots (lower C/N) decomposed faster than coarse roots (higher C/N). There was no evidence that soil inorganic N addition affected root decomposition. Incubation studies are an excellent way to determine the effects of plant litter chemistry on decomposition since environmental factors are held constant.

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Winter Annual Legume Cover Crop Root Decomposition as a Function of Spring
Termination Approach and Root Morphology

by
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DEDICATION

In loving memory of my mentors, Captain Thomas Sankara and Amilcar Cabral, fearless champions of the voiceless and men whose courage was surpassed only by their immeasurable love and hope for the exploited and forgotten of society

BIOGRAPHY

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CHAPTER 1: WINTER ANNUAL LEGUME COVER CROP ROOT DECOMPOSITION UNDER TRADITIONAL AND NOVEL TERMINATION APPROACHES: A REVIEW

Winter Annual Legume Cover Crops in Sustainable Agriculture

Benefits of incorporating legume cover crops into farming systems

Winter annual legume cover crops are an integral component of soil fertility management programs in organic cropping systems. Producers depend on these cover crops, which include Austrian winter pea (*Pisum sativum*), crimson clover (*Trifolium incarnatum*), and hairy vetch (*Vicia villosa* Roth), to furnish essential plant nutrients, particularly nitrogen (N), for subsequent cash crops (Ranells and Wagger 1993; Wagger et al. 1998). Integration of legume cover crops into farming systems enhances long-term agronomic productivity, decreases reliance on mineral fertilizers, contributes sequestration of atmospheric CO₂, and improves farmer livelihood (Puget and Drinkwater 2001; Kong and Six 2010).

Utilization of legume cover crops in farming systems has long been associated with net soil N mineralization (Fornara et al. 2009). Crimson clover residue released 63% of residue N within four weeks after termination in maize systems (Waggoner 1989). Legume cover crop-derived N can be a significant source N for subsequent cash crops. Sullivan (2003) reported that Austrian winter pea, crimson clover, and hairy vetch accumulate 144, 115, and 141 lbs N acre⁻¹, respectively of which approximately 50% is utilized by subsequent cash crops (Gaskell et al. 2007). This provision of N reduces farmer dependence on mineral fertilizers.

Estimates of legume cover crop contribution to soil organic carbon (SOC) have generally not included roots, which have led to an incomplete understanding of total legume cover crop contribution to long-term SOC. Roots have been reported to account for at least 30% of total legume cover crop biomass (Puget and Drinkwater 2001), and it is also widely reported that roots decompose at a slower rate than shoots across several plant functional groups (Balesdent and Balabane 1996; Lu et al. 2003; Abiven et al. 2005; Williams et al. 2006). It is, therefore, crucial to include roots into estimates of legume cover crop residue contribution to soil in order to gain greater insight into the potential of legume cover crops to build long-term SOC.

Soil organic carbon is critical to ensuring high levels of agricultural productivity due to its positive effect on soil physical and chemical properties (Sullivan 2003). SOC is associated with greater soil biodiversity, increased soil water and nutrient holding capacity, decreased crusting and erosion, and improved soil aggregate stability, which leads to deeper and more prolific root systems and greater agricultural production (Sullivan 2003; Lal 2008; Alcántara 2011). The objective of this literature review is to discuss root decomposition dynamics of winter annual legume cover crops common in Southeastern US farming systems. Specifically, it will discuss factors contributing to legume cover crop decomposition and introduce how cover crop termination method and root morphology may potentially affect decomposition dynamics.

Factors Affecting Legume Cover Crop-Derived SOC Retention in Soils

Climatic controls on SOM decomposition

Residence time of cover crop-derived SOC is influenced by several abiotic and biotic factors including climate and soil environmental conditions (Schmidt et al. 2011; Dungait et al. 2012), plant litter biochemical composition (Fujimaki et al. 2008), soil physical and chemical properties (Sanaullah et al. 2011; Rasse et al. 2005), decomposer organism and catalytic enzyme accessibility to plant biomass (Georgieva et al. 2005), and soil nutrient status, particularly N (Fog 1988; Mary et al. 1996). Models of SOC decomposition dynamics, such as the CENTURY model, suggest that climate, particularly temperature and moisture, is the single best predictor of crop residue decomposition across large geographical regions (Gijsman et al. 1997; Berg and McLaugherty 2008; Fujimaki et al. 2008; Wang et al. 2010). For example, constant freezing temperatures in permafrost regions lead to low plant residue decomposer activity, which explains why permafrost soils store as much SOC as that believed to exist in all the other soils in the world (Schmidt et al. 2011). Craine et al. (2010) reported temperature sensitivity, not SOC biochemical quality, to be the primary control on SOC decomposition in soils from a wide range of sites. Raising temperature in incubation studies, for example, has significantly increased decomposition rate of rice root and shoot residues (Lu et al. 2003). Andrén et al. (1992) found soil moisture to be the major limiting factor influencing decomposition of barley straw and root biomass under field conditions, while Gijsman et al. (1997) reported similar results for root decomposition of grasses.

It is likely that relative importance of moisture and temperature on crop residue decomposition depends on existing climatic conditions. In humid regions, for example, decomposition responds to elevated temperature, while in arid or semi-arid regions decomposition responds primarily to moisture (Gill and Burke 2002). This temperature effect could potentially accelerate legume cover crop decomposition in the very humid Southeastern US.

The significance of this climatic effect on litter decomposition is more pronounced at the soil surface than within the soil profile (Fujii and Takeda 2010). Within the confines of a specific climatic zone, variables such as temperature and moisture, have a greater effect on decomposing surface litter compared to roots since decomposer communities within the soil are buffered from extremes in temperature and precipitation relative to surface litter (Silver and Miya 2001; Fornara et al. 2009). Climatic factors, especially temperature and moisture, play a crucial role in controlling rate of plant litter decomposition and must be considered when investigating plant litter decomposition dynamics.

Plant litter biochemical composition and decomposition

Plant biochemical composition has traditionally been viewed as a major determinant of plant litter decomposition and models suggest that litter quality is second only to climate as the main predictor of plant litter decomposition rates (Berg et al. 1987; Silver and Miya 2001; Fujimaki et al. 2008). Plant litter can be grouped into easily decomposable, intermediately decomposable, and slowly decomposable fractions based in part on carbohydrate (soluble cell materials, cellulose, hemicelluloses, and starches), protein, lignin, and nutrient contents

(Paul and Clark 2007; Sylvia et al. 2004; Abiven et al. 2005). There is great variability in decomposition rates of different carbohydrate components ranging from a few hours (simple sugars and fructans) to several weeks (cellulose) with hemicelluloses having intermediate decomposition rates (Berg et al. 1987; Gunnarsson and Marstorp 2002). Generally, higher concentrations of soluble cell components (simple sugars), hemicelluloses, and protein favor more rapid decomposition rate of plant materials, while elevated lignin decreases decomposition rates (Herman et al. 1977; Sylvia et al. 2004). Plant biochemical composition helps to explain why shoot and root residues from legume cover crops such as white clover and crimson clover (high protein, low lignin) decompose more rapidly than ryegrass (low protein, high lignin) (Buchanan and King 1993; Rasmussen et al. 2010). Variation in decomposability of different carbohydrate components is an important factor to consider as it may influence N mineralization in plant proteins (Gunnarsson and Marstorp 2002).

The importance of initial C, N, and lignin content on plant biomass decomposition has long been recognized (Urquiaga et al. 1998; Abiven et al. 2005; Soto et al. 2005). Measurements of C/N and lignin/N ratios can predict cover crop residue decomposition (Puget and Drinkwater 2001; Berg and McLaugherty 2008; Fujii and Takeda 2010) whereas plant litter decomposition has been positively correlated with tissue N status across a plethora of species (Fornara et al. 2009; Dodd and Mackay 2011). Lignin, however, is hydrophobic and has a nonspecific structure, which makes it difficult for catalytic enzymes to attack (Metting, Jr. 1993; Paul 2007). It is usually chemically-connected to carbohydrates such as cellulose and hemicelluloses and it provides resistance to decomposition of these carbohydrates (Sylvia et al. 2004).

Lignin has traditionally been viewed as a major component of plant-derived SOC, influencing both its pool size and its turnover, although recent findings suggest this lignin effect may be over-estimated (Thevenot et al. 2010). During decomposition of plant litter, lignin concentration generally increases as readily decomposable carbohydrates are consumed (Berg et al. 1987; Abiven et al. 2005). Generally as lignin/N and C/N ratio increases, plant litter rate of decomposition and nutrient mineralization decreases. However, Palm and Sanchez (1991) reported that polyphenol and polyphenol/N ratio served as the best predictors of tropical legume shoot N mineralization. Decomposition and N mineralization is typically predicted if $C/N < 25/1$ (Soto et al. 2005). For example, white clover roots ($C/N = 15$) have been shown to decompose twice as fast as rye grass roots ($C/N = 38$) under field conditions (Rasmussen et al. 2010). Buchanan and King (1993), however, have reported significantly greater decomposition rates for crimson clover shoots ($C/N = 14$) compared to roots ($C/N = 17$), which may be explained by roots significantly higher lignin/N ratio compared to shoots, 5.3 and 2.5, respectively. Lu et al. (2003) reported similar results with rice straw and root residues. Plant biochemical composition, therefore, serves as a valuable aid in predicting and explaining decomposition patterns in cover crop biomass, especially when root biochemical composition is included in the model.

Rate of plant litter decomposition

The general pattern of decomposition for plant biomass over time assumes a first- order rate of decay and is influenced by the C, N, and lignin content of the plant biomass (Gijsman et al. 1997; Sylvia et al. 2004; Soto et al. 2005; Paul 2007). This pattern can best be described

as an initial exponential decomposition rate followed by a much slower phase (Andrén et al. 1992; Lu et al. 2003). For example, Berg et al. (1987) reported an initial rate (first two weeks) of red clover root decomposition that was 40 times greater than decomposition at later stages. During this initial rapid decomposition period, the easily decomposable materials including simple carbohydrates, some hemicelluloses, and proteins are easily metabolized by soil microorganisms (Metting, Jr. 1993; Paul and Clark 2007).

Decomposition then slows considerably after this period as the proportion of lignin and lignin-complexes increases (Berg et al. 1987; Abiven et al. 2005). In fact, C/N ratio, which is a good predictor of early rates of decomposition, is often not correlated with decomposition rates during later stages (Mary 1996; Gorissen and Cotrufo 2000; Schmidt et al. 2011; Creamer et al. 2013). Plant lignin concentration plays a more pivotal role than litter C/N ratio during later stages of decomposition (Herman et al. 1977; Mary et al. 1996). In terms of litter biochemical properties, lignin likely plays a greater role in controlling decomposition during these later stages because easily decomposable C sources have been consumed by decomposer communities.

While plant litter biochemical composition is an important determinant of plant litter decomposition, a growing body of empirical evidence suggests that it plays a secondary role in determining long-term SOC residence time, and that soil C stability is more closely associated with external abiotic and biotic controls (Hobbie et al. 2008; Schmidt et al. 2011; Dungait et al. 2012). This new insight into plant litter decomposition underscores the importance of adopting a more holistic perspective that considers not only the drivers, but also how they interact.

Soil physical properties and decomposition

Soil physical properties such as texture and structure can have a profound effect on decomposition both directly and through their interactions with other drivers of decomposition. Soil texture and structure are unlikely to significantly affect surface litter decomposition due to limited surface litter-soil contact, but decomposition of belowground plant biomass is likely to be heavily influenced by these soil physical properties (Gijssman et al. 1997). Soil texture directly influences soil water holding capacity and drainage, soil surface area, and overall surface charge (Coyne 1999; Schmidt et al. 2011). Furthermore, these properties vary with depth within the soil profile leading to site-specific rates of decomposition (Gill and Burke 2002; Sanauallah et al. 2011). Finer textured soils have smaller average pore size and have higher water content for a given soil water potential, which is conducive to high cover crop biomass productivity and subsequent SOC accumulation (Gijssman 1997; Sylvia et al. 2004). The high negative charge associated with the soil clay fraction in systems without pH-dependent charge allows for greater sorption of nutrients and organic molecules, which helps support microbial decomposer communities (Paul and Clark 2007). Interestingly, C mineralization rates, however, are often lower in fine-textured soils compared to coarse-textured soils. For example, Matus et al. (2008) reports that SOC is correlated to clay and silt content in both forest soils and cultivated lands. Sorption of SOC molecules onto clay surface minerals confers its preservation, and SOC in coarse-textured soils is more vulnerable to environmental disturbances such as tillage since it

does not readily complex to the coarse mineral fraction due to its low surface charge (Creamer et al. 2012).

Soil physical, chemical, and biological properties interact to influence soil structure and subsequently aggregate stability (Daynes et al. 2013), which aids in the protection of newly added carbon materials. A well-aggregated soil allows for increased water entry, air flow, water-holding capacity, and enhanced SOC protection (Sullivan 2003; Sullivan 2004). Greater microbial activity and SOC mineralization is observed in larger pores because larger size facilitates decomposer microorganism accessibility (Aanayeva et al. 2012). Within soil aggregates oxygen concentration generally decreases further into the interior, which limits aerobic decomposition and favors SOC stabilization (Coyne 1999). Decomposer organisms are often physically separated from SOC leading to greater persistence of SOC over time (Schmidt et al. 2011; Dungait et al. 2012). This separation occurs because SOC acts as a binding agent in aggregate formation and the protection of SOC within aggregates diminishes its availability to decomposer organisms and enzymes (Sanaullah et al. 2011). Soils with more stable aggregates, therefore, more efficiently store SOC than poorly aggregated soils. Incorporation of SOC within microaggregates is an especially important mechanism for long-term SOC storage since these smaller aggregates are more challenging for decomposer organisms to penetrate (Lu et al. 2003; Williams et al. 2006; Kong and Six 2010). Well-aggregated soils are in large part responsible for long-term residence time of SOC.

Soil inorganic N status and decomposition

Legume cover crops deposit large quantities of N into the soil as they decompose and evidence suggests a positive relationship between soil inorganic N status and plant litter decomposition rate (Herman et al. 1977; Fog 1988; Mary 1996). This fertility effect, however, has not always been empirically supported (Recous et al. 1995; Gorissen and Cotrufo 2000; Dodd and Mackay 2011). Nitrogen additions to soil are known to stimulate microbial activity and subsequent plant litter decomposition (Dignac 2002). Soil inorganic N is utilized by soil microorganisms in decomposition of residues, but if soil inorganic N is insufficient, catabolized litter will be inefficiently assimilated into microbial biomass (Paul and Clark 2007; Sylvia et al. 2004; Williams et al. 2006). This principle of soil inorganic N limitation on plant litter decomposition is observed across plant functional groups. In both grass and legume systems, for example, C/N ratio of soil has been shown to be positively related to total living root biomass and negatively related to root biomass loss (Fornara et al. 2009). Soil inorganic N status must be taken into account when seeking to predict or explain decomposition of plant litter.

Soil inorganic N concentration varies considerably during plant litter decomposition due to constant N flux between organic and inorganic forms. Plant-derived N is mineralized, immobilized, and remineralized concurrently by microorganisms and the rate of these processes depends partly on the quality of residue and stage of litter decomposition (Gunnarsson and Marstorp 2002; Mary et al. 1996; Fornara et al. 2009). As decomposition progresses and the proportion of recalcitrant plant litter increases, microbial biomass

decreases leading to remineralization of plant-derived immobilized N into the soil (Malpassi et al. 2000). Remineralization of immobilized NO_3^- is most rapid when soil inorganic N is not a limiting factor to microbial growth (Zagal and Persson 1994). In other words, under high soil inorganic N conditions, immobilized N is quickly released back into the soil inorganic N pool. This pattern does not hold true under low soil N conditions. Under low soil inorganic N concentration, decomposition rate of plant residues decreases, but doesn't stop and immobilization intensity is reduced leading to delay in N remineralization (Mary et al. 1996). Through an understanding of leguminous cover crop residue quality and its associated changes on a temporal scale as well as soil inorganic N status, it is possible to gain better insight into the decomposition dynamics of plant litter.

Decomposition Dynamics of Root Material

Historical bias toward only quantifying shoot material

Decomposition and nutrient mineralization dynamics of winter annual leguminous cover crop aboveground residue has been investigated extensively (Ranells 1992; Buchanan and King 1993; Puget and Drinkwater 2001; Kong and Six 2010), while belowground biomass (i.e. roots) has traditionally received far less attention in decomposition studies. This occurs in part due to difficulties in excavation and inaccessibility, which takes considerable time. It also is more difficult to obtain root biomass in its entirety, as fine roots are often lost in the sampling process due to their small size (Lu et al. 2003). Fine roots also have quick turnover

rates (Fornara et al. 2009; Wang et al. 2010) which could lead to underestimates of total root production at sampling.

Apart from these logistical difficulties in collecting roots, the historical perception has also been that leguminous cover crop roots represent only a small fraction (5-15%) of total plant N and that shoot N can provide a reasonable estimate of total cover crop N (Peoples et al. 2009). Recent evidence, however, suggests that this perception of roots has been erroneous and roots can furnish large quantities of N agro-ecosystems (Silver and Miya 2001; Kähkölä et al. 2012). Peoples et al. (2009) report that total N inputs associated with or derived from nodulated roots can account for 30-50% of total plant N in pasture and crop legumes. Despite this evidence for leguminous cover crop root relevance from a nutrient cycling perspective, understanding of leguminous cover crop root detritus N dynamics is still very limited.

Root contributions to long-term SOC

Root biomass, in particular, decomposes slowly relative to aboveground biomass and roots have a relatively greater influence than aboveground biomass on soil C pools (Lu et al. 2003; Williams et al. 2006; Kong and Six 2010). This slow decomposition rate might be useful in contributing soil C to aid in atmospheric CO₂ sequestration to mitigate the threat of global climate change. Soil is an enormous C sink in the global C cycle and it functions as the third largest global C pool sequestering two-thirds of C in the form of SOC (Lal 2008). In some forest ecosystems, root litter inputs contribute to SOC and soil nutrient pools to the same magnitude as foliar materials (Berg 2008; Wang et al. 2010). In agro-ecosystems, maize

roots have been reported to contribute 1.5 times that of stalks and leaves to SOC and this is attributed to relatively high rhizo-deposition and slower rates of root decay (Balesdent and Balabane 1996).

