6-2013

Decomposition and Nutrient Release of Different Cover Crops in Organic Farm Systems

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DECOMPOSITION RATES AND NUTRIENT RELEASE OF DIFFERENT COVER CROPS IN ORGANIC FARM SYSTEMS

by

Jianru Shi

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Natural Resource Sciences

Under the Supervision of Professor James R. Brandle

Lincoln, Nebraska

June, 2013
Cover crops act as green manure adding organic matter to agricultural-soils. For legume green manures to be an effective nitrogen (N) source for organic farming systems, their N release must be in synchrony with crop N demand. The objectives of this study were 1) determine the decomposition rates of three common cover crops (white clover, *Trifolium repens*, L) red clover (*Trifolium pratense* L.) and soybean (*Glycine max* L) in order to determine when most N was released and its synchrony with subsequent corn crop uptake; 2) we focused on the effect of cover crops on soil N levels. This study was conducted in a certified organic field, near Mead, NE. We assessed the decomposition rates and chemical composition of three cover crops with different incorporation time (fall and spring). Cover crop samples were taken and air dried in fall and spring. Litterbags containing plant samples were buried at a depth of 15 cm in December, 2011 and March, 2012. The nine extraction times for fall treatment were 0, 12, 16, 20, 24, 28, 32, 40, 48 weeks after burial. For spring treatment, samples were dug up every four weeks. Soil samples from each experimental unit were taken at the same time. Extracted litterbags were oven-dried and samples were analyzed for biomass fractions (soluble, hemicellulose, cellulose, and lignin) and total C and N content. As conclusion, decomposition rates of five treatments follow the order: white clover incorporated in spring > red clover
incorporated in spring> white clover incorporated in fall> red clover incorporated in fall> soybean incorporated in fall. Mass loss, nutrient content, and litter quality were all changed mostly in the first 0-3 months, which indicates that early stage of cover crops incorporation is critical to cover crop management. To describe the decomposition process, asymptotic models are more appropriate. Cover crops killed in spring have a better synchrony with corn uptake curve. After growing season, soil following red clover had greater level of soil nitrate-N.
Acknowledgements

I would like to express my deepest appreciation to all those who provided me the possibility to complete this thesis. A special gratitude I give to my committee, Dr. James Brandle, Dr. Charles Shapiro, Dr. Terry Loecke, and Dr. Erin Blankenship, for their support and guidance. Thank you to their patient help.

The good advice, support and friendship of my colleague, Katja Koehler-Cole, has been invaluable on both an academic and a personal level, for which I am extremely grateful. I would also like to acknowledge with much appreciation staff of our department. Including Jeremy Hiller, who helped me with the technical problems; Karen and Charles Messenger, who drove me to Mead before I got the driver license; and our technicians Bruce Bolander and Doug Watson, who helped me a lot on the farm.

I am thankful to all my friends here and in China, for their support and encouragement throughout. Special thanks to Jinfu Leng, Ya Li, Can Liu, Yao Li, Chao Tai, and Jianwen He. They are always there for me, and I feel lucky to have their support.

At last, I owe my deepest gratitude to my parents, Mr. Shaojun Shi, and Mrs. Yanping Fan. They provided one hundred percent support for my study. Words cannot express how much their love and understanding meant to me.
Table of Contents

Acknowledgements.................................................................................................................. i

Table of Figures ........................................................................................................................ iv

Table of Tables .......................................................................................................................... vi

1 Introduction ............................................................................................................................ 1

2 Literature Review ................................................................................................................... 5
   2.1 Cover crops application on organic farm .............................................................. 5
   2.2 Litter decomposition in soil .................................................................................. 6
   2.3 Factors that affect decomposition ...................................................................... 8
      2.3.1 Plant quality ......................................................................................... 8
      2.3.2 Soil properties ...................................................................................... 12
      2.3.3 Weather condition ............................................................................... 15
   2.4 Synchrony of nitrogen release and crop uptake ........................................... 16
      2.4.1 Nitrogen release during decomposition ............................................... 16
      2.4.2 Crop nitrogen uptake curve ................................................................. 18

3 General materials and method ......................................................................................... 25
   3.1 Experiment site .................................................................................................. 25
   3.2 Litter bag test .................................................................................................. 26
   3.3 Plant Materials ................................................................................................. 26
   3.4 Data Analysis .................................................................................................. 27
   3.5 Missing Data in this study ............................................................................... 28