Drivers of long-term root-derived C storage in soil

The primary drivers responsible for greater root contributions to long-term SOC, relative to shoots are the rhizosphere environment and differences in root biochemical composition compared to shoots. Plant litter physical properties such as particle size (root thickness and mean straw length) have traditionally not been included in decomposition models (Andr n et al. 1992). This exclusion has been especially problematic for predicting root decomposition since this process correlates very strongly to root particle size (Hobbie et al. 2008; Rasmussen et al 2010). Models that simply rely on initial C/N ratio to predict decomposition have proven inadequate in predicting root decomposition for not considering unique chemical characteristics of root tissue (Abiven et al. 2005). Silver and Miya (2001) assert that models utilized to describe aboveground biomass decomposition may not accurately describe root decomposition dynamics due to the different environments these two plant components occupy. These two unique environments generate different physicochemical conditions and associated decomposer communities (Fujii and Takeda 2010). Root-derived SOC is preferentially stored within soil aggregates due to root-soil contact during aggregate formation (Sanaullah 2011). The unique physicochemical characteristics of the soil environment and the long-term association of roots with those characteristics leads to a differential pattern in decomposition of root litter compared to shoot litter.

Longer-term residence time of root-derived SOC compared to shoot-derived SOC is most often attributed to greater recalcitrance of root litter compared to shoot litter (Buchanan and King 1993; Lu et al. 2003). Roots exist in a very different environment from shoots and they perform fundamentally different physiological functions, which could explain some of these differences in chemical composition (Silver and Miya, 2001; Horwath et al., 2007). Roots contain a high concentration of suberin, for example, which complexes with lignin to create structurally dynamic, impermeable barriers that protect the plant from harmful substances diffused from the soil (Abiven et al. 2005). This suberin-lignin complex is highly resistant to decomposition. Results of a legume cover crop decomposition study by Kong and Six (2010) reveal the importance root recalcitrance. In the investigation, 52% of hairy vetch-derived SOC remaining at the end of the maize growing season compared to just 4% of shoot-derived residue using ^{13}C tracers. Root biomass, if considered across all particle sizes, generally has biochemical properties that lend it more relevant in determining long-term SOC pools (Balesdent and Balabane 1996; Abiven et al. 2005; Fujii and Takeda 2010).

Thorough analysis of rhizosphere ecology provides greater insight into the significance of root litter biochemistry in determining SOC pools. Roots death and decomposition occurs continuously in the rhizosphere. The rhizosphere has an abundance of readily available C-rich substrates through sloughed off plant cells and compounds released by plant roots (Sylvia et al. 2004; Paul and Clark 2007; Cardon and Whitbeck 2007). Van der Krift et al. (2002) found that living roots of grass species accelerated dead root decomposition through the former's stimulation of decomposer microbial biomass via C exudates. The rhizosphere is buffered from climatic extremes and supports a vibrant

decomposer microbial community, which creates an environment conducive to rapid decomposition. Root biomass, however, contributes greater amounts to SOC than shoots, which underscores the importance of root biochemical composition in controlling decomposition.

Considering that root and shoot materials have different biochemical properties, it is within reason to expect that they would contribute differently to the long-term biochemical composition of SOC. Abiven et al. (2005) reported different C and N mineralization dynamics for leaf, stem, and root decomposition across a wide range of species, but did not investigate how these plant components affected SOC composition. In forest systems, fine root components have been shown to contribute more than foliar material to specific protein markers during initial stages of decomposition (Mambelli et al. 2011). Clemente et al. (2012) found that changes in SOC composition over time relates to plant biochemical composition and that maize roots contributed greater quantities of microbe-derived SOC than maize leaf and stem materials. These investigations provide insight into the role that root and shoot components have in influencing the composition of SOC.

Decomposition dynamics of coarse and fine roots

Adding further complexity to root litter decomposition is the influence of root morphology on decomposition rate. Root morphology refers to root physical properties such as total root length, diameter, total surface area, and total number of root tips (Magalhaes et al. 2011; Nichols et al. 2007). Coarse roots are generally reported as those having root diameter > 2-mm, while fine roots are < 2-mm diameter (Fujimaki et al. 2007; Fornara et al. 2009; Hobbie

et al. 2010). Strict definitions, however, should be avoided due to heterogeneity between species (Berg 2008). Fine roots, for example, have also been reported as < 1-mm (Berg et al. 1987).

Fine roots often have quick turnover rates and play a pivotal role in nutrient cycling (Silver and Miya 2001; Fornara et al. 2009). Rasmussen et al. (2010) asserts that in ryegrass and clover mixes, coarse roots determine SOC pool build-up, while fine roots determine soil N pool build-up. Decomposition behavior of coarse and fine roots is often explained by reference to tissue biochemistry. Fine roots have biochemical composition that is more conducive to decomposition than coarse roots. Legume cover crops have been reported to contain 1.1 – 1.3% and 3.4 – 3.7% N in coarse and fine roots, respectively (Gardner and Sarrantonio 2012). Fine roots of several forest species have also been reported to have significantly higher % N and lower C/N than corresponding coarse roots (Fujimaki et al. 2008). Wang et al. (2010) and Hobbie et al. (2010) have reported correlations between fine roots and leaf litter in nutrient content as well as specific root length and specific leaf area in forest systems, but similar correlations of leaf and fine root decomposition have been inconsistent.

The effects of root diameter on winter annual leguminous cover crop decomposition dynamics has not been investigated extensively. Berg et al. (1987) followed decomposition behavior of red clover fine (< 1-mm), intermediate (1-4-mm), and coarse (> 4-mm) roots over 196 days, but did not find significant differences in rate of mass loss. It is important to consider the effect of soil inorganic N status on legume cover crop root decomposition. In the weeks immediately following legume cover crop termination, soil inorganic N content

reaches its peak, but the effect that higher soil inorganic N content has on decomposition of legume cover crop roots is poorly understood.

Winter Annual Legume Cover Crop Spring Termination

Traditional and novel termination methods in sustainable cropping systems

In organic farming systems of the Southeastern US, producers use traditional and novel methods to terminate winter annual leguminous crops. Disking is a traditional cover crop termination strategy common in organic cropping systems in which a mechanical disk incorporates shoot residues into the soil. This method leads to stimulation of microbial activity due to improved soil aeration and an infusion of cover crop substrate materials (Georgieva et al. 2005; Paul and Clark 2007) and results in rapid residue decomposition (Buchanan and King 1993). Termination methods such as herbicide spraying or flailing produce surface mulch that decomposes slower due to less residue soil contact. During residue decomposition in disked systems, larger quantities of cover crop N are mineralized and at a faster rate compared to traditional herbicide spray systems (Sullivan 2003).

While disked leguminous cover crop residues furnish large quantities of plant available N, the potentially long-term deleterious effects that disking has on soil structure must also be considered. Disking leads to long-term reduction in SOC, alters food webs by degrading microbial biomass, and weakens soil structure (DuPont et al. 2010). Disking also weakens the integrity of soil macro-aggregates leading to release of occluded SOC (Cardon and Whitbeck 2007) and thereby negating the benefits of atmospheric CO₂ sequestration.

The end result is greater uniformity in pore size distribution with a dominance of medium sized pores and the absence of smaller pores that are most effective at sequestering long-term SOC (Ananyeva et al. 2013).

Terminating cover crops with a tractor-mounted implement called a roller-crimper is a novel approach that has been gaining popularity due to its proven effectiveness in weed suppression (Ashford and Reeves 2003; Mirsky et al 2011). The implement is a cylindrical drum with chevron shaped blades that rolls over and crimps cover crop stems when the crop is in full bloom leaving plants flattened on the soil surface as protective mulch (Davis 2010; Parr et al. 2011). Mulching correlates strongly with improved soil water holding capacity, enhanced soil aggregate stability, and long persistence of SOC (Buchanan and King 1993; Metting, Jr. 1993; Kornecki et al. 2009). Small grains or other horticultural crops can be directly planted into crimped fields. Crimping reduces or eliminates herbicide usage (Mirsky et al. 2009; Mirsky et al. 2011) offering the possibility of no-till organic farming systems. If the roller-crimper is going to be utilized as a cover crop termination strategy, it is crucial to ensure that cover crops are not left standing, which may entail multiple passages over a field. Persistence of leguminous cover crops such as hairy vetch in improperly crimped systems has led to reduced maize stand counts and yields (Mirsky et al. 2009; Parr et al. 2011).

In traditional no-till systems that utilize herbicides for cover crop termination, less plant available N is provided for subsequent cash crops compared to systems where cover crops are disked (Waggoner 1989; Sullivan 2003). Investigations directly comparing soil inorganic N dynamics in roller-crimped and disked systems from the same study site are lacking. Complicating this matter further is the great variability reported in total N (10 to

217 kg ha⁻¹) furnished by different legume cover crops in roller-crimped systems (Parr et al. 2011). Considering the different soil physical and environmental conditions that are generated in disked and roller-crimped systems in addition to potential variability in soil N cycling within each system, it is within reason to expect that legume cover crop root residues would decompose differently under these different management strategies.

Conclusions

Winter annual legume cover crops such Austrian winter pea (*Pisum sativum*), crimson clover (*Trifolium incarnatum*), and hairy vetch (*Vicia villosa* Roth) are known to improve both short-term and long-term agricultural productivity through provision of essential crop nutrients, particularly N, and abundant crop residues which potentially contribute to long-term SOC depending on decomposition dynamics. It is likely that estimates of legume cover crop residue contribution to long-term SOC pools has been significantly underestimated due to an incomplete understanding of root biomass contributions in legume cover crop systems (Rasmussen et al. 2010; Ranells and Wagger 1993). While numerous factors influence legume cover crop root litter decomposition, it is crucial to realize interactions of such factors and that root system decomposition is an intrinsic ecosystem property (Schmidt et al 2011; Dungait et al. 2012).

Differences in biochemical composition between root size fractions have been attributed to their different decomposition characteristics (Kong and Six 2010; Rasmussen et al. 2010). Despite their importance in organic farming systems, decomposition of leguminous cover crop root size fractions has not been studied extensively. Research that

has occurred (Berg et al. 1987) has not considered the relevance of soil inorganic N status on root decomposition. Considering that the soil environment in the weeks following kill may be N-rich after input of legume biomass, rates of decomposition are expected to vary.

Legume cover crop kill method is likely to affect short-term N dynamics and thus long-term SOC buildup. Disking is likely to augment short term soil N, but perhaps at the expense of long-term soil quality (Sullivan 2003; Cardon and Whitbeck 2007). Using the roller-crimper termination method may have a more positive effect on long-term SOC and soil structure compared to disking (Sanford et al. 2012). Knowledge of legume cover crop root system decomposition and contribution to long-term SOC is limited in both of these systems. Considering the different readily available soil N contents associated with both systems, study of root contributions to long-term SOC in these systems would be a worthy pursuit.

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CHAPTER 2: WINTER ANNUAL LEGUME COVER CROP DECOMPOSITION DYNAMICS UNDER TRADITIONAL AND NOVEL TERMINATION APPROACHES

Introduction

Winter annual legume cover crops furnish essential plant nutrients, particularly N, to subsequent cash crops as well as plant residues, which can contribute to long-term soil organic carbon (SOC) in the form of soil organic matter (SOM) (Ranells and Wagger 1993; Wagger et al. 1998). Mineralization and decomposition dynamics of aboveground legume cover crop residues have been investigated extensively (Ranells 1992; Buchanan and King 1993; Kong and Six 2010). Legume cover crops are generally used to furnish N for subsequent cash crops and have not traditionally been viewed as a source of long-term SOC (Sullivan 2003). However, estimates of legume cover crop contribution to SOC have generally not included roots, which have led to an incomplete understanding of total legume cover crop contribution to long-term SOC. Roots have been reported to account for at least 30% of total legume cover crop biomass (Puget and Drinkwater 2001), and it is also widely reported that roots decompose at a slower rate than shoots across several plant functional groups (Balesdent and Balabane 1996; Lu et al. 2003; Abiven et al. 2005; Williams et al. 2006). Including roots into estimates of legume cover crop contribution to SOC will provide new insight into the potential of legume cover crops to build long-term SOM.

Factors known to influence residue decomposition include climate and environmental conditions, plant litter biochemical composition, soil physical and chemical properties, decomposer accessibility to crop residues, and soil inorganic N status (Mary et al. 1996;

Georgieva et al. 2005; Schmidt et al. 2011; Dungait et al. 2012). The observed longer residence time of root-derived SOC compared to aboveground litter is influenced by soil climatic conditions and root biochemical composition (Kong and Six 2010). For example, the soil environment generates different physiochemical conditions and associated decomposer communities compared to the soil surface (Silver and Miya 2001; Fujii and Takeda 2010). Smaller roots also penetrate developing soil aggregates and become occluded, which confers physical protection from decomposer communities (Kong and Six 2010; Sanaullah et al. 2011). The importance of initial C, N and lignin content on plant residue decomposition has also long been recognized (Urquiaga et al. 1998; Abiven et al. 2005; Soto et al. 2005). Roots generally have lower N content, higher C/N ratios, and greater amounts of recalcitrant materials such as lignin and suberin compared to aboveground residues leading to slower rates of root decomposition (Puget and Drinkwater 2001; Abiven et al. 2005; Berg and McLaugherty 2008; Lindedam et al. 2009; Fujii and Takeda 2010).

Disking is a common legume cover crop termination method that results in rapid cover crop aboveground residue decomposition due to greater residue-soil contact compared to surface mulch systems and elevated soil oxygen content, both of which stimulate microbial activity (Georgieva et al. 2005; Paul and Clark 2007; White and Rice 2007). Such faster rates of decomposition and associated nutrient mineralization in disked systems also leads to greater soil inorganic N content compared to termination methods that leave cover crop residues as surface mulch (Sullivan 2003). Soil inorganic N has been identified as a limiting factor in aboveground plant litter decomposition by several investigators (Fog 1988; Mary et al. 1996), but similar effects on root decomposition are poorly understood.

Termination of legume cover crops with a roller-crimper, a tractor-mounted cylindrical drum with chevron-shaped blades is a novel approach that is gaining popularity due to its effectiveness in weed suppression (Ashford and Reeves 2003; Sullivan 2003). Roller-crimping affords farmers the opportunity to implement no-tillage practices without use of herbicides (Mirsky et al. 2009; Mirsky et al. 2011). The implement rolls over cover crops disrupting vascular flow and leaving aboveground litter as surface mulch, which correlates strongly with improved soil water holding capacity (Kornecki et al. 2009). Surface mulching in no-tillage systems is associated with greater soil aggregate stability and longer persistence of SOC (Buchanan and King 1993; Metting, Jr. 1993; Smith et al. 2007). Such benefits have not been investigated extensively in roller-crimped systems, but it is reasonable to speculate surface mulch in this system provides similar benefits.

In traditional no-tillage systems that utilize herbicides for cover crop termination, less plant available N is provided for subsequent cash crops compared to systems where cover crops are disked (Wagger 1989; Sullivan 2003). Investigations directly comparing soil inorganic N dynamics in roller-crimped and disked systems are lacking. Complicating this matter further is the great variability reported in total N (10 to 217 kg ha⁻¹) furnished by different legume cover crops in roller-crimped systems (Parr et al. 2011). Considering the different soil physical and environmental conditions that are generated in disked and roller-crimped systems in addition to potential variability in soil N cycling within each system, it is within reason to expect that legume cover crop root residues would decompose differently under these different management strategies.

Puget and Drinkwater (2001) and Kong and Six (2010) followed hairy vetch root decomposition dynamics in traditional disked systems, but the effect of roller-crimped approaches on legume cover crop root decomposition is poorly understood. In the present study, we investigated the effect of termination method (disking vs. roller-crimping) on decomposition of Austrian winter pea, crimson clover, and hairy vetch root litter over 16-weeks at two locations (Goldsboro and Kinston, North Carolina) planted to corn after cover crop termination. The objectives of this study were to: (i) compare the decomposition rate of Austrian winter pea, crimson clover, and hairy vetch roots after termination (ii) follow cover crop root litter C and N dynamics over time and relate changes to observed patterns in root decomposition and (iii) determine the relationship between soil inorganic N status and rate of cover crop root decomposition. We hypothesized that cover crop roots would decompose fastest in disked plots due to the soil's higher inorganic N content compared to rolled plots.

Materials and Methods

Field Site and experimental setup

Legume cover crop root decomposition was investigated in Goldsboro (35°39'N, 78°03'W) and Kinston (35°26'N, 77°65'W), NC. In Goldsboro, the study occurred at the Center for Environmental Farming Systems (CEFS) on Wickham loamy sand (fine loamy, mixed, thermic, Typic Hapludult), while in Kinston, the study was located at the North Carolina Department of Agriculture & Consumer Services Caswell Research Farm on Portsmouth loam (fine loamy fluviomarine deposits over sandy skeletal fluviomarine, mixed, thermic

Typic Umbraquult). Treatments included three cover crop species of Austrian winter pea (AWP) (*Pisum sativum*), crimson clover (CC) (*Trifolium incarnatum*), hairy vetch (HV) (*Vicia villosa* Roth) and termination treatments of roller-crimped, disked, flail mowed and herbicide sprayed. For this study only two of the four termination treatments (roller-crimped and disked) were used. All cover crops were planted at both sites in a randomized complete block split-plot design. Main plots (termination method) were 12 x 27 m² in Goldsboro and 18 x 30.5 m² in Kinston, while split plots (cover crop species) measured 6 x 14 m² in Goldsboro and 6 x 15 m² in Kinston. Within each main plot, one (Goldsboro) and two (Kinston) split plots were not planted to cover crops and served as bare controls. A schedule of field activities is presented in Table 2-1. In Goldsboro, certified organic plots prohibited application of mineral fertilizers, thus, only negative controls without N addition (N00) were established. In Kinston, mineral fertilizer was used and bare plots served as zero-N or positive N bare controls. Positive bare controls received 150 kg N ha⁻¹ as urea-ammonium nitrate immediately preceding corn planting. Plots where cover crops were grown are collectively referred to as “cover crop fertility plots”, while controls are designated as N00 or N150 plots (Table 2-2). Monthly means of daily maximum air temperature as well as total precipitation during the study period for Goldsboro and Kinston are presented in Figs. 2-1 and 2-2.

Root litter collection and burial

In April 2012 prior to cover crop termination in Goldsboro and Kinston, root litter (including both coarse and fine roots) from living AWP, CC, and HV was collected to 15 cm depth at

both sites and processed separately. Roots were soaked and washed thoroughly using tap water, air-dried at room temperature on greenhouse benches until constant weight was achieved, and cut into 3-5 cm pieces (Berg et al. 1987). The litterbag method (Bocock and Gilbert 1957) was used to determine the effect of termination treatment on rate of root decomposition in the field. Root litter was placed in nylon bags (9 x 9 cm², 1 mm mesh) in the following quantities: 0.8 g bag⁻¹ for AWP and CC, 0.7 g bag⁻¹ for hairy vetch as less hairy vetch roots were available than AWP and CC. Root litterbags were then buried between rows to 15-cm depth in plots terminated by disking or roller-crimping. Roots were buried in each of their corresponding cover crop fertility plots, and roots from all species were buried in N00 and N150 control plots. In Kinston, for example, hairy vetch roots were buried in disked and roller-crimped plots where hairy vetch was previously sown, plus separate litterbags for roots of AWP, CC, and HV were buried in N00 and N150 control plots. Litterbags were retrieved 2, 4, 6, 8, 12, and 16 weeks after burial (Table 2-1) and were oven-dried at 70°C for 48 hours. Oven-dried roots were removed from litterbags and a brush was used to remove adhering soil, capturing all material for further analysis. Forceps were used to retrieve displaced roots (Wang et al. 2010). Oven-dried root weight was recorded.

Root litter chemical analysis

Subsamples of root litter were collected prior to placement in litterbags and ground in a laboratory mill (Cyclotec 1093 Sample Mill) equipped with a 1-mm mesh screen to determine initial C, N, and lignin content. Initial root C and N content was measured using a Perkin Elmer 2400 CHNS/O Elemental Analyzer (Norwalk, CT, USA) in the Environmental

and Analytical Testing Service (EATS) facility at North Carolina State University (NCSU). Lignin was measured by Dairy One (Ithaca, NY). At each litterbag retrieval date during the study, root subsamples were taken from the bags to determine ash, C and N content after litterbag content oven-dried weight was determined. Remaining ash represented the soil mineral contaminant of root litterbag contents and was determined through combustion in a muffle furnace. To determine root mass loss at each collection date, subsample weight used for ash determination was recorded before and after combustion. It was assumed that differences in weight resulting from combustion were due to root organic C loss as CO₂. The percent root in subsamples is equal to the root subsample weight before combustion minus root subsample weight after combustion divided by the root subsample weight before combustion. The proportion of originally buried root mass remaining in retrieved litterbags at time (t) is equal to the collected oven-dried litterbag content weight multiplied by the percent root mass of the subsample and divided by the original weight of the buried root mass.

To determine root N mass in litterbags, corrections were made for litterbag soil contamination. Root litterbag content mass was multiplied by percent N obtained from litterbag subsamples to determine litterbag content N mass. Subsample percent ash was multiplied by litterbag content to determine soil contaminant. Four random soil samples were collected 15 cm depth using a 2.54 cm (one-inch) diameter soil probe from each site and averaged within site to determine percent total soil N. Percent total soil N was multiplied by litterbag soil contaminant to determine soil-derived N mass. Root N mass was determined by subtracting soil-derived N mass from litterbag content N mass.

Soil inorganic N analysis

At each root litterbag retrieval date, soil samples were collected from each corresponding treatment plot to 15 cm depth using a 2.54 cm (one-inch) diameter soil probe. Ten subsamples were taken per plot and homogenized manually then bulked. Soils were dried at 40°C to constant mass, then ground to pass 1 mm mesh screen. Samples were extracted with 1 M KCl with shaking for 1 hour. Samples were then allowed to settle for 20 minutes before filtration through #42 Whatman filter paper. Extracts were refrigerated at 4°C until analysis for NH_4^+ and NO_3^- on a QuikChem 2000 flow injection autoanalyzer (Lachat Instruments, Loveland CO) (Parr et al. 2013).

Statistical analyses

In this study, analysis of variance with the PROC MIXED procedure (SAS Institute Inc., Cary, NC) was used to measure the following response variables: percent root mass remaining, root C and N mass and percent remaining, and soil inorganic N content. Termination method, cover crop fertility, and time were fixed effects, while block was treated as a random effect. Multiple comparisons between treatment means were made with Tukey-Kramer HSD ($P < 0.05$) on least squared means.

Plant litter decomposition is often best described by first order decomposition kinetics characterized by initial rapid mass loss followed by a slower rate of decay (Gill and Burke 2002; Paul and Clark 2007). Buchanan and King (1993) have reported that CC root decomposition follows an exponential function and similar decomposition dynamics may be

expected for AWP and HV. Percent root mass remaining was regressed with time from all treatment plots using nonlinear regression procedures of SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA) to find the most suitable exponential model to describe decomposition. The following exponential models were fit to root mass loss: i) single, two-parameter model (Eq. [1]); ii) single, three-parameter model (Eq. [2]); and iii) double, four-parameter model (Eq. [3]). The single, two-parameter model assumes an equal rate of residue decomposition throughout the experimental period, while the single, three-parameter model assumes a portion of litter does not decompose during the experimental period. The double, four-parameter model assumes that litter decomposes at two different rates during the experimental period (Lopp and Guillard 2004; Soto et al. 2005). Model selection to best describe decomposition was based on adjusted coefficient of determination values (adjusted R^2) produced using nonlinear regression. The exponential models used are as follows:

$$f = ae^{-kx} + \varepsilon \quad [1]$$

$$f = a + be^{-kx} + \varepsilon \quad [2]$$

$$f = ae^{-k_1x} + be^{-k_2x} + \varepsilon \quad [3]$$

where f is the percent mass remaining at time x (weeks), a and b represent percent root mass, k is the decomposition rate constant, and ε is the random error term.

Results

Root decomposition dynamics

In Goldsboro, there was a three-way interaction of termination method x cover crop fertility x time effect on root decomposition rate. However, termination method, cover crop fertility, and time as well as interactions of termination method x cover crop fertility and cover crop fertility x time did not affect root decomposition during the study (Fig. 2-3). As expected, a strong effect of time was observed in Goldsboro across all treatments and was characterized by an initial rapid rate of mass loss (weeks 0-2), followed by a slow decomposition period (weeks 2-8), and concluded by another accelerated rate of decay (weeks 12-16). This decomposition pattern is presented for root litter in AWP, CC, and HV cover crop fertility plots averaged across termination methods in Fig. 2-4.

In Kinston, two-way interactions of termination method x cover crop fertility and cover crop fertility x time affected root decomposition rate. The main effects of cover crop fertility and time also were significant. In Kinston cover crop fertility plots, CC roots decomposed at a faster rate than AWP and/or HV averaged across termination methods for 5 of 6 sampling periods and at an increasingly larger rate from weeks 8 -16 (Fig. 2-4). Root decomposition in these plots was characterized by an initial rapid rate of mass loss through week 4, followed by a slower rate of decay for the remainder of the experiment. After 4 weeks, roots lost 60-66% of their original mass. Over the next 12 weeks, there was an additional 14 to 19% loss in root mass. The accelerated rate of mass loss observed in Goldsboro during week 12-16 was not evident in Kinston.

It also was observed that CC roots buried in CC fertility plots decomposed faster than CC roots buried in bare N00 and N150 plots averaged across termination methods, but this phenomenon was not consistently observed for other species (Fig. 2-5). Like Goldsboro, there also was no evidence to support the effect of termination method on root decomposition in Kinston (Fig. 2-6). At both locations, it was found that the rate of decomposition across all treatments was best described by a double exponential four parameter model (Fig. 2-7).

Root chemistry

The initial C/N ratio of roots prior to litterbag burial was significantly higher for CC compared to AWP and HV at both sites (Table 2-3). Due to insufficient root biomass at both locations, only single initial lignin measurements were taken and multiple comparisons were not possible. At each litterbag collection, C/N ratio of root litter from treatment plots was determined to provide insight into observed root decomposition dynamics. Nitrogen mass loss from root biomass was also determined to gain a greater understanding of root N mineralization dynamics. In both Goldsboro and Kinston, CC root C/N ratio from both cover crop fertility and control plots decreased over time across termination methods, while C/N ratio did not fluctuate for AWP and HV (Fig. 2-8). Although CC root C/N ratio was initially higher than AWP and HV at both sites, all three root types had similar C/N ratios by the end of the study. In Goldsboro, cover crop fertility treatments affected root N mass loss with HV roots releasing inorganic N at a faster rate than other species through week 8 across termination methods (Fig. 2-9). In all cover crop fertility and control plots, N mass loss did not vary from weeks 4 to 8. Within N00 plots, N mass loss was similar between species. In

Kinston, root N mass loss varied considerably between species across termination methods (Fig. 2-9). No termination effect on N mineralization was observed in Kinston. In cover crop fertility plots, N mass loss was most rapid through week two and was insignificant from weeks 4 to 12 (AWP, CC, N00), weeks 4 to 16 (HV), and weeks 4 to 8 (N150). Within N00 and N150 plots, N mass loss was similar between species.

Soil inorganic N content

At both Goldsboro and Kinston sites, there was no evidence to support a relationship between soil inorganic N status and root decomposition rate. In Goldsboro, the effects of termination method, cover crop fertility, and time as well as the two-way interactions of termination method x cover crop fertility, termination method x time, and cover crop fertility x time affected soil inorganic N status throughout the root decomposition period. Disked cover crop plots generally had higher soil inorganic N content than corresponding rolled plots and cover crop fertility plots also had higher soil inorganic N content than N00 plots (Fig. 2-10). In Goldsboro, although soil inorganic N fluctuation was highly variable, AWP and HV plots generally contained greater soil inorganic N than CC plots under both rolled and disked termination methods throughout the decomposition period (Fig. 2-11). Rolled cover crop fertility plots in Goldsboro peaked in soil inorganic N content at week four, while results varied with disked treatments.

In Kinston, only cover crop fertility and time affected soil inorganic N status. Two-way interactions of termination method x time and cover crop fertility x time as well as the three-way interaction of termination method x cover crop fertility x time also were observed.

N150 plots had significantly higher soil inorganic N concentrations at litterbag burial (week 0), but at subsequent soil sampling dates soil inorganic N from N150 plots content decreased to similar levels of other fertility plots (data not shown). Soil inorganic N content in Kinston did not differ between cover crop species significantly in cover crop fertility plots, except for week 0 (Fig. 2-12) when cover crop fertility plots peaked in soil inorganic N approximately 10 to 14 days after termination. A second smaller peak occurred at week 8. Bare (N00) control plots peaked in soil inorganic N at week 8 and had smaller peaks at week 0 and 16. Bare (N150) positive control plots peaked in N content at cover crop burial (week 0), which corresponded to mineral fertilizer application (Table 2-1). Positive control plots contributed heavily to the fertility effect on soil N concentration. It is also evident that soil inorganic N was greater in Goldsboro than in Kinston throughout the 16-week study (Fig. 2-11 and 2-12).

Discussion

Root decomposition

Our initial hypothesis that higher soil inorganic N content in disked plots would accelerate cover crop root decomposition was not supported and other factors may have been more important determinants of decomposition. Weed pressure was high in both rolled and disked plots in Goldsboro and may have contributed to soil environmental conditions that were indistinguishable between all treatments, perhaps leading to the lack of termination effect. Climatic factors, particularly temperature and moisture, are often reported as the best predictors of plant litter decomposition (Fujimaki et al. 2008; Wang et al. 2010). Treatment

plots in Kinston did not appear to be limited by soil moisture, and weed pressure also was generally low in both disked and rolled plots. Soil temperature, although not recorded, may have been initially higher in disked plots due to a lack of mulching effect. However, after corn canopy development temperature differences likely were minimal. These similar soil environmental conditions in both disked and rolled cover crop treatment plots likely led to the indistinguishable decomposition rates that were observed in Kinston.

The double exponential four parameter model provided an excellent fit for all treatments at both sites indicating that root litter decomposed at different rates at different times during the study. Weider and Lang (1982) have also suggested that in litterbag decomposition studies, double exponential models best describe root mass loss. However, Soto et al. (2005) and McGrath et al. (2000) found single three-term exponential and single two-term exponential models, respectively, to provide the best fit for aboveground peach palm residue decomposition. Observed differences in best model fits may relate to frequency of decomposition measurements, cropping systems (i.e. monocultures vs. intercropping, agroforestry systems, among others), and criteria used in model selection (Soto et al. 2005).

The initial rapid decline in root biomass observed across cover crop fertility and termination treatments over the first two weeks in both Goldsboro and Kinston was expected, and likely due to microbial consumption of easily decomposable root constituents such as soluble cell components, simple carbohydrates, and proteins (Gijssman et al. 1997; Sylvia et al. 2004; Paul and Clark 2007). The slower, yet steady rate of mass loss observed from weeks 2-4 was most likely the result of decomposition of moderately decomposable root constituents such as hemicelluloses and cellulose. Gunnarsson and Marstorp (2002) have

reported on decomposition rates of several plant litter types that ranged from as little as a few hours (simple sugars and fructans) to several weeks (cellulose) with hemicelluloses having intermediate decomposition rates. The much slower rate of mass loss observed in Kinston over the final 12-weeks of the study is possibly due to the predominance of recalcitrant materials (lignin, suberin, and cutin) and holocellulose-lignin complexes in root biomass after the liberation of easily and moderately decomposable materials (Andrén et al. 1992; Fujimaki et al. 2008; Fujii and Takeda 2010). Although recalcitrant materials, hemicelluloses, and cellulose were not measured throughout this experiment, Berg et al. (1987) followed decomposition for red clover and found a similar pattern of decomposition, which was attributed to high initial concentration of water soluble substances (31%) and potentially higher concentrations of lignin-carbohydrate complexes at later stages of the decomposition process.

However, the accelerated pattern of decomposition observed in Goldsboro across treatments during the final eight weeks of the study is unusual in investigations of plant litter decomposition (Buchanan and King 1993; Abiven et al. 2005; Soto et al. 2005). The final root mass remaining in Goldsboro when considered across all treatments was 12.44%, which was considerably lower than both the Kinston site (23.16%) as well as that observed in other published winter annual legume cover crop studies (Berg et al. 1987; Puget and Drinkwater 2001; Kong and Six 2010). Plant litter decomposition rate has been strongly correlated to soil moisture (Andén et al. 1992; Gijsman et al. 1997), but during this study period total precipitation was low (321 mm) and during the final period of rapid decomposition in question (weeks 8 – 16) precipitation was less than 130 mm. For comparison, precipitation

in Kinston over the final eight weeks of the study totaled approximately 380 mm, which resulted in only an additional 11.56% mass loss compared to 35.28% for Goldsboro suggesting that rainfall and resulting changes in soil moisture were not contributing factors toward the accelerated rate of decomposition during the final eight weeks of the Goldsboro study. Differences in soil texture also likely did not contribute to this rapid later period of decomposition, as several investigators have reported similar patterns of decomposition to that observed in Kinston across soil texture classes (Andr n et al. 1992; Gijssman et al.1997; Soto et al. 2005). One possible explanation for this rapid rate of decomposition during the latter part of the study relates to weed pressure. In Goldsboro, severe grass and broadleaf weed pressure was observed in the majority of plots. Weed growth was especially prolific during July, which corresponded to the beginning of the period of accelerated root mass loss. Readily decomposable C exudates in the rhizosphere derived from living roots are known to enhance soil biological activity (Renella et al. 2006; Cardon and Whitbeck 2007), and Van der Krift et al (2002) and Kuzyakov et al (2007) have provided empirical evidence to show that C exudates from living roots enhance plant litter decomposition. Van der Krift et al. (2002) investigated exudates derived from roots of several grass species, which also comprised a large portion of weed biomass in Goldsboro.

The pattern of faster root decomposition in cover crop fertility plots compared to N00 and N150 plots across termination methods observed in Kinston (Fig. 2-5) may have been related to increased SOC levels, microbial biomass C, and microbial enzyme activity (Feng et al. 2003; Zhang et al. 2012) that is associated with farming systems that utilize legume cover crops. These improved soil quality indicators are conducive to healthy soil biological

activity leading to more rapid rates of root litter decomposition and associated nutrient mineralization. These observations have very practical implications for farmers from a nutrient management perspective as improved soil quality indicators associated with implementation of legume cover crops into farming systems may hasten cover crop-derived nutrient availability over time.

Root litter chemistry

In this study, root litter C/N ratio measurements taken with each litterbag recovery date did not provide insight into patterns of root decomposition. Crimson clover roots contained significantly higher initial C/N ratios at both sites, but decomposed similarly to other species in Goldsboro, and faster than other species in Kinston. Lignin may have played a greater role in influencing rate of decomposition. It is well-established that as readily decomposable plant components are exhausted the proportion of lignin in plant material increases over time and plays a critical role in determining long-term residence time of plant litter (Abiven et al. 2011). Crimson clover had a trend of lower lignin content than other species, based on a single sample analysis. However, the lack of an increased decomposition rate at one of the two sites indicates that lignin may not have been a stronger driver of decomposition than environmental variables. While shoot and root litter biochemistry has successfully predicted decomposition in other investigations (Herman et al. 1977; Buchanan and King 1993; Rasmussen et al. 2010), it is more insightful to view plant litter decomposition dynamics holistically accounting for interactions with other abiotic and biotic factors that influence decomposition. Since plant litter decomposition is influenced by physicochemical and

biological factors from the surrounding environment some researchers have recommended that this process be viewed as an ecosystem property (Silver and Miya 2011; Schmidt et al. 2011).

Root N mass loss proceeded rapidly in this study compared to other investigations. Malpassi et al. (2000), for example, traced N release from rye and oat roots in a controlled incubation and reported less than 55% of root N becoming available for subsequent cash crop uptake during 112 days. In this investigation, approximately 87% and 69% of root N was released from Goldsboro and Kinston sites, respectively during 16 weeks. These reported differences in N release may relate to different environmental conditions and/or plant lignin content. Malpassi et al. (2000) did not report lignin content, but Sullivan (2003) has reported higher lignin content for rye compared to AWP, CC, and HV. The results from this investigation indicate that the majority of legume cover crop root N is released during cash crop growth.