4 Decomposition Study of Three Cover Crops ............................................................. 34
   4.1 Introduction ....................................................................................................... 34
   4.2 Materials and Method ...................................................................................... 35
      4.2.1 Site Statement ..................................................................................... 35
      4.2.2 Litter Bags Tests Experiment Design ............................................... 35
      4.2.3 Plant Properties Analysis .................................................................. 35
      4.2.4 Statistical method .............................................................................. 37
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>Results and discussion</td>
<td>37</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Decomposition Rate of Cover Crops</td>
<td>37</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Nutrient Change Pattern during Litter Decomposition</td>
<td>39</td>
</tr>
<tr>
<td>4.4</td>
<td>Conclusion</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>Nitrogen Release and Crop Uptake</td>
<td>60</td>
</tr>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>60</td>
</tr>
<tr>
<td>5.2</td>
<td>Materials and Method</td>
<td>61</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Soil sampling</td>
<td>61</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Soil properties test</td>
<td>61</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Data analysis</td>
<td>62</td>
</tr>
<tr>
<td>5.3</td>
<td>Results and discussion</td>
<td>62</td>
</tr>
<tr>
<td>5.4</td>
<td>Conclusion</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>General Discussion</td>
<td>66</td>
</tr>
<tr>
<td>6.1</td>
<td>Cover Crops Replacement for Chemical Fertilizer</td>
<td>66</td>
</tr>
<tr>
<td>6.2</td>
<td>Limitation of this study</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>Reference</td>
<td>69</td>
</tr>
</tbody>
</table>
# Table of Figures

- **Figure 2.1** Established decomposition pattern ............................................. 21
- **Figure 2.2** General Nitrogen Cycle (Taitt) ....................................................... 22
- **Figure 2.3** A conceptual diagram depicting the factors that control rates of nutrient release from organic matter. (Prescott, 2005a) ...................................... 23
- **Figure 2.4** Generalized Nitrogen Uptake Pattern in Corn (Hergert, et al.) .......... 24
- **Figure 3.1** Farm management timeline for the crop rotation on the organic fields at ARDC ............................................................................................................ 30
- **Figure 3.2** Google view of field W789 ................................................................ 31
- **Figure 3.3** Plot map on Google view .................................................................. 32
- **Figure 3.4** Weather stations on Google view .................................................... 33
- **Figure 4.1** Sampling plots on Google view .......................................................... 47
- **Figure 4.2** Remaining mass of red clover, white clover and soybean during the decomposition time (weeks) for fall incorporation .............................................. 48
- **Figure 4.3** Remaining mass of red clover and white clover during the decomposition time (weeks) for spring incorporation .............................................. 49
- **Figure 4.4** Application of the single exponential model for different treatments.. ......................................................................................................................... 50
- **Figure 4.5** Application of the asymptotic exponential model \( (Mt = A + Br^t) \) for different treatments. ......................................................................................................................... 51
- **Figure 4.6** Application of the asymptotic exponential model \( (Lt = m(1 - e^{-ktm})) \) for different treatments ......................................................................................................................... 52
- **Figure 4.7** Nitrogen contents change after incorporated ....................................... 53
- **Figure 4.8** C/N ratio change after incorporation .................................................... 54
- **Figure 4.9** Soluble substrates content changes after incorporation ....................... 55
Figure 4.10 Lignin Content Changes after Incorporation..........................56
Figure 4.11 Percentage of mass left as a function of time......................57
Figure 4.12 Degree days as a function of time for decomposition study ..........58
Figure 4.13 Percentage of mass left as a function of degree day. ..................59
Figure 5.1 Soil nitrate-N concentration after incorporate through the first 10
months of 2012 for different treatment. ..................................................64
Figure 5.2 Soil nitrate-N concentration after fall and spring incorporation. ....65
Figure 6.1 Comparison of soil mineral N content in fertilizer system with cover
crop system. (Crews and Peoples, 2005)..................................................68
Table of Tables
Table 1.1 Thesis Structure ................................................................. 4
Table 3.1 Litter bag extraction timeline in decomposition study .......... 29
Table 4.1 Treatment design for litter bag study .................................. 42
Table 4.2 ANOVA analysis of treatment and incorporation time as it affects the mass left percentage ........................................................................................................ 43
Table 4.3 Models used to describe the decomposition of litter ............ 44
Table 4.4 A comparison of decomposition parameters estimated in different models ...................................................................................................................... 45
Table 4.5 Initial nutrients contents ....................................................... 46
1 Introduction