Soil inorganic N content

The lack of termination effect in Kinston was not expected since disking legume cover crop residues into the soil is associated with accelerated decomposition and subsequent nutrient mineralization (Buchanan and King 1993). Sullivan (2003) reports that 60% of legume cover crop N is mineralized for subsequent cash crops in disked systems compared to 40% for no tillage surface mulches. The growth cycle of CC is also very rapid in spring compared to AWP and HV as CC reaches maturity and peak dry matter production 3 to 4 weeks ahead of AWP and HV (Hoyt et al. 2004). It is recommended that winter annual legume cover crops

be terminated at flowering to optimize N provision for subsequent cash crops. In this study, cover crops were terminated based on AWP and HV flowering times and CC was terminated several weeks after its optimal kill date. Shoot biomass of CC had a higher C/N ratio and lignin content compared to AWP and HV at both sites (Brown 2013), which likely led to slower rates of CC-derived N mineralization and subsequent lower soil inorganic N status in CC plots. Parr et al. (2011) has even reported CC to have N immobilization effects on soil inorganic N if terminated several weeks beyond optimal kill date. Rate of N mineralization of these cover crops is expected to increase over a 3 to 6 week period following termination before returning to pre-kill levels by week 6 to 8 (Gaskell et al. 2007; Parr et al. 2011). This general pattern was observed in Goldsboro. The N dynamics associated with all cover crop species showed no correlation to root decomposition.

Peak inorganic N in Kinston occurred at two weeks after termination and a second similar peak was observed 10 weeks after termination (8 weeks after litterbag burial). This initial earlier than usual peak may be associated with high soil moisture (178-mm precipitation in May) accelerating N mineralization. This moisture effect, however, would not explain the second peak as this was preceded by 4 weeks of very low soil moisture (18-mm). Soil inorganic N furnished by cover crop species was also similar in Kinston, despite the suboptimal kill date for CC. Variability in soil inorganic N dynamics across study sites was expected due to differences between sites in terms of soil texture and climate. Leaching potential of soil inorganic N was greater in Kinston due to the higher sand content in the soil and greater precipitation compared to Goldsboro. The leaching factor was most evident in bare, N150 control plots where soil inorganic N content decreased to similar levels as bare

N00 plots within two weeks after application. It also is reasonable to conclude that some soil inorganic N from cover crop fertility plots also leached beyond 15 cm depth and was, therefore, could not be accounted for in root decomposition and soil inorganic N measurements.

Lack of evidence to support a relationship between soil inorganic N content, termination method, and root decomposition rate has important management implications for organic farmers that rely heavily upon winter legume cover crops as both a nutrient and SOM source (Gaskell et al. 2007). It is well established that disked legume cover crop shoot residues decompose more rapidly and are associated with higher soil inorganic N status compared to mulched residues (Buchanan and King 1993; Smith and Collins 2007).

Termination approach and soil inorganic N did not determine root decomposition in this investigation, which provides organic farmers with a more accurate understanding of total legume cover crop contribution to SOC pools. For example, Parr et al. (2011) reported great variability in total legume cover crop-derived N in roller-crimped systems, and organic farmers, who are reliant on maintaining SOM for soil fertility management, need not consider soil N as a factor contributing to legume cover crop root decomposition. Recent efforts have also been made to quantify the value of C stored in agricultural soils (Sparling et al. 2006) and it is possible that future C credit legislation that rewards farmers for growing cover crops will be enacted. Under such a scenario it is critical to have an accurate understanding of total contribution of legume cover crops to SOC pools.

Conclusions

Our original hypothesis that cover crop root litter in disked plots would decompose faster than root litter in roller-crimped plots due to higher soil inorganic N status in disked plots was not supported and there was no evidence that soil inorganic N status affected root decomposition rate. Interestingly, C/N ratio of root material also did not provide insight into observations of decomposition for cover crop species providing further evidence for the emerging view that plant litter decomposition must be viewed holistically taking into account the importance of biotic and abiotic influences from the surrounding environment (Schmidt et al. 2011, Dungait et al. 2012). There was evidence to suggest that roots from different legume cover crop species decomposed at different rates with crimson clover having decomposed fastest in Kinston. Considering the importance of sequestering atmospheric CO₂ from the perspective of global climate change, it is very insightful to have a clearer understanding of root contribution to total legume cover crop biomass production (Silver and Miya 2001). Further investigations are needed to determine nutrient mineralization dynamics during root decomposition for these species as well how root morphological differences (i.e. total root length, fine root length, and surface area) between species may affect decomposition rate.

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Goldsboro climatic data

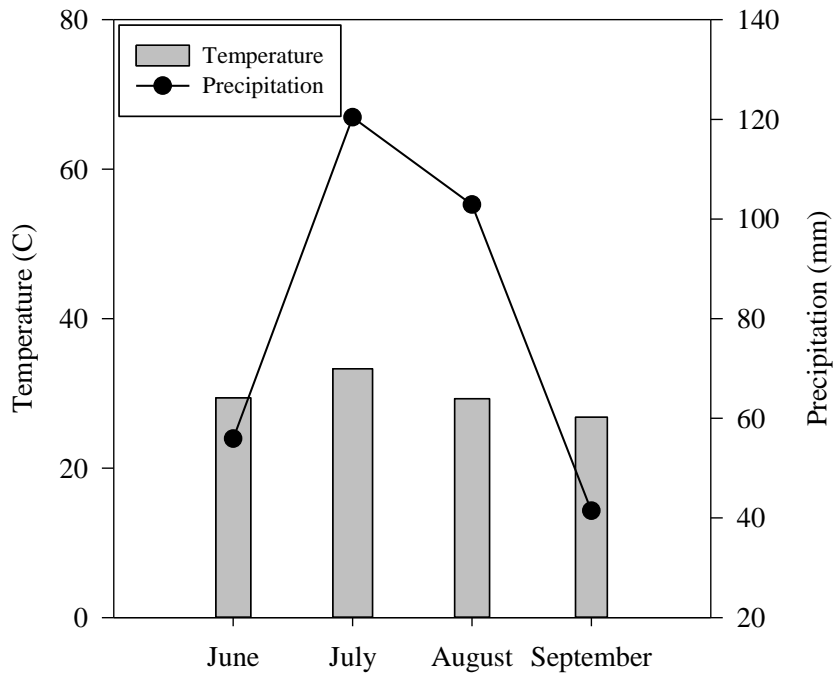


Figure 2-1 Monthly means of daily maximum air temperature (C°) and total monthly precipitation for CEFS study site in Goldsboro, NC site. Data from May 25-31 were added to June. September 13, 2012 was the last sampling date.

Kinston climatic data

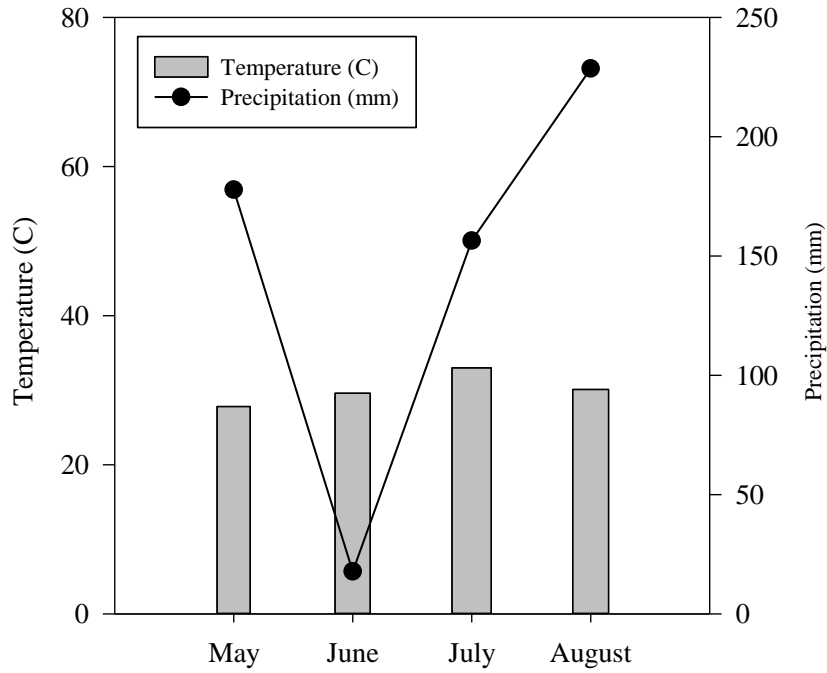


Figure 2-2 Monthly means of daily maximum air temperature (C°) and total monthly precipitation for Caswell Research Farm study site in Kinston, NC. Data were taken May 2 to August 23.

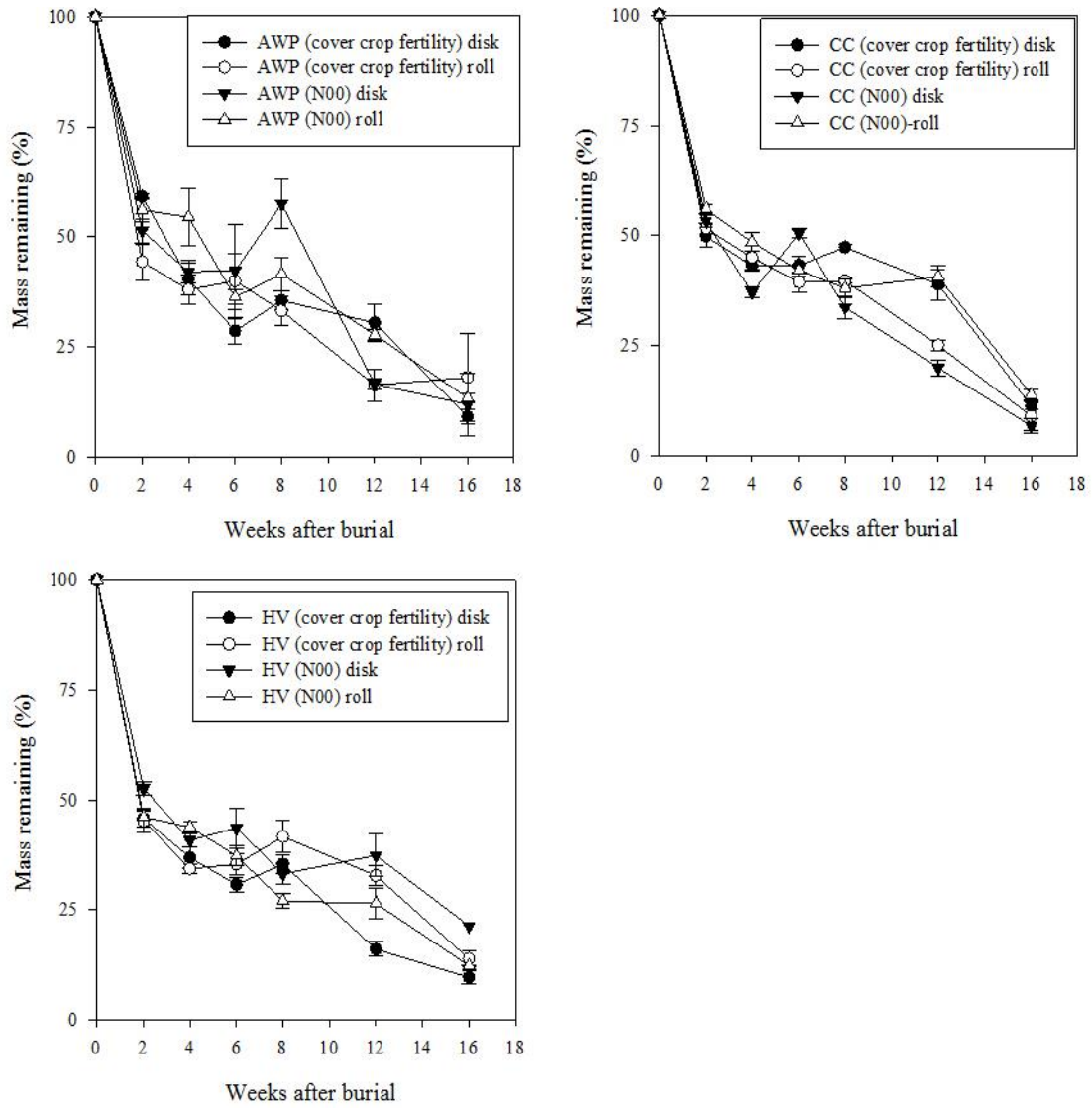


Figure 2-3 Root decomposition in cover crop fertility and bare, control (N00) plots as a function of termination method at Goldsboro site. Error bars represent one standard error of the mean.

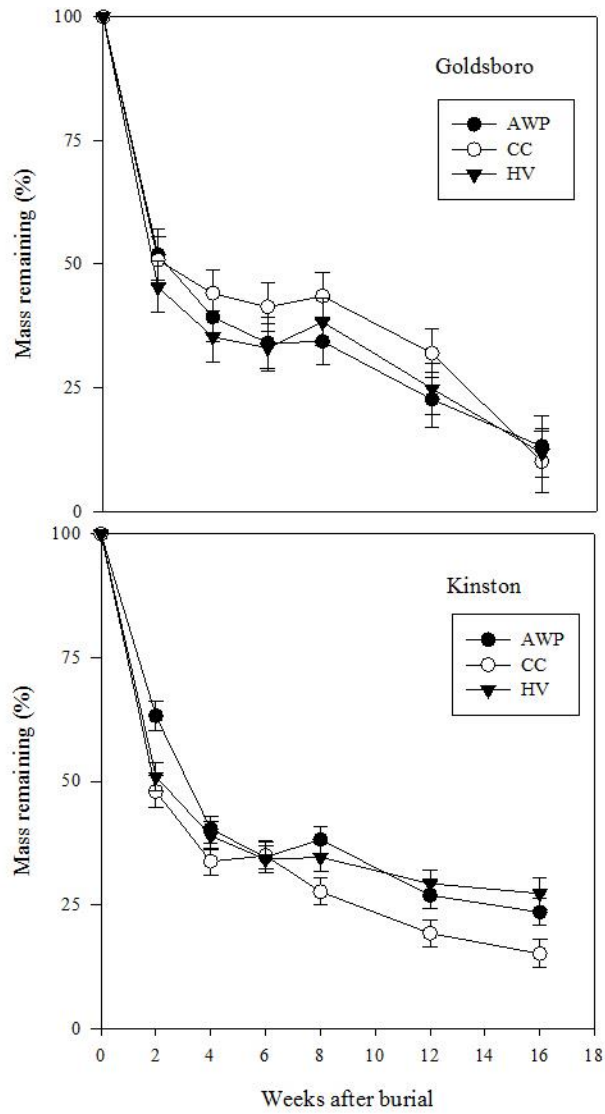


Figure 2-4 Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) root decomposition in Goldsboro (top) and Kinston (bottom) cover crop fertility plots averaged across termination methods. Error bars represent one standard error of the mean.

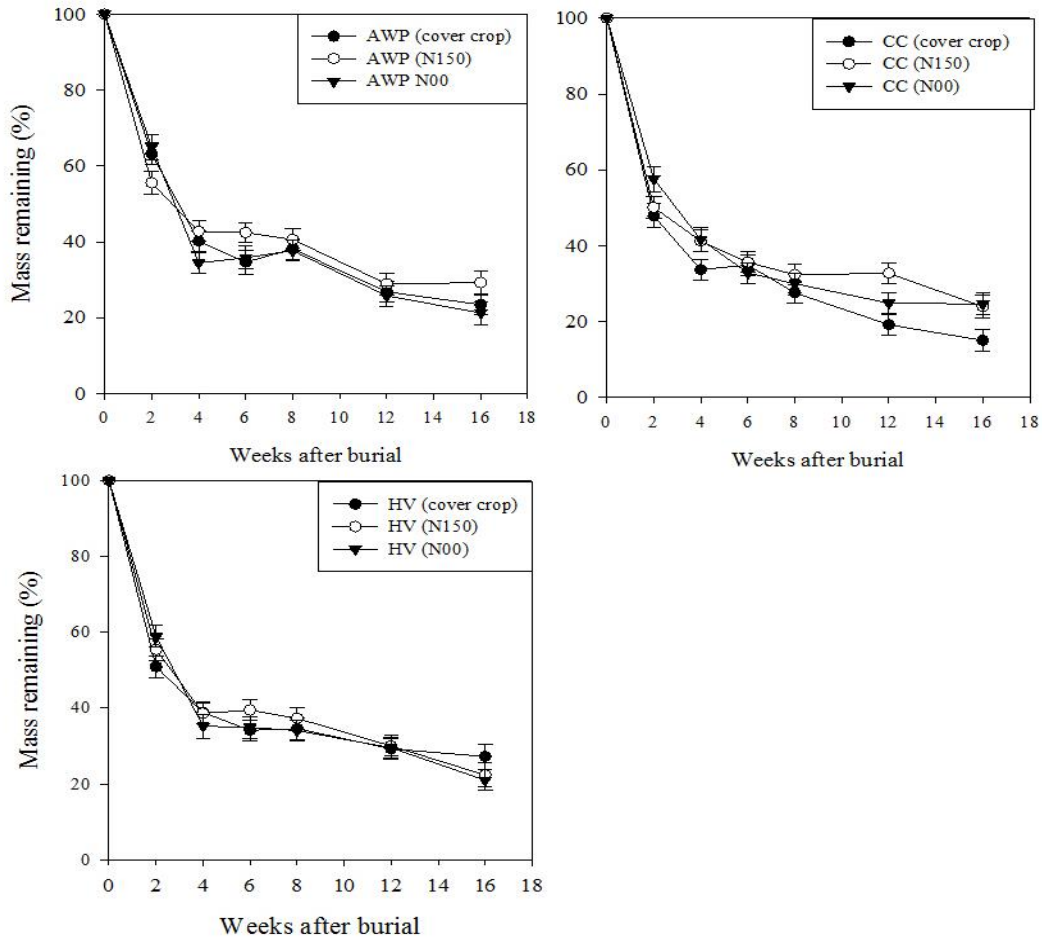


Figure 2-5 Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) root decomposition in cover crop fertility and control (N00, N150) plots, Kinston site. Error bars represent one standard error of the mean.

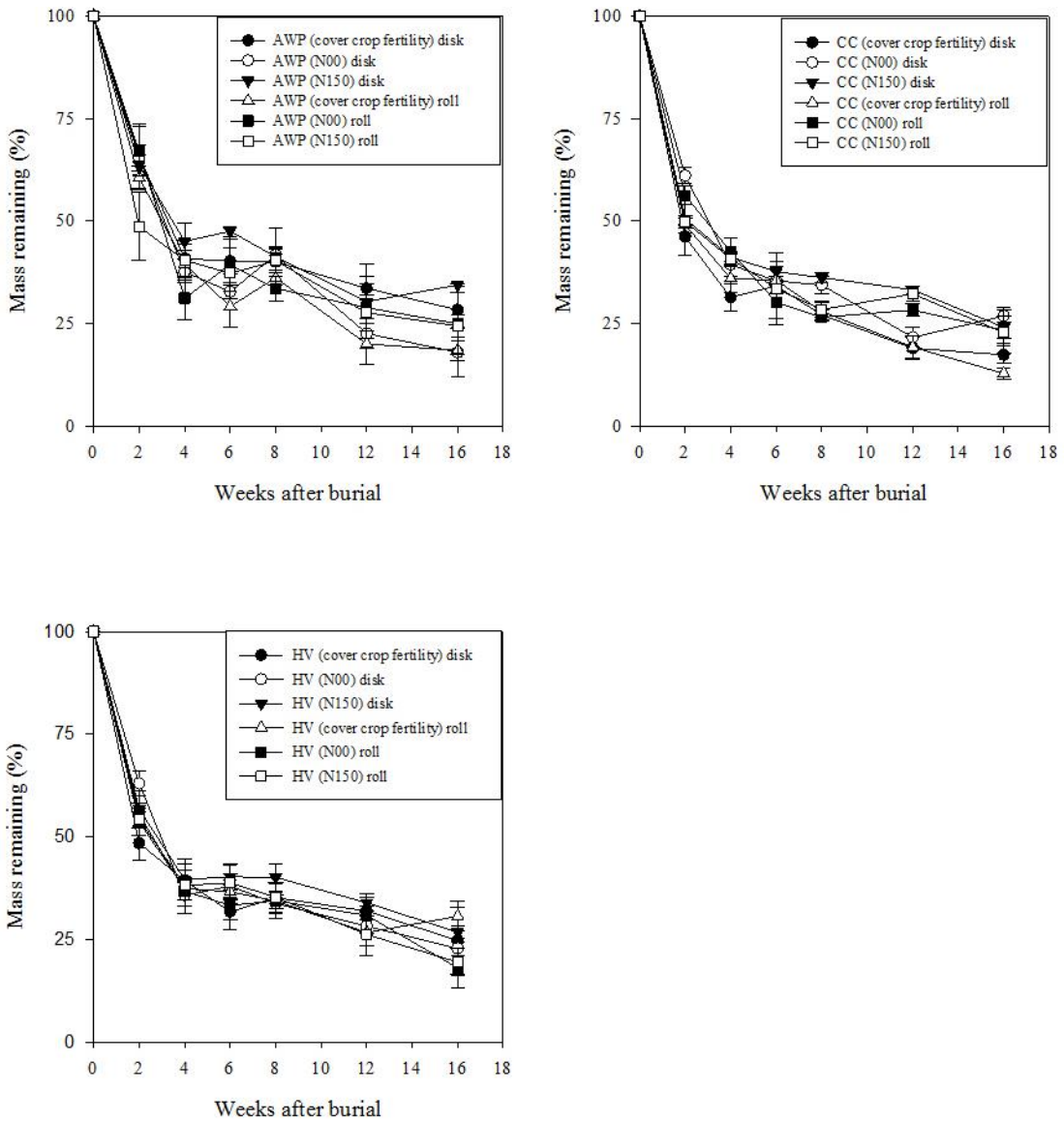


Figure 2-6 Root decomposition in cover crop fertility and bare, control plots as a function of termination method at Kinston site. Error bars represent one standard error of the mean.

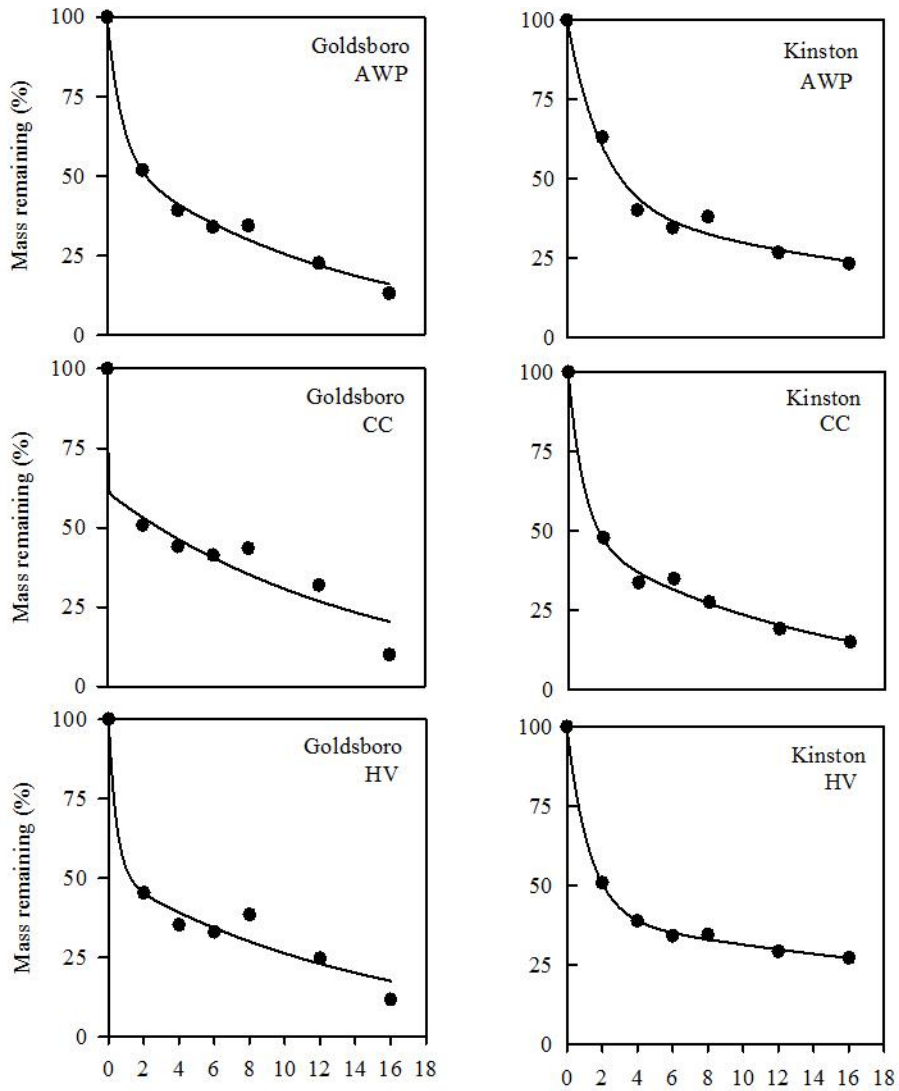


Figure 2-7 Percent root mass remaining over time averaged across termination method in Goldsboro and Kinston cover crop fertility plots. Symbols correspond to means fit to a double exponential four term parameter model. Error bars represent one standard error of the mean.

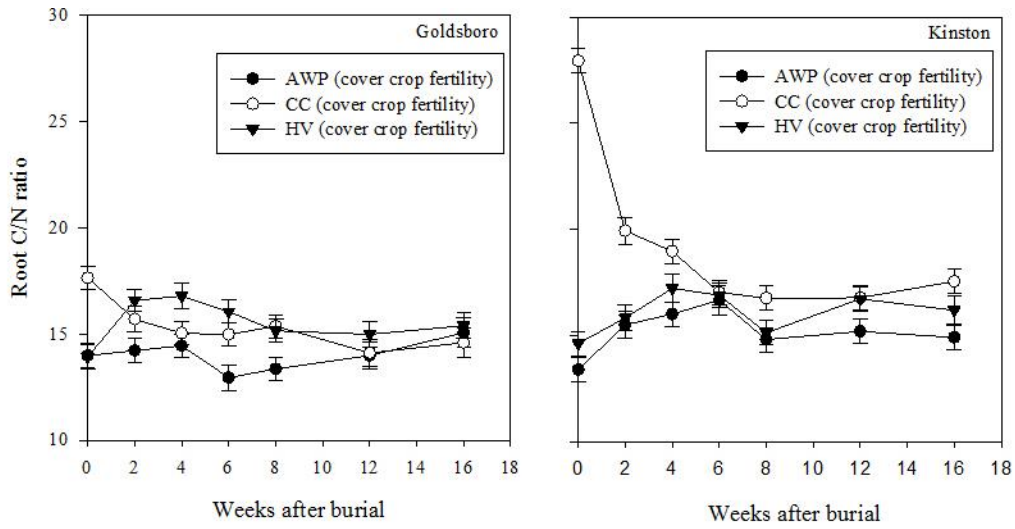


Figure 2-8 Austrian (AWP), crimson clover (CC), and hairy vetch (HV) root C/N ratio dynamics from cover crop fertility plots and averaged over kill methods in Goldsboro (left) and Kinston (right). Error bars represent one standard error of the mean.

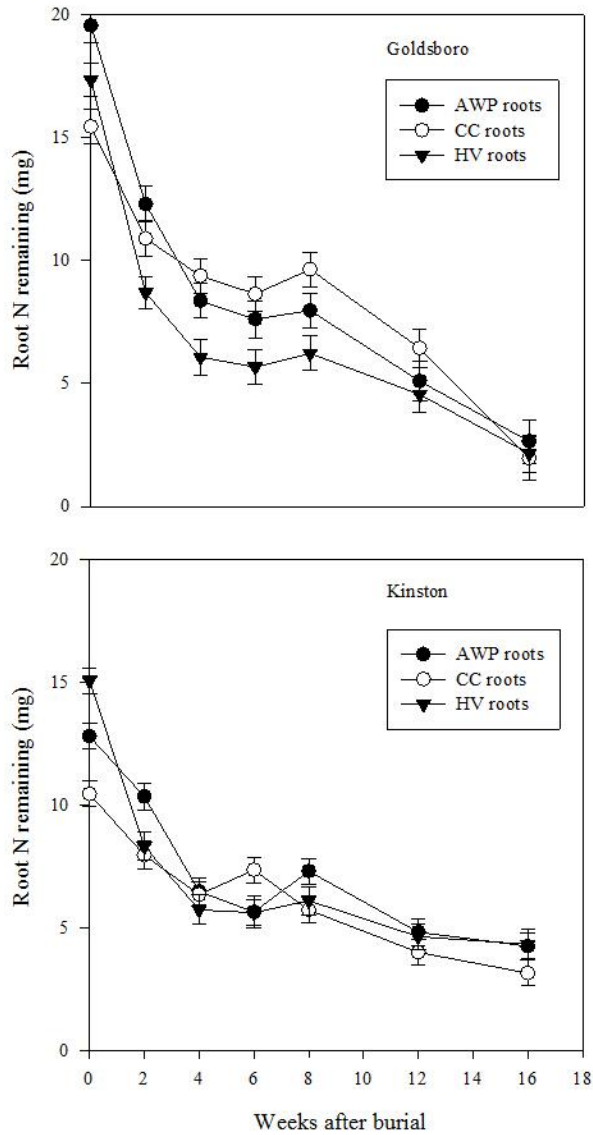


Figure 2-9 Root N remaining in Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) cover crop fertility treatment plots averaged over termination methods in Goldsboro (top) and Kinston (bottom) sites. Error bars represent one standard error of the mean.

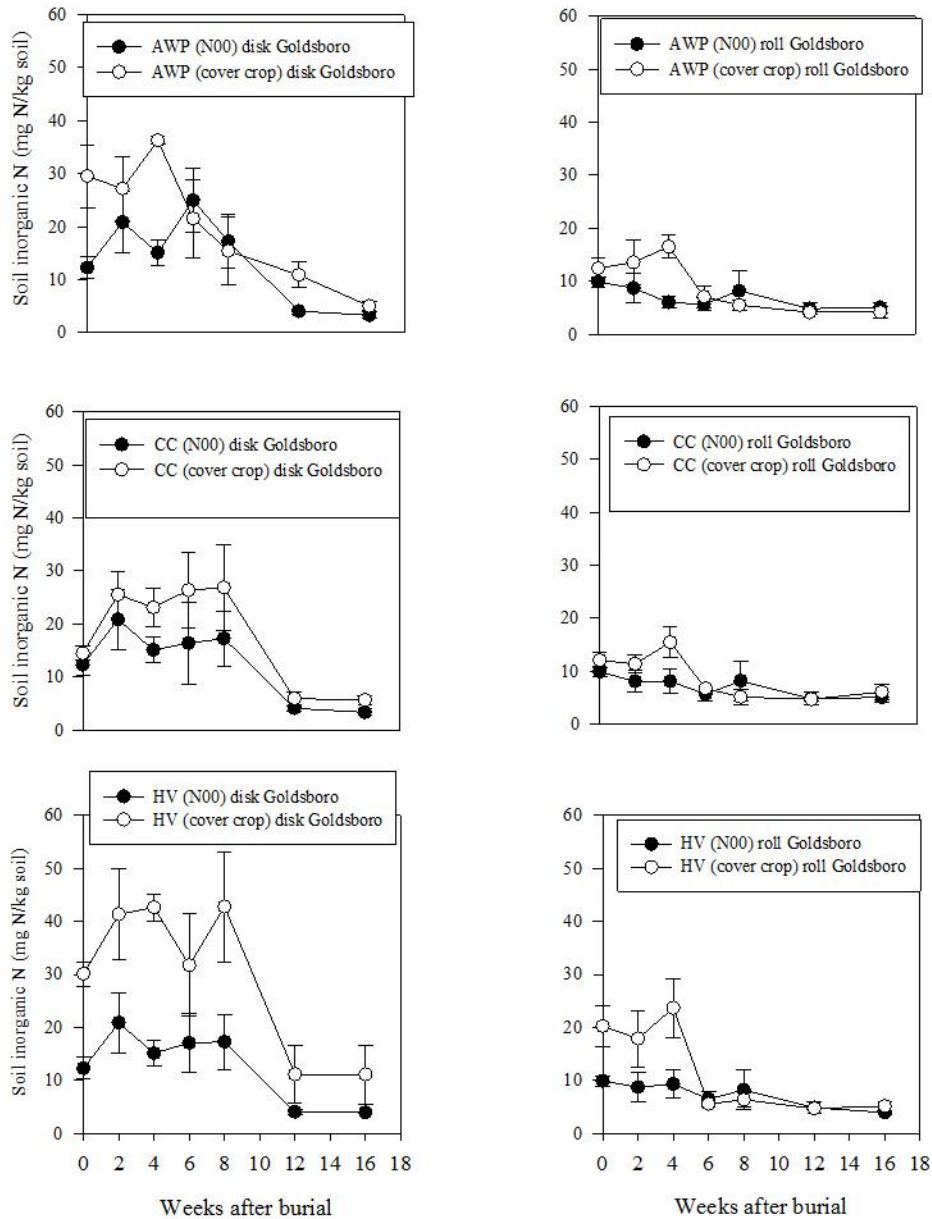


Figure 2-10 Soil inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) change over time as a function of termination method in control and treatment plots for Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) at Goldsboro site. Zero-N (N00) plots were bare without a cover crop, while cover crop plots had cover crop residue. Error bars represent one standard error of the mean.

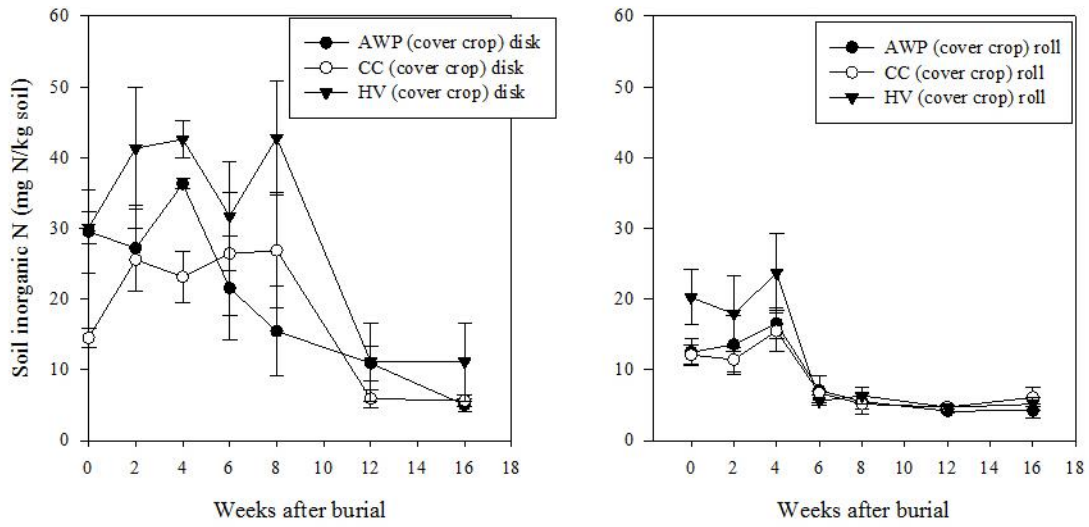


Figure 2-11 Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) soil inorganic N (mg N/kg soil) change over time as a function of termination method in cover crop fertility plots in Goldsboro. Error bars represent one standard error of the mean.

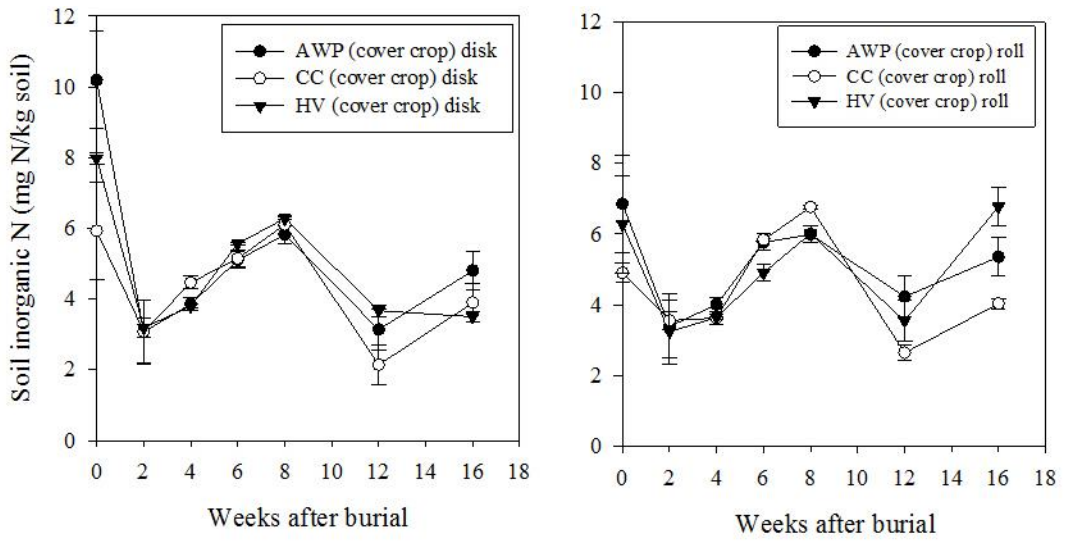


Figure 2-12 Soil inorganic N (mg N/kg soil) fluctuation over time as a function of cover crop fertility treatment and termination method in Kinston. Error bars represent one standard error of the mean.