Agriculture built a bridge between humans and nature. During thousands of years of agricultural history, agricultural practices changed as civilization developed. After the industrial revolution, farmers began to use chemical fertilizers and pesticides to increase the productivity of land in response to exponential growth of world population. The dependence on nonrenewable fossil fuels increased stress on the agroecosystem. Additionally, world population will have reached 9 million by 2050; soil degradation is regarded as an obstacle to meet the increasing need for world food supply. Land degradation, global warming and food security problems indicate that the conventional farming system cannot be sustainable for the long-term (Gliessman, 2007).

Organic agriculture developed as an effective way to decrease environmental damage from farming activities and ensure long term food security. Organic farming systems combine scientific knowledge of ecology and technology with traditional farming practices based on naturally occurring biological processes. Instead of using synthetic pesticides and water-soluble synthetically purified fertilizers, organic farmers are restricted by regulations to using natural pesticides and fertilizers. The central idea of organic management is using the natural environment to enhance agricultural productivity. The principal methods of organic farming include crop rotations, cover crop green manures, and integrated biocontrols. Studies show that organic farming systems can reduce soil erosion (Reganold, et al., 1987), enrich biodiversity of agricultural systems (Hole, et al., 2005), and enhance soil fertility (Watson, et al., 2002).

However, lower productivity of organically farmed land is often used as an argument against organic agriculture. Research from central Europe showed crop
yield to be 20% lower in the organic system (Mäder, et al., 2002). In contrast, after completing a 22-year study on organic farming, Cornell University published a report in 2005, claiming that organic farming produced the same soybean and corn products as the conventional methods (Pimentel, et al., 2005). Moreover, in recent decades, more and more farmers and researchers have been involved in the study of organic farming systems and their research demonstrates the more positive effects of organic agriculture on the environment, such as maintaining biodiversity and species abundance (Bengtsson, et al., 2005), increasing carbon storage and preventing nitrogen leaching (Drinkwater, et al., 1995). Badgley (2007) concluded that organic agriculture has the potential to contribute quite substantially to the global food supply, while reducing the detrimental environmental impacts of conventional agriculture.

Soil fertility is a decisive factor in determining the productivity of all farming systems (Badgley, et al., 2007). Nitrogen is commonly considered to be the key factor limiting crop growth in organic systems (Berentsen, et al., 1998, Möller, et al., 2008, Thorup-Kristensen, et al., 2003). The amount and timing of mineralization determines N availability. Different from conventional farming systems, which dependent on chemical fertilizers, organic farming systems rely on the management of soil organic matter to optimize crop production (Watson, et al., 2002). Hence, addition of plant residues has become a pivotal strategy for soil fertility improvement and sustainable of land use. Cover crop composition and its breakdown rate affect soil physical, chemical, and biological properties. In order to optimize the benefits of plant residue on soil quality improvement, it is critical to synchronize the release of nutrients from residue decomposition with patterns of plant nutrient uptake, which may minimize the loss of available nutrients via leaching, runoff and erosion.
To study cover crop effects on soil fertility, especially nitrogen contributions to the next crop in the rotation, this research was carried out on a certified organic farm at the University of Nebraska’s Agricultural Research and Development Center (ARDC) near Mead, NE. Our first research objective was to examine the decomposition rates of three common cover crops (white clover, \textit{(Trifolium repens,L.)} red clover \textit{(Trifolium pratense L.)} and soybean \textit{(Glycine max L.)} in order to determine when most N was released and its synchrony with subsequent corn crop uptake. Second, we focused on the effect of cover crops on soil N levels.