Table 2-1 Field activities for root decomposition study

	Goldsboro	Kinston
Previous crop	Corn preceded by winter legume cover crop	Corn
Cover crop planting	September 30, 2011	September 29, 2011
Root collection for use in decomposition study	April 20 – 30, 2012	April 1 – 10 , 2012
Cover crop termination	May 10, 2012	April 19, 2012
Fertilizer application to N150 plots*	X	April 30, 2012
Corn planting	May 25, 2012	May 1, 2012
Root litterbag burial	May 25, 2012	May 2, 2012
Root litterbag collection	June 8, 22; July 6, 20; Aug 17; Sept 13	May 16, 30; June 13, 27; July 25; Aug 23

* In Goldsboro, there were no N150 control plots.

Table 2-2 Factors investigated at Goldsboro and Kinston, NC study sites

Factors	Levels
Cover crop fertility ^a	AWP, CC, HV, N00, N150 ^b
Termination method	Disking, roller-crimping
Time	0, 2, 4, 6, 8, 12, 16 weeks after burial

Root type is nested within cover crop fertility. AWP = Austrian winter pea, CC = crimson clover, HV = hairy vetch, N00 = bare plots without N addition, N150 = bare plots with 150 kg N/ha equivalent. ^a N150 fertility treatments were only applied at Kinston location.

Table 2-3 Initial chemical characteristics and final root N mass remaining for Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) root biomass used in Goldsboro and Kinston field sites

	%C	%N	C/N	lignin	lignin/N	*N ₀ (mg)	**N ₁₆ (mg)
Goldsboro							
AWP	41.9a	3.0a	14.0a	13.0	4.3	19.6a	2.6a
CC	40.6a	2.3b	17.7b	10.9	4.7	15.5b	2.0a
HV	38.9a	2.8a	13.9a	15.4	5.5	17.3ab	2.2a
Kinston							
AWP	29.7a	2.2a	13.8a	13.7	6.4	12.8abc	4.3a
CC	39.9b	1.4b	28.1b	11.2	7.9	10.5c	3.2a
HV	36.5b	2.5a	14.6a	15.0	6.0	15.1a	4.3a

Values with the same letters are not significantly different at $p < 0.05$ (Tukey-Kramer's HSD). Lignin was not analyzed for significance. Only species from the same site were compared. *N₀ is root mass N at week 0. **N₁₆ is root mass N at week 16.

CHAPTER 3: WINTER ANNUAL LEGUME COVER CROP COARSE AND FINE ROOT DECOMPOSITION AS A FUNCTION OF SOIL INORGANIC NITROGEN

Introduction

Among their other benefits, use of cover crops in agricultural systems is a recommended practice to enhance sequestration of atmospheric CO₂ and, mitigate the severity of global climate change (Lal 2008). Winter annual legume cover crops are highly valued for their N contribution to subsequent cash crops (Ranells 1992; Sullivan 2003; Parr et al. 2011), but are often not viewed as a source of long-term soil organic carbon (SOC) due to the rapid decomposition of their shoot material (Wagger et al. 1998; Gaskell et al. 2007). However, root biomass has been reported to decompose at a slower rate relative to shoot biomass for legume cover crops such as crimson clover and hairy vetch (Buchanan and King 1993; Puget and Drinkwater 2001; Kong and Six 2010) and has accounted for up to 30% of total hairy vetch biomass (Puget and Drinkwater 2001). It is reasonable to expect that if root residues are included in estimates of legume cover crop-derived SOC, then legume cover crops may play a much greater role in long-term sequestration of atmospheric CO₂.

While legume cover crop roots have been reported to decompose slower than associated shoots, it is still poorly understood how root morphology affects rate of decomposition in legume cover crop species. Root morphology, which refers to structural properties including total root length, root diameter class, and total root surface area (Magalhaes et al. 2011; Nichols et al. 2007), has been shown to influence root decomposition rate in several plant species (Gordon and Jackson 2000; Fujimaki et al. 2008; Pohl et al.

2011). Coarse roots, generally defined as greater than 1 or 2-mm diameter, have been observed to decompose slower than fine roots (< 1 or 2-mm diameter) in many plant species (Berg et al. 1987; Jackson and Mooney 1997; Fornara et al. 2009; Hobbie et al. 2010). Due to their slower rate of decomposition, coarse roots have been closely linked to SOC accrual, whereas fine roots are thought to play a more pivotal role in nutrient cycling (Silver and Miya 2001; Fornara et al. 2009; Rasmussen et al. 2010; Kähkölä et al. 2012).

Differences in decomposition dynamics between coarse and fine roots relate primarily to root litter biochemical composition (Fujimaki et al. 2008; Hobbie et al. 2010). Fine root tissue chemistry is generally more conducive to rapid decomposition and in some respects resembles leaf litter more than it does coarse roots (Berg and McClaugherty 2008; Fujii and Takeda 2010; Wang et al. 2010). Fine roots of many plant species have been reported to have significantly higher N, P, and Mg content, and lower C/N and lignin/N ratios compared to coarse roots (Gordon and Jackson 2000; Gardner and Sarrantonio 2012) and these properties can lead to accelerated fine root decomposition (Herman et al. 1977).

Past investigations have suggested a positive relationship between soil inorganic N status and rate of residue decomposition in many plant species (Herman et al. 1977; Fog 1988; Recous et al. 1995; Dignac et al. 2002; Lindedam et al. 2009). Mary et al. (1996) reported that when soil inorganic N status is limiting, decomposition rate of plant residues decreases. Legume cover crops deposit large quantities of N into soil and the rate of N mineralization of these cover crops is expected to increase over a 3 to 6 week period following termination before returning to pre-kill levels by week 6 to 8 (Gaskell et al. 2007;

Parr et al. 2011). The effect of this elevated soil inorganic N status on legume cover crop root decomposition is poorly understood.

Berg et al. (1987) followed decomposition dynamics of red clover roots taking morphology into account, but did not consider soil inorganic N effects. This fertility factor may alter overall soil N status, changing decomposition rate of the root material remaining when cover crops are terminated. In the present study, we performed root morphological analysis on greenhouse-grown Austrian winter pea (AWP), crimson clover (CC), and hairy vetch (HV) and quantified characteristics relevant to root decomposition. This morphological study was followed by an incubation study in which coarse (> 1-mm diameter) and fine (< 1-mm diameter) root decomposition of CC and HV under natural and elevated soil N (~ 200 kg/ha) levels was measured in terms of mass loss over a 12-week period. A four-factorial experiment was designed (cover crop species x root size fraction x N addition x time). Specific objectives were to (i) compare decomposition rate of CC and HV coarse and fine root fractions (ii) measure root N and C/N compositional shifts over time, and (iii) determine soil inorganic N effects on fine and coarse root decomposition. We hypothesized that N addition would accelerate decomposition of all roots and fine roots would decompose faster than coarse roots for all species.

Materials and Methods

Root morphology analysis

A greenhouse experiment was carried out at the North Carolina State University Method Road Greenhouse Facility in Raleigh, North Carolina (35.81°N, 78.64°W) (Table 3-1). Austrian winter pea (AWP) (*Pisum sativum*), crimson clover (CC) (*Trifolium incarnatum*), and hairy vetch (HV) (*Vicia villosa* Roth) were grown for 16 weeks (February 8, 2012 to May 30, 2012) in 1.5 m tall, 10 cm diameter Polyvinyl chloride cylinders in an adaptation of methods described by Jaramillo et al. (2013). Cylinders were arranged in a completely randomized design with four replications for each species (12 cylinders total). Cylinders were used instead of traditional greenhouse pots to allow for extensive root system development and were supported in a wooden frame and enclosed using foam insulation to moderate temperature. Transparent plastic sleeves (101 µm thickness) were cut to 1.7 m length, sealed with an electric bag sealer, and placed inside cylinders. Slits were cut at the bottom of sleeves to allow for drainage. Plants were grown in sleeves to facilitate harvest at which time sleeves were easily removed. Growing medium consisted of 50% sand, 35% vermiculite, 10% top soil, and 5% perlite. Medium was placed in cylinders and pre-watered (~ 500 ml/day) with deionized water for four days. Five seeds were sown in each cylinder and thinned to one plant per cylinder 10 days after planting. Plants were irrigated and fertilized daily using 200 ml N-free Hoagland nutrient solution (Hoagland and Arnon 1950). Greenhouse temperature was uncontrolled during the study and fluctuated from 17° to 47°C. At harvest, sleeves were pulled out of cylinders and cut open along the length. A gentle hose

spray was used to wash off attached medium particles from roots to avoid loss of smaller diameter roots (Van Groenigen et al. 2005). Washed roots were then analyzed for total root length, total fine roots, percent fine roots, and total root surface area using WinRhizo™ 2012b (Regent Instruments, Québec, Canada).

The root morphology study was repeated with modifications from November 9, 2012 to February 1, 2013. Plants were grown for 12 weeks in a growth medium that consisted of 55% sand, 35% vermiculite, and 10% topsoil (Table 3-1), as perlite was found to adhere to the roots and interfere with fine root removal. Greenhouse temperatures during the study fluctuated from 10° to 31°C. Design consisted of six replications for each species in a completely randomized arrangement. All other conditions were consistent with the previous root morphology study.

Root collection and initial chemistry analysis for incubation study

In February 2013, CC and HV roots were collected to 15-cm depth from separate plots established in September 2012 at the Center for Environmental Farming Systems (CEFS) in Goldsboro, (N 35.39°N, 78.02°W), NC (Table 3-1). Roots were gently rinsed with tap water to remove adhering soil particles and partitioned into coarse and fine root fractions.

Partitioned roots were air-dried on greenhouse benches to 5-8% moisture and dry mass was determined at 105°C. Subsamples of coarse and fine roots were ground through a laboratory mill (Cyclotec 1093 Sample Mill) and analyzed for ash, lignin, and C and N content. Ash content represents soil contaminant and was determined through combustion at 550°C in a muffle furnace. Initial root lignin was determined by Dairy One (Ithaca, NY). Root C and N

content was determined by North Carolina State University Environmental and Agricultural Testing Services using a Perkin-Elmer PE 2400 CHN Elemental Analyzer (Norwalk, CT, USA).

Soil collection

Soil used for this experiment was Wickham loamy sand (fine loamy, mixed, thermic, Typic Hapludult) collected at CEFS in January 2013 from bare plots that received no fertilizer addition over the previous year. Soil was sieved (2-mm) while moist and stored at 4°C until ready for use.

Soil water container capacity determination

Soil water container capacity is the maximum amount of water that soil can hold after gravitational water has drained and is analogous to field capacity. Soil water container capacity was determined on duplicate CEFS soil samples (Fig. 3-1). Ten polyvinyl chloride rings were stacked and secured using duct tape (Howard et al. 2010). Soil was added to stacked rings until full and then weighed. Deionized water was added to the ring structure to approximate saturation for the top 5 rings and allowed to drain for 24 hours. Subsamples of 20 g were taken from each of the top five rings and dried at 105°C to determine gravimetric water content. Soil water container capacity was determined as the mean water content for each of the top two rings (242 ml) of soil (Montalvo 2008).

Experimental setup

Air-dried coarse and fine root fractions from CC and HV were placed in nylon litterbags (6 x 6 cm², 1-mm diameter) in the following proportions: coarse roots = 250 mg/litterbag, fine roots = 500 mg/bag. A four-factorial experiment was designed: cover crop species (crimson clover, hairy vetch) x root type (fine, coarse) x N content (N addition, no addition), and time (6 retrieval dates). Styrofoam cups were filled with 200 ml of soil and watered to 80% soil water container capacity in order to assure prevention of anaerobic, denitrifying conditions. Cups were weighed daily and watered when necessary to achieve weights corresponding to 80% water container capacity. After seven days of pre-incubation, 100 ml of soil was removed per cup and one root litterbag was placed flat on top of the remaining 100 ml of soil. Removed soil was then placed back in each cup. Reagent grade urea (CH₄N₂O) was applied at the rate of 133 µg N cm⁻³ of soil (200 kg N ha⁻¹) into the top 2 to 3 cm of soil that was placed on top of the root litterbag for those treatments receiving N to simulate the input of legume biomass. Cups were destructively sampled 1, 2, 3, 4, 8, and 12 weeks after burial and soil and root litterbags were harvested.

Root processing

Root litterbags were oven-dried at 70°C for 48 hours at each sampling time. Litterbag content was ground through the laboratory mill and subsamples of the ground product were used to determine root mass loss using the combustion process described earlier. Subsample weights before and after combustion were recorded and differences were assumed to be due

to root organic C loss as CO₂. The percent root in subsamples is equal to the root subsample weight before combustion minus root subsample weight after combustion divided by the root subsample weight before combustion and multiplied by 100. The percent of originally buried root mass remaining in retrieved litterbags at time (t) is equal to the collected oven-dried litterbag content weight multiplied by the percent root mass of the subsample and divided by the original weight of the buried root mass and multiplied by 100.

To determine root N mass in litterbags, corrections were made for litterbag soil contamination. Root litterbag content mass was multiplied by percent N obtained from litterbag subsamples to determine litterbag content N mass. Subsample percent ash was multiplied by litterbag content to determine soil contaminant. Four random soil samples were collected 15 cm depth using a 2.54 cm (one-inch) diameter soil probe from each site and averaged within site to determine percent total soil N. Percent total soil N was multiplied by litterbag soil contaminant to determine soil-derived N mass. Root N mass was determined by subtracting soil-derived N mass from litterbag content N mass.

Soil inorganic N measurements

Soils from cups were dried at 40°C to constant mass, then ground to pass 1 mm mesh screen. Samples were extracted with 1 M KCl with shaking for 1 hour. Samples were then allowed to settle for 20 minutes before filtration through #42 (2.5µm) filter paper. Extracts were refrigerated at 4°C until analysis for NH₄⁺ and NO₃⁻ on a QuikChem 2000 flow injection autoanalyzer (Lachat Instruments, Loveland CO) (Parr et al. 2013).

Environmental conditions and statistical analysis

The root decomposition study occurred in a climate-controlled Environmental Growth Chamber at the NCSU Phytotron facility. Daily temperature was 22.5 °C and night temperature was 18°C. Relative humidity was 40 – 60%. A randomized complete block design with four blocks and four replications per treatment was employed. Each block contained 48 experimental units (Styrofoam cups). Blocks were located on carts and were rotated weekly to avoid blocking effects. Analysis of variance with the PROC MIXED procedure (SAS Institute Inc., Cary, NC) was used to test the following response variables: percent root remaining, soil inorganic N content, and root C/N ratio. Block was a random effect, while cover crop species, root type, initial soil N addition, and time were fixed effects. Multiple comparisons were made using Tukey-Kramer's HSD at $p < 0.05$.

Results

Root morphological analysis

In the spring root morphology study (February 8, 2012 to May 30, 2012), fine roots comprised an overwhelming majority of total roots across species ($\geq 79\%$). However, differences in total root length, total fine root length, percent fine roots, and surface area between species were not observed (Table 3-2). Excessively high temperatures induced death of two HV replications and AWP displayed signs of heat-related stress during the final two weeks of the study. Large variability in measurements of total root length, fine root length, and surface area within species were observed.

In the winter 2013 root morphological study, the majority of roots for both AWP and HV were also in the fine fraction ($\geq 70\%$). Replications of CC were severely weakened by disease three weeks after germination and reliable data could not be gathered from this species. AWP and HV, however, performed well under the cooler growing conditions. There was no evidence to support significant differences in total root length, total fine root length, total root surface area, and percent fine roots between species (Table 3-2).

Root decomposition and chemistry

For the root incubation study, fine roots decomposed faster than coarse roots for both CC and HV at both N levels (Fig. 3-2). Differences in coarse and fine root decomposition rates were not observed between species. Total root mass loss was greatest during the first week of the study for all treatments, but was insignificant from weeks 1 to 4 ($p < 0.05$). A gradual increase in rate of mass loss for all treatments during the final 8 weeks of the study was also observed (Fig. 3-2). At the end of the incubation period, 9 and 14% of buried HV and CC fine roots remained averaged across N treatments, while 12 and 19% of HV and CC coarse roots averaged across N treatments remained. Initial soil inorganic N addition did not have a consistent effect on the rate of root decomposition across treatments (Figures 3-3 and 3-4).

Initial root litter chemical characteristics for CC and HV coarse and fine root fractions are reported in Table 3-3. Root biomass in the coarse fraction for both species was insufficient to analyze multiple samples for lignin measurements. Soil inorganic N addition did not affect root C/N ratio. For all treatments, C/N ratios decreased during the study and were characterized by an initial steep decline during the first two weeks followed by a

gradual decline thereafter (Fig. 3-5). Crimson clover C/N ratio decreased more rapidly than HV across N levels during the first two weeks after litterbag burial. Similar C/N values for both CC and HV roots averaged across N levels were observed for all sampling dates, except weeks 1 and 12. Coarse roots were characterized by higher C/N compared to fine roots through the first three weeks of the study. After this period, C/N ratios of coarse and fine root fractions for CC and HV were similar. Decline in C/N for coarse roots averaged over N treatments was also more rapid during the first week after burial, but followed a similar rate of decrease during the remainder of the study (Fig. 3-6). The period characterized by the most rapid rate of root decomposition for all treatments (week 1) also corresponded to the period of greatest decline in root C/N across all treatments (Fig. 3-7).

Initial root mass N at burial was highest for fine roots of both CC and HV due to the larger fine root mass buried (0.5 g/cup) compared to coarse roots (0.25 g/cup). However, by week 2 of the incubation, root N mass remaining was similar for all species (Fig. 3-8). Only time was observed to have a significant effect on root N release. For all treatments, rate of root N release decreased after week one and was insignificant from weeks 1 to 4. After 12 weeks, CC and HV roots from coarse and fine root fractions released 66-77% and 77-82% of N, respectively (Table 2-3).

Soil inorganic N measurements

Treatments that included addition of inorganic N at litterbag burial, maintained higher soil inorganic N concentration throughout the study compared to treatments without N addition (Fig. 3-8). There was very little fluctuation in soil inorganic N over time within individual

treatments. In positive treatments, for example, soil inorganic N status was similar at all collection periods, except for week one. In treatments without added N, measurements of soil inorganic N status were statistically similar at all collection periods except when comparing weeks one to 12. Soil inorganic N concentration over time was not affected by cover crop species, root type or their interaction (Fig. 3-9).

Discussion

Root litter chemistry and decomposition

The faster rate of fine root decomposition compared to coarse roots was likely related to differences in root litter chemistry between root types and possibly greater surface area of fine roots compared to coarse roots. Initial measurements indicating higher C/N ratios for coarse roots compared to fine roots has been observed in other legume, grass, and forest species (Fujimaki et al. 2008; Rasmussen et al. 2010). The range of initial C/N ratios (Table 3-3) observed for CC and HV roots resembles values reported in other legume cover crop root decomposition investigations (Puget and Drinkwater 2001; Gardner and Sarrantonio 2012). Numerous studies have pointed to the overwhelming effect of climatic and environmental factors on controlling decomposition in situ (Gijsman et al. 1997; Berg and McLaugherty 2008; Fujimaki et al. 2008; Wang et al. 2010). However, to determine the effects of plant litter chemical composition on decomposition rate, controlled incubations are the best approach to take (Abiven et al. 2005). In this study, climatic factors (temperature, moisture, and relative humidity) were held constant and root litter chemistry likely

determined observed differences in decomposition between treatments. Coarse roots had the highest C/N ratio and decomposed slower than fine roots with lower C/N values. However, for both CC and HV coarse and fine root C/N ratios were below 25 indicating decomposition rate was expected to proceed rapidly for root types from both species (Soto et al. 2005). Although surface area measurements were not taken for root material used in the incubation study, data from the root morphology study indicates that CC and HV fine roots have greater surface area than coarse roots, which may be associated with more abundant microbial colonization.

Other root compositional components such as lignin likely played a more pivotal role in controlling rate of decomposition. While insufficient root material prevented statistical analysis of coarse-root derived lignin, it is plausible that lignin was at least partially responsible for observed differences in decomposition considering that slower rates of decomposition corresponded to higher lignin content in root material. Lignin also may have played a greater role in determining later stages of decomposition. Gorissen and Cotrufo (2000) and Van Ginkel et al. (1997) observed that initial C/N ratio accurately predicts early plant litter decomposition, but does not correlate with decomposition during later stages. Models of decomposition based on initial C/N ratio of plant litter have also proven unreliable when estimating root decomposition because roots have unique chemical characteristics that cannot be easily simulated with models (Abiven et al. (2005). In this study, C/N ratios of coarse and fine root material were statistically similar after week three, but coarse roots still decomposed significantly slower until week 12 indicating a factor other than C/N ratio influenced decomposition.

Lignin/N ratio is another parameter that is commonly used to describe observed patterns of litter composition. As lignin/N ratio increases, rate of plant litter decomposition decreases (Gijsman et al. 1997; Soto et al. 2005). Both CC and HV fine roots decomposed in a similar fashion and had similar lignin/N ratios. Considering the essential role that lignin plays in controlling plant litter decomposition, lignin/N ratio is a very relevant parameter to consider when analyzing root decomposition dynamics.

Root N release from all treatments was greatest during the first week of incubation, and $\geq 66\%$ of root N was released from all treatments during the 12-week incubation providing evidence that legume cover crop root-derived N may be available for subsequent cash crops. These results are in contrast to those reported by Malpassi et al. (2000) who found that less than 55% of oat and rye root-derived N is available for subsequent cash crops. These differences are likely related to the relatively higher C/N ratio and lignin content in oat and rye roots compared to the leguminous species used in this study (Sullivan 2003).

Root morphology analysis

Although root morphological differences between species were not observed in either study, greater insight into root morphological characterization for common legume cover crop species was achieved, which can provide insight into decomposition and C sequestration dynamics associated with legume cover crop roots. Fine roots in this study were conservatively defined (< 1-mm diameter) compared to other studies (< 2-mm diameter) on root decomposition (Fujii and Takeda 2010; Fornara et al 2009; Wang et al. 2010), but still comprised an overwhelming proportion of all roots in both studies. Considering the rapid

turnover of fine roots and their importance in soil C and nutrient cycling (Jackson and Mooney 1997), it is critical to have a greater understanding of their decomposition characteristics and contribution to SOC.

The spring 2012 root morphology study was beset by excessive temperatures that likely affected root growth and subsequent morphological analysis. Excessive soil temperature beyond the optimal range of a species has been reported to hinder nutrient uptake, decrease branching and reduce root growth (Nature 1966; McMichael and Burke 1998). Cylinders were not rotated and plants located on the edge of the design performed especially poorly with two hairy vetch plants dying before the conclusion of the experiment and two Austrian winter pea plants displaying signs of heat stress that resulted in lower values for total root length, fine root length, and surface area. Austrian winter pea and hairy vetch have been reported to be particularly susceptible to temperature extremes (Hannaway & Larson 2004; SARE 2007). Observed edge effects combined with elevated temperature caused great variability in morphology measurements and subsequently high error values.

The inclusion of perlite in the growing medium also decreased the accuracy of root morphology measurements. Smaller diameter roots often penetrated perlite particles and removal was not always feasible. Roots occluded in perlite could not be analyzed with WinRhizo™ software. The combination of excessively high growing temperatures and underestimation of root morphological parameters due to root occlusion in perlite weakened the validity of root morphological data in the spring 2012 study. Plant growth during the winter study was not impeded by temperature extremes and the absence of perlite in the growing medium lead to more accurate analysis of root morphological characteristics.

Soil inorganic N measurements

The lack of inorganic N fluctuation across treatments after week 1 indicates that leaching was insignificant, which was expected since soil was maintained at 80% water container capacity throughout the study. There also was minimal loss of inorganic N through denitrification since this process is inhibited by the presence of O₂ (Paul and Clark 2007; Robertson and Groffman 2007). All treatments were also characterized by an initial increase in soil inorganic N content before N stabilization for the remainder of the study. This initial increase in soil inorganic N corresponded to the period of rapid root decomposition and could be attributed in part to mineralized root N. Montalvo (2008), similarly, reported initial increase in net inorganic N content in incubated soils with different organic fertility treatments and attributed this to readily degradable organic forms of N.

Conclusions

Common winter annual legume cover crops are dominated by the fine root fraction and having a greater understanding of fine and coarse root decomposition provides insight into legume cover crop contribution to SOC and nutrient cycling. In controlled incubation studies, plant litter chemistry likely plays a greater role in determining decomposition rate. In this investigation initial C/N measurements accurately predicted short-term decomposition, but failed to provide insight into later stages of decomposition. We can speculate that lignin played a greater role in controlling longer-term decomposition rate. Our hypothesis that soil inorganic N status would increase decomposition was not supported.

Root C/N ratio for all species and root types was under 25/1, which supports mineralization. Soil inorganic N likely was not a limiting factor in microbial-mediated root decomposition. This study underscores the importance of root litter chemistry in determining decomposition when climatic variables are held constant.

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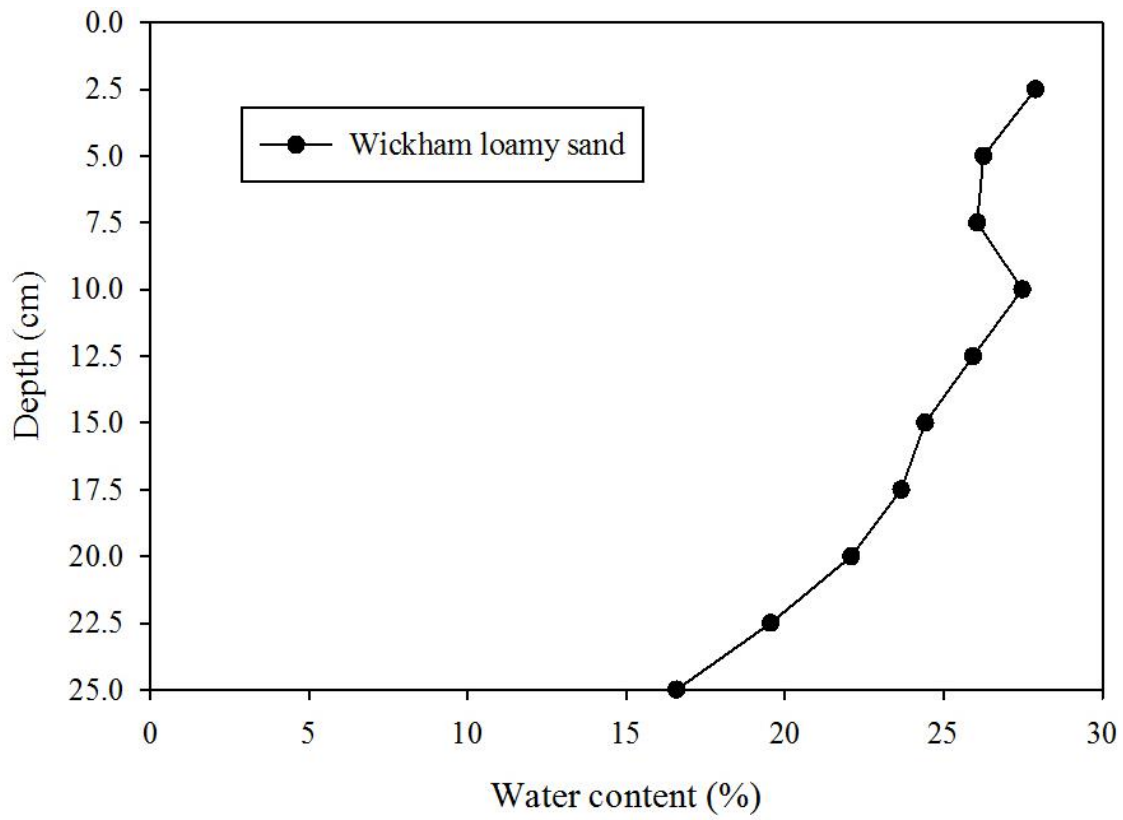


Figure 3-1 Volumetric soil water content as a function of cylinder depth for Wickham loamy sand (sieved to pass 2-mm) used in incubation study.

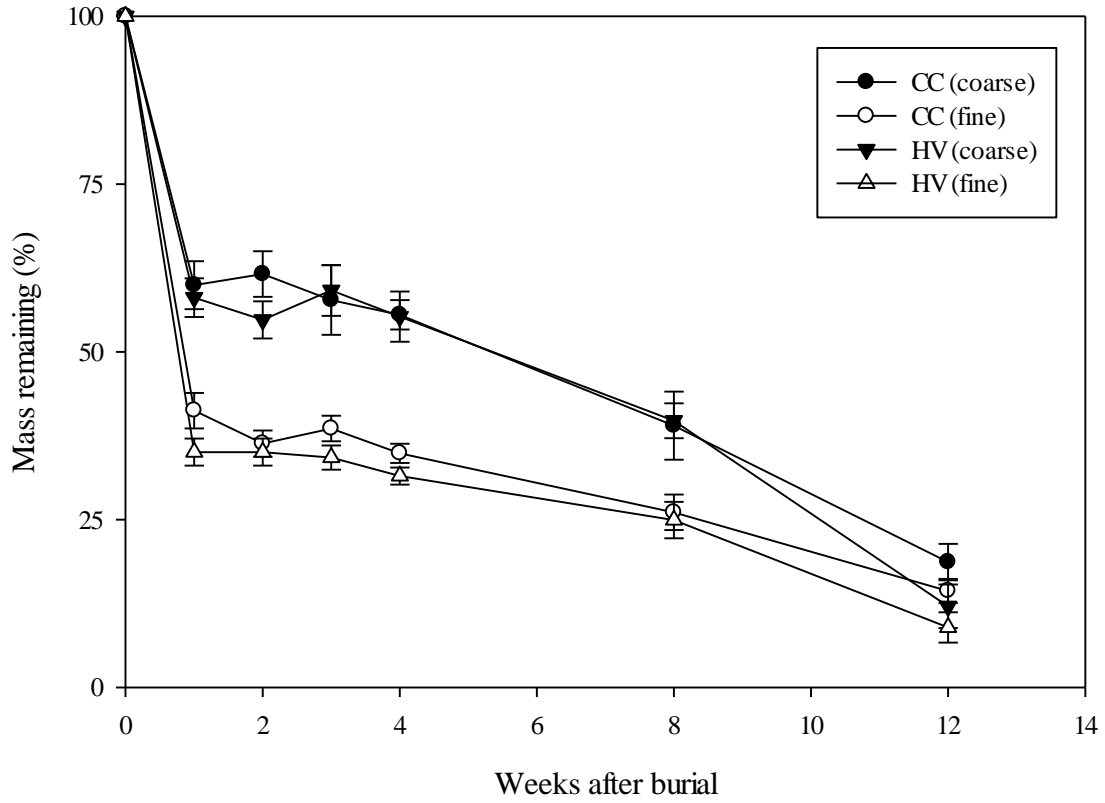


Figure 3-2 Decomposition of crimson clover (CC) and hairy vetch (HV) coarse and fine root fractions averaged over N treatments. Error bars represent one standard error of the mean.

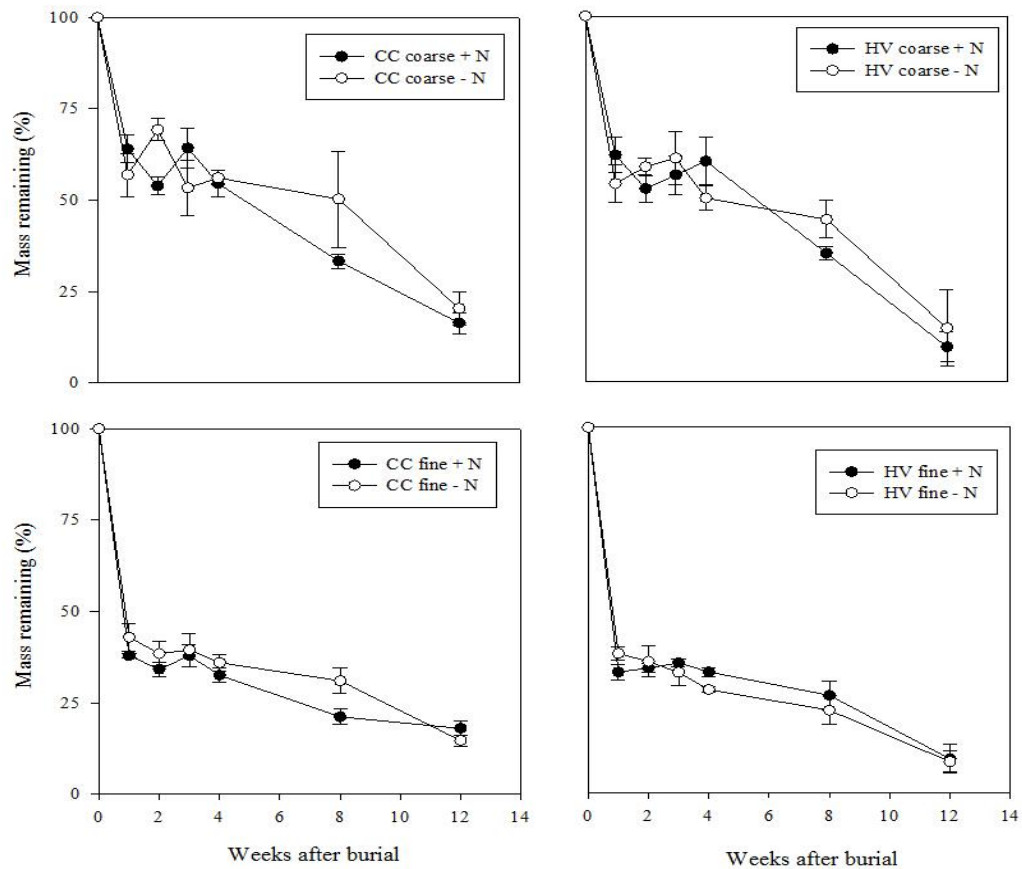


Figure 3-3 Effect of initial soil inorganic N addition (200 kg/ha) on decomposition of crimson clover (CC) and hairy vetch (HV) coarse and fine root fractions. Error bars represent one standard error of the mean.

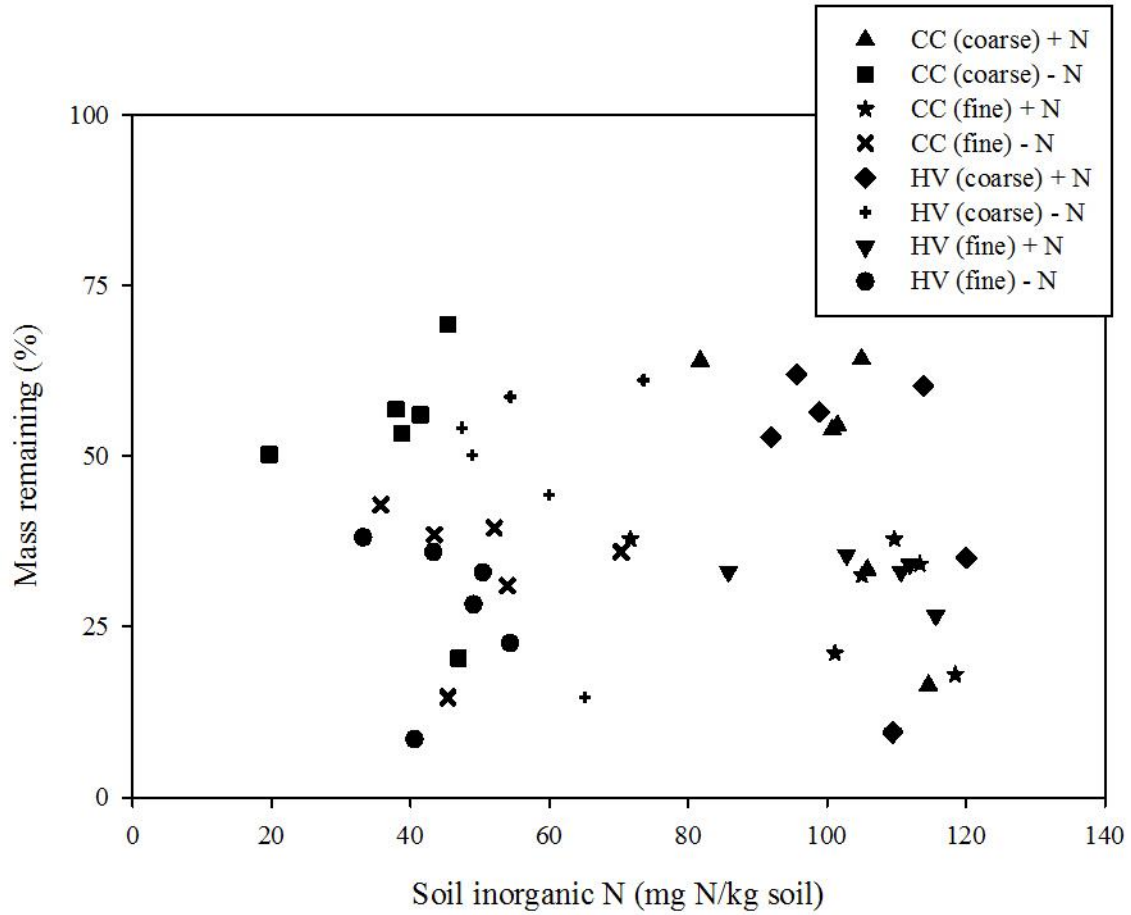


Figure 3-4 Relationship between soil inorganic N content and root mass decomposition reported as percent of original mass remaining for crimson clover (CC) and hairy vetch (HV) coarse and fine root fractions with (+ N) and without (- N) initial N additions over 12-week study period.