As shown in Table 1.1, in chapter 1, I stated the motivation and objectives of this study. In chapter 2, I summarized some background knowledge and conclusions from previous studies. Chapter 3 is the general materials and methods. We can tell from my thesis title that my thesis consists of two important parts, one is the decomposition study which is discussed in chapter 4, and the other is the cover crops nitrogen contribution to soil, which is discussed in Chapter 5. Chapter 6 is for general discussion.
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1</td>
<td>General Introduction</td>
</tr>
<tr>
<td></td>
<td>Literature review on litter decomposition pattern and factors affecting it</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Experimental design and general statistical analyze methodology</td>
</tr>
<tr>
<td></td>
<td>Decomposition study and the effects of plants chemical properties and temperature on decomposition</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>The effect of cover crops on soil nitrogen content</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>General discussion</td>
</tr>
</tbody>
</table>
2 Literature Review

2.1 Cover crops application on organic farm

Cover crops are crucial parts of organic farm management. They are helpful for building soil health and preventing soil erosion. Cover crops are also important method to improve soil nutrition while controlling weeds and insects. Cover crops that have been used as green manures are commonly leguminous. There are about 18,000 species in 670 to 750 genera of legumes (Raven and Stirton, 1981).

Soybean (*Glycine max* L.) is often grown in rotation with corn. The nitrogen contribution potential of legume crops has been proven in previous studies. For example, Rembon and MacKenzie (1997) reported 26 to 75 lbs. (11.8 to 34 kg) more soil NO$_3$-N in the spring after soybean than after corn (no N applied). Another soil organic N study to a 6-inch depth in Iowa showed that soil N is supplemented during soybean growth. Martens et al. (2006) reported an average decrease of 328 lbs. (148.8 kg) N/A between early spring and fall harvest of a corn crop while soybean production resulted in a 286 lbs. (130 kg) N/A enrichment the following season.

Red clover (*Trifolium pratense* L.) is a dependable and low-cost cover crop for many areas in the U.S. It creates loamy topsoil, adds soil nitrogen content, helps to suppress weeds and breaks up heavy soil. It is usually overseeded or frostseeded into standing crops. As a cover crop, red clover is used primarily as a legume green manure killed ahead of corn or vegetable crops planted in early summer (Clark, 2008). Full-season, over-wintered red clover can produce 2 to 4 tons dry matter per acre, and fix 70 to 150 lbs. (31.8 to 68 kg) N/A. In Ohio, over-wintered mammoth and medium red clover contained about 75 lbs. (34 kg) N/A by May 15, increasing to 130 lbs. (59 kg) N/A by June 22 (Schmidt, et al., 2001). A two-year study in Wisconsin showed that corn following red clover yielded the same as corn supplied with 160 lbs. (73 kg)
N/A. At the same time, cover crop application reduced the risk of post-harvest N leaching. Soil testing also showed that 50% of the cover crop N was released in the first month after incorporation, which correspond well with corn’s nitrogen demand (Stute and Posner, 1995).

White clover (*Trifolium repens*, L) is a persistent, widely adapted perennial legume. It grows just 6 to 12 inches tall, has tough stems and a dense shallow root mass to protect soil from erosion and help to suppress weeds. A healthy stand of white clover can produce 80 to 130 lbs. (36 to 59 kg) N/A when killed the year after establishment. In established stands, it also may provide some N to growing crops when it is managed as living mulch between crop rows. Because it contains more of its total N in its roots than other legumes, partial tilling is an especially effective way to trigger N release. The low C:N ratio of stems and leaves causes them to decompose rapidly to release N (Clark, 2008).

### 2.2 Litter decomposition in soil

Decomposition is one of most important processes that accounts for carbon and nutrient cycling on planet Earth. Decomposition or litter mass loss is regarded as the sum of CO₂ release and leaching of compounds, including both C compounds and nutrients. Organic compounds in residues are classified into soluble components, hemicellulose, cellulose, and lignin based on molecular size, solubility and primary constituent.

Litter decomposition is complex and involves physical, chemical, and biological processes. It generally follows a sequential pattern with different classes of organic compounds dominating the decay process as it proceeds. When plant parts fall onto the soil surface, soluble organic substances such as sugars, phenolic, hydrocarbons, and glycerides are leached rapidly by water. This process is highly affected by climate
conditions. At the same time, under soil microbial effects and external forces (such as wind, rain and cultivation), large pieces of plant residues are physically broken down into small bits, which provide greater surface area for microbial colonization and attack. Under the action of rapidly growing microorganisms, some sugars, low-molecular-weight phenolics, and some nutrients are readily lost from the litter. Following this, the plant detritus (cellulose, hemicellulose, and lignin) undergoes chemical alteration by microbes, encompassing both fungi and bacteria (Fioretto, et al., 2005).

Figure 2.1 Established decomposition pattern. The + and − signs indicate positively and negatively related effects, respectively, to increased concentrations of nutrients and lignin (Berg and McClougherty, 2007) describes an established decomposition pattern. In this pattern, there are three stages (early stage, late stage, and humus near stage) before humus is formed. In the early stage, decomposition of soluble and unshielded cellulose and hemicellulose takes place. In this stage the process is influenced by climate. In the late stage, the influence of climate on decomposition gradually decreases to essentially zero. In the same late stage, N may have a negative influence on lignin degradation through a repression of de novo ligninase synthesis and by creating a barrier based on chemical bonds between lignin remains and N.

Plant residue composition changes during decomposition. The final products of plant residue decomposition include carbon dioxide, water, energy, microbial biomass, inorganic nutrients and re-synthesized organic carbon compounds such as humus, phenolics, celluloses, hemicelluloses and lignin. Under aerobic conditions, microbial decomposition results in a release of CO₂. Under anaerobic or oxygen-limited conditions, anaerobic decomposers produce organic acids.
2.3 Factors that affect decomposition

Decomposition is primarily driven by microbial activities and can be best predicted by environmental factors such as temperature and precipitation, as well as litter quality, but soil chemistry and physical conditions can also influence the rate of litter decomposition (Liu, et al., 2006). Study of the factors that affect litter decomposition is fundamental to quantitative analysis of nutrient cycling in terrestrial ecosystems. Plant litter qualities, soil qualities, and environmental conditions are discussed as main impact factors of decomposition process in this section.

2.3.1 Plant quality

Decomposition rates and patterns are directly determined by the quality of plant residue. Quality can be described as the suitability of the substrate as a carbon, energy or nutrient source to the organisms that degrade it. Physical nature and biochemical composition are two main characteristics to consider in the breakdown rates of plant residue.

2.3.1.1 Biochemical Composition

Biochemical properties of plant residue and their relationship with decomposition have been well studied. A wide range of residue chemical components have been found to be good indicators of the decomposition processes. According to their different responses to degradation, chemical components of plant residue can be classified into three groups: 1) easily decomposable sugars and amino acids; 2) slowly decomposable compounds including hemicellulose and cellulose; 3) recalcitrant material such as lignin. Many studies have attempted to relate chemical quality to decomposition and mineralization. Chemical quality components such as initial C or N content, polyphenols, cellulose and lignin content are good indicators for plant residue quality and residue decomposition rates.
Soil microorganisms that decompose organic matter use carbon as a source of energy and nitrogen for building cell structure. These organisms use about 30 parts carbon for each part of nitrogen, so an initial C/N ratio of 30 promotes rapid decomposition. University of California studies on materials with an initial C/N ratio varying from 20 to 78 and nitrogen contents varying from 0.52% to 1.74% indicated that initial C/N ratio of 30 to 35 was optimum. A majority of investigators believe that for C/N ratios above 30 there will be little loss of nitrogen. According to Baldock (2007), plant residues with a high C/N ratio (>40) are mineralized far more slowly than residues with the C/N less than 40. If there is too much carbon, decomposition slows when the nitrogen is used up and some organisms die. Instead, low C/N plant residue will meet the N requirements of soil microbial organisms and extra N will be released and becomes available for plant uptake.

Polyphenols are plant tannins that are relatively resistant to decomposition. The polyphenol content of plant material may vary within a source of plant material (Haynes, 1986). Sivapalan et al. (1985) found that plant residue decomposition rate decreases as the concentration of polyphenols, cellulosics, and waxes increases because of enzyme inhibition and binding of mineralized N to insoluble organic compounds. Palm and Sanchez (1991) found that N mineralization was negatively correlated with polyphenol concentration (r=-0.63) and polyphenol/N ratio (r=-0.75), and concluded that plant residues high in polyphenols have low N mineralization due to the formation of stable polymers between polyphenol and amino groups. Similarly, Oglesby and Fownes (1992) found that the initial polyphenol/N ratio was the best chemical index of N mineralization. Bending et al. (1998) showed that N mineralization was correlated with phenolic content especially in the early stages.
Lignin has traditionally been considered as a recalcitrant compound that retards biotic breakdown of organic matter. Thus we can deduce that lignin’s control effect on decomposition rate may take place in the late stages of decomposition. From the analysis of litter mass-loss rate as compared with lignin concentration, Berg et al. (1987) concluded that lignin concentration tends to increase during litter decomposition and high lignin concentrations are related to lower decomposition rate. More specific, they indicated that the effect of lignin concentration on litter mass-loss rates may be described as a negative linear relationship in the later decomposition stages (Berg and Mc Claugherty, 2007).

It is obvious that lignin content can influence decomposition rate of litter, but there’s limited evidence to prove lignin alone can be an efficient indicator of decomposition rate. Lignin degradation is made by microorganisms like fungi and bacteria. Phanerochaete Chrysosporium has been one of the most intensively studied white rot fungi, which can produce a variety of extracellular lignin peroxidase (Leštan, et al., 1994). Research done on the lignolytic enzyme system of the fungus Phanerochaete Chrysosporium led to the conclusion that even low levels of either ammonium or some amino acids could reduce both the formation of lignin-degrading enzymes and the rate of lignin degradation (at least for this fungus) (Melillo, et al., 1989). Briefly, N slows lignin decay rate in laboratory studies. Thus it is more reasonable to consider nitrogen content as well as lignin content when predicting litter decomposition rate. Many studies took lignin: N ratio as an efficient indicator of litter decomposition rate.

However, Taylor et al. (1989) were able to find only limited support for the lignin: N ratio as an index of substrate quality. Under most circumstances N content alone provided better prediction of decomposition rate than did the lignin/N ratio. They also
concluded that: 1) for high-lignin pine needles, regressions based on lignin or the lignin/N ratio were more accurate predictors of decomposition rate than those based on nutrients or total C; 2) in low-lignin substrates such as herbs, lignin control of decay begins only after the very large pool of labile matter is exhausted, which may be close to 50% mass loss and even when lignocellulose decomposition has begun, the low lignin content means that its retarding influence is not strong; 3) for litter of medium lignin content the lignin/N ratio is expected to be a good predictor of decay rate. In other words, lignin control of decomposition rate will be stronger in high-lignin litter than in low-lignin litter.

2.3.1.2 Physical Nature

Compared to chemical quality, there’s much less attention paid to the physical quality of plant residues. Plant physical properties include particle size, toughness, and surface properties. These properties have the potential to affect the accessibility of substrates to soil organisms, and thus alter rates of colonization and patterns of decomposition and mineralization.

Particle size is the physical quality parameter that has received most attention in nutrient cycling studies. Some studies showed that particles with a smaller size decompose faster than larger particles. For example, Singh et al. (2004) showed that the particle size of canola ((Brassica napus L.) residue had a significant effect on mineral N immobilization, but did not significantly affect the C mineralization rate. Particle size influences the soil microbial activities in a number of ways. First, particle size controls the surface area available for colonization by the soil microbes, and will influence exchange of water, nutrients and oxygen between the substrate and the soil matrix (Swift et al., 1979). Additionally, particle size will influence contact of the material with clay and silt particles, which can protect organic materials from
microbial attack (Hassink, 1997). Bending and Turner (1999) demonstrated that particle size of crop residue materials influences the activities of the soil microbial population after being incorporated into soil, and that the nature of the effects depend on the biochemical quality of the plant material and the stage of decomposition. They concluded that the relative importance of these influences will depend on the biochemical and physical composition of the organic material, together with the physical and chemical environment of the soil.

2.3.2 Soil properties

2.3.2.1 Chemical properties

Soil chemical properties include pH, organic matter content, and nutrient availability. All of these properties can influence the composition of the microbial community.

Soil pH affects directly the kind, density and the activity of fungi, bacteria and actinomycetes involved in the process of decomposition and thereby the rate of decomposition of organic matter. The rate of decomposition is greater in neutral soils than in acidic soils. Therefore, treatment of acid soils with lime can accelerate the rate of organic matter decomposition. Baath et al. (1980) found that acidification lowered the decomposition rate of needle and root litter, and there were significant changes in the functional characteristics of the bacterial population due to the acid treatment. Similarly, Dancer’s study on influences of soil pH on ammonification and nitrification of N showed that soil pH did not affect rates of ammonification appreciably; however it had a significant effect on nitrification rates. Length of the delay period was increased and rate of NO₃⁻ accumulation decreased with a decrease in soil pH (Dancer, et al., 1973).
To maintain their life activities, decomposer microbes utilize nutrients from either litter material or surrounding soils (Ocio et al., 1991; Sinsabaugh et al., 1993). Thus soil nutrient availability has been suggested as one of the controlling factors affecting the rate of litter decomposition for a long time (Swift et al., 1979). However, results of the studies concerning the effects of increased soil N and P on the rate of litter decomposition and nutrient dynamics to date have been controversial. For example, some studies (Hunt et al., 1988; Fenn, 1991; Ostertag and Hobbie, 1999) found that increased N and P could stimulate litter decomposition, while others found no (i.e. Prescott et al., 1999; Dukes and Field, 2000) or depressing effects (Söderström et al., 1983; Magill and Aber, 1998). Berg et al. (1982) and Berg and Matzner (1997) found a positive response to N in the initial decomposition phase but a negative response in the later stages. Kwabiah et al. (1999) suggested that responses of plant litter decomposition to soil nutrients were determined by litter quality. Such inconsistency in the relationships between litter decomposition and soil nutrients, therefore, calls for continued investigations of the subject.

2.3.2.2 Physical Properties
Among all soil physical properties, such as density, porosity, structure, consistency, and resistivity, texture is perhaps the most important. It influences nutrient and water dynamics, surface area, and other physical properties. To get a better understanding of the relation between soil texture and soil organic matter dynamics, some recent studies focused on determining the possible mechanisms of soil textural controls on soil organic matter dynamic.

Six et al. (2002) proposed the protected SOM theory, which conceptualized a model of SOM dynamics based on four measurable pools: (1) a biochemically-protected C pool, (2) a silt- and clay-protected C pool, (3) a
microaggregate-protected C pool, and (4) an unprotected C pool. Among them, the silt and clay protected C pool is the C that is protected by association with the mineral particles and is by definition hydrolyzable. The stabilization of organic C and N are dependent on the clay content and clay type (i.e. 2:1 versus 1:1 versus allophanic clay minerals).

Another theory is related to soil water dynamics. Scott et al. (1996) studied effects of both soil texture and soil water potential on the decomposition of wheat litter. While texture had no impact on short term decomposition rates there was an interaction between soil texture and water potential. They also reported there was an impact on litter decomposition of the older C that was already in the soil. They conclude that soil texture is more important for long-term organic matter dynamics than for initial phases of decay. Water levels generally influence decay relatively little on sandy soils, but have a significant effect on loams or finer-textured soils, because finer-textured soils will interact with water more than do coarser textured ones.

Mtambanengwe et al. (2004) examined two mechanisms that could explain the effect of soil texture on organic matter decomposition: 1) the protective action by clays against organic matter degradation through the formation of complexes between metal ions associated with large clay surfaces and high CEC; and 2) accessibility by soil microbes. By testing carbon mineralization of tobacco starch (Nicotiana tobacum) and barley straw (Hordeum vulgare) in soils of different textures, the study provides empirical evidence to support the theory that decomposition of fresh organic matter is governed by its physical accessibility by microbes as determined by soil texture and pore size distribution.
2.3.3 Weather condition

Decomposition and nitrogen releasing processes are biological, so they are sensitive to environment conditions such as temperature and moisture. Environmental control of decomposition depends on the stage of decomposition.

In early decomposition stages, water availability can influence the rates of litter decomposition and nutrient release through its effects on the activities of the decomposer communities (Liu, et al., 2006). Water supply in the form of rainfall can also affect decomposition by facilitating leaching and breakdown of surface litter (Swift, et al., 1979). Substantially greater rates of decay have been reported following irrigation of dry forests (Raison, et al., 1990). Moreover, water availability may affect litter decomposition indirectly by altering the litter quality in terms of lignin and nutrient content of plants (Prescott, 2005). In general, litter moisture content in excess of 150% or below 30% (dry weight basis) tend to slow litter decomposition (Haynes, 1986). Within this range, decomposition rates will increase with increasing moisture if temperature is adequate (Bunnell, et al., 1977).

It is difficult to separate the effects of temperature on decomposition from that of many other environmental factors at the early decomposition stages. Soil temperature often co-varies with other factors that also affect decomposition. Moreover, decomposer organisms have a wide range of optimal temperatures from 0 to 45°C (Paul, 2006), even though their activities often show a positive correlation with increased temperature (Swift, et al., 1979). Numerous manipulative experiments, including artificial warming of litter layers, demonstrate that increased temperature results in higher rates of CO₂ evolution and mass loss (Prescott, 2005). At the late stages of decomposition