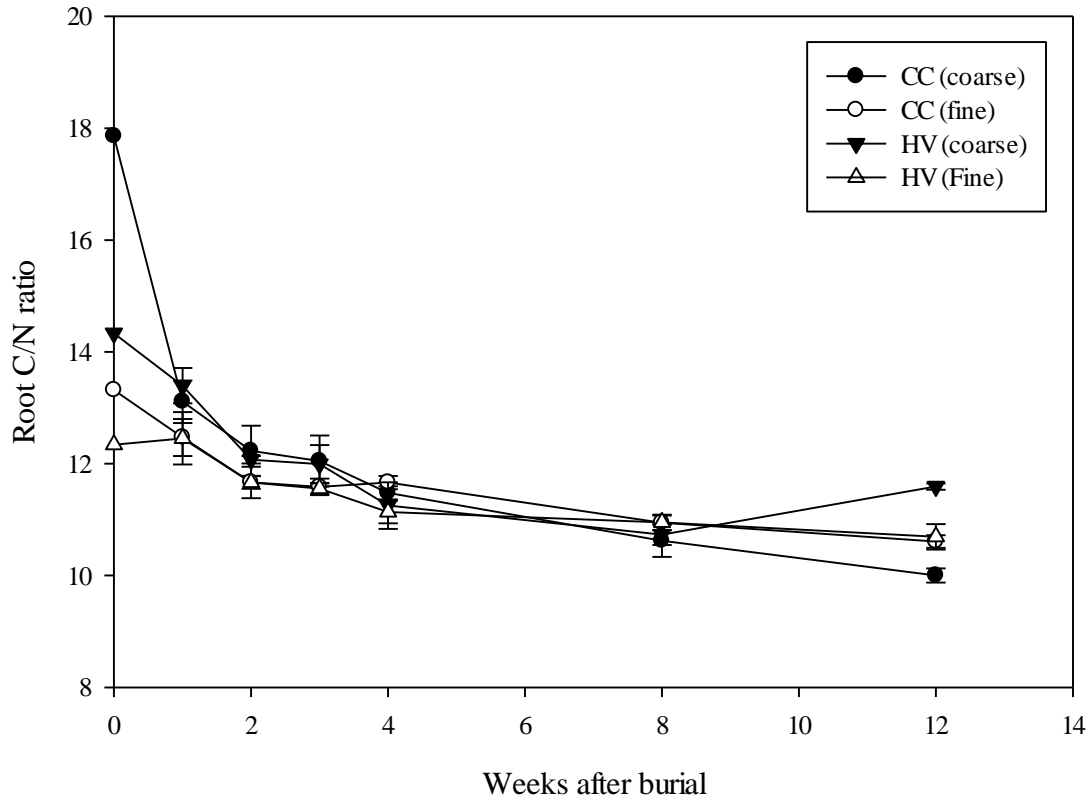


Figure 3-5 Root C/N ratio fluctuation over time for crimson clover (CC) and hairy vetch (HV) coarse and fine root fractions. Error bars represent one standard error of the mean.

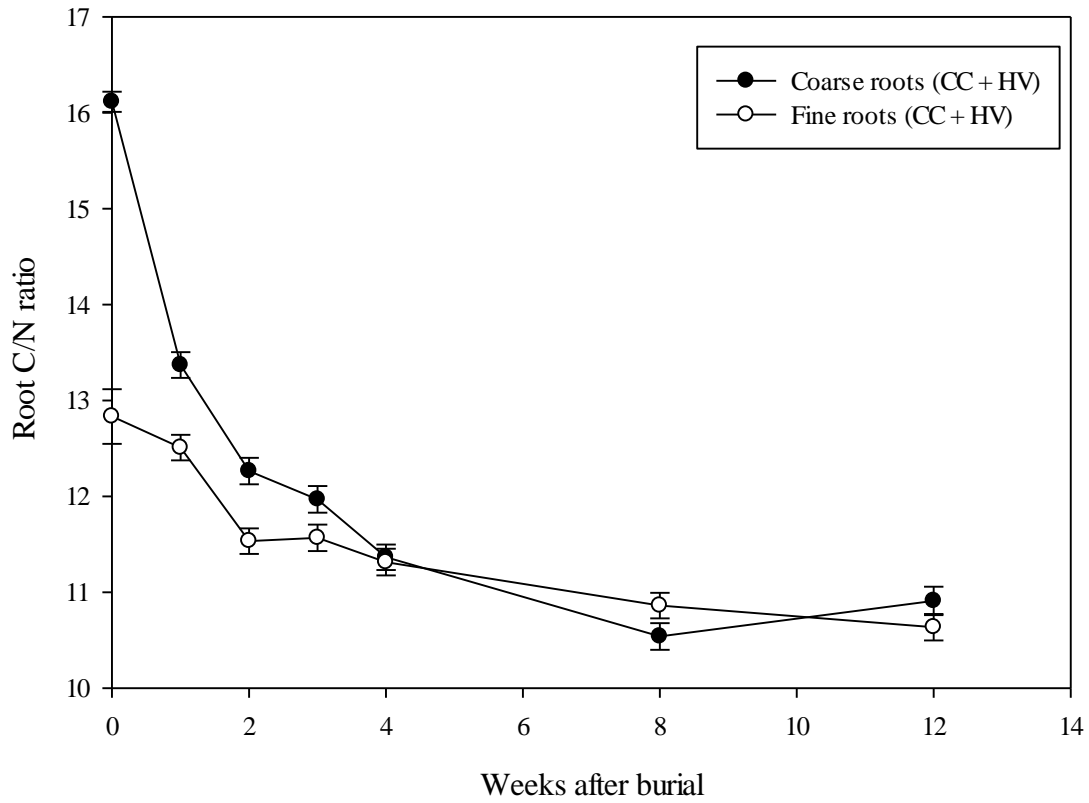


Figure 3-6 Coarse and fine root C/N dynamics averaged across species and N treatments as a function of time. Error bars represent one standard error of the mean.

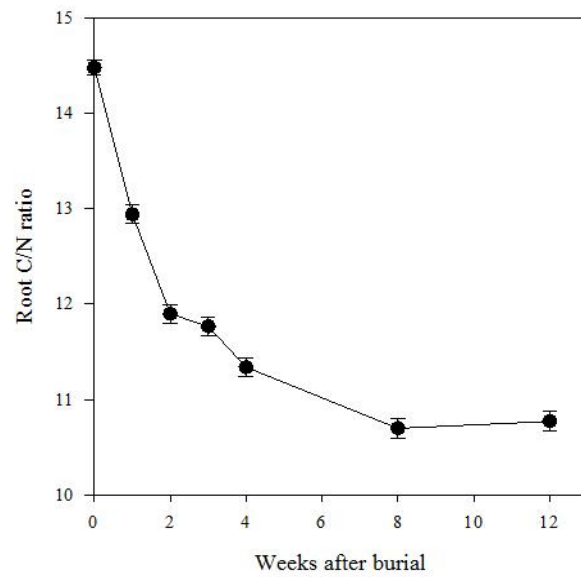
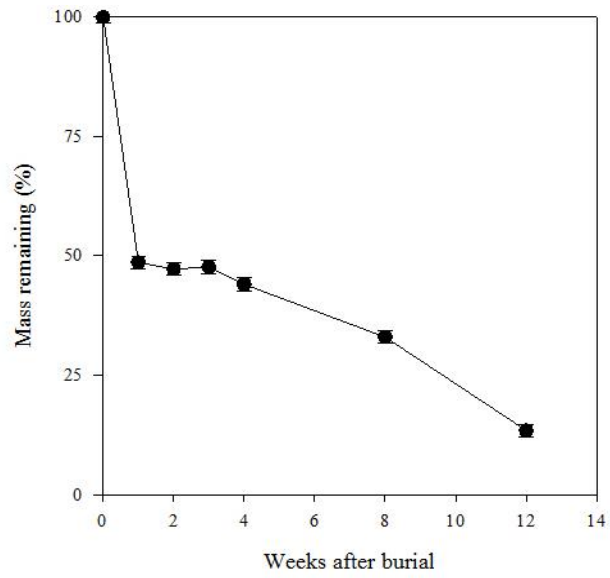


Figure 3-7 Comparison of root mass loss and C/N fluctuations averaged across all treatments as a function of time. Error bars represent one standard error of the mean.

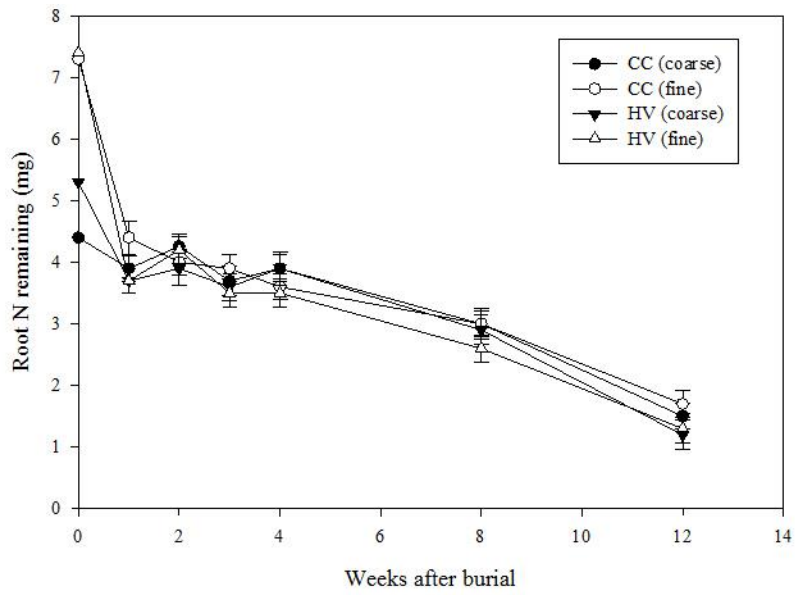


Figure 3-8 Root N release (mg) from crimson clover (CC) and hairy vetch (HV) coarse and fine root fractions averaged over N treatments. Error bars represent one standard error of the mean.

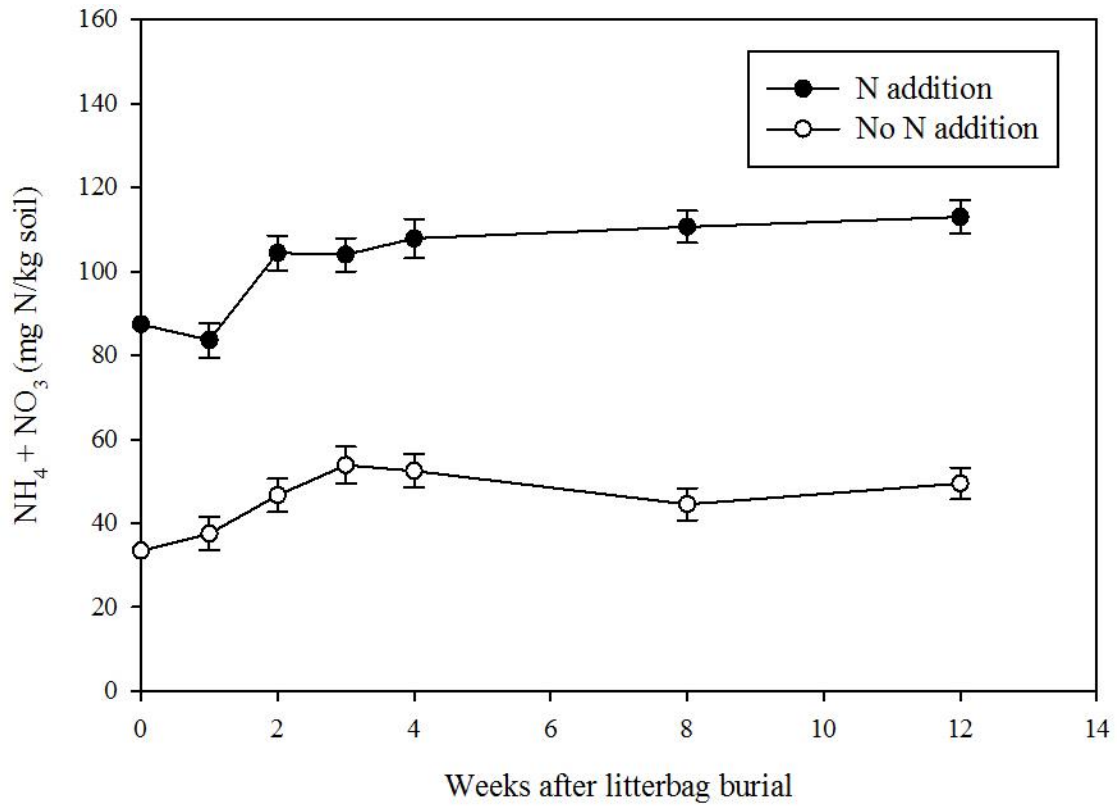


Figure 3-9 Soil inorganic N fluctuation over time for treatments with and without initial N addition. Error bars represent one standard error of the mean.

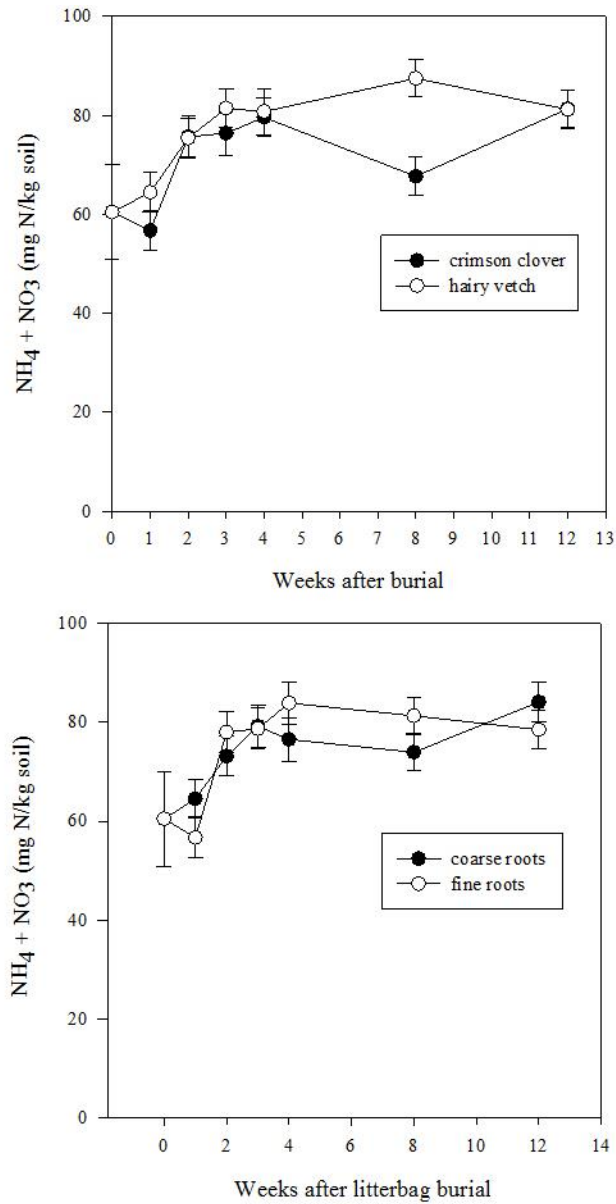


Figure 3-10 Soil inorganic N fluctuation as a function of cover crop species (top) and root type (bottom). Error bars represent one standard error of the mean.

Table 3-1 Root morphology and incubation study activities

Activity	Timetable
Spring root morphology study	February 8 – May 30
Winter root morphology study	November 9, 2012 – February 1, 2013
Soil collection (CEFS) for incubation study	January 2013
Root collection (CEFS) for incubation study	February 2013
Soil pre-incubation	February 22 – March 1, 2013
Incubation study	March 1 –May 24, 2013
Incubation study collection dates	March 8, 15, 22, 29; April 26; May 24

Table 3-2 Legume cover crop root morphological characteristics relevant to decomposition

	Total root length (m)	Fine root length(m)	% fine roots	Total root surface area (cm ²)
<u>Spring 16-week study</u>				
Austrian winter pea	24.1a	19.3a	81.0a	618.4a
Crimson clover	39.7a	32.3a	81.4a	943.8a
Hairy vetch	32.4a	25.9a	79.3a	899.2a
<u>Winter 12-week study</u>				
Austrian winter pea	13.6a	10.5a	76.0a	371.1a
Hairy vetch	13.0a	9.2a	70.7a	442.4a

Mean values are presented in the table. Means with the same letter are not significantly different using Tukey-Kramer's HSD ($p < 0.05$).

Table 3-3 Initial root litter chemical characteristics and root mass N dynamics for crimson clover (CC) and hairy vetch (HV) coarse and fine root fractions

	%C	%N	C/N	*lignin	lignin/N	**N ₀ (mg)	***N ₁₆ (mg)
CC-coarse	37.6a	2.1a	17.9a	11.9	5.7	4.4a	1.5a
CC-fine	30.6b	2.3a	13.3b	8.0a	3.5a	7.3b	1.7a
HV-coarse	37.4a	2.6a	14.4b	9.1	3.6	5.3a	1.2a
HV-fine	28.5b	2.3a	12.4b	7.7a	3.3a	7.4b	1.3a

*Lignin reported on dry matter basis. Means with same letters are not significantly different using Tukey-Kramer HSD. Coarse roots were not statistically analyzed for % lignin. **N₀ is root N (mg) at week 0. ***N₁₆ is root (mg) at week 16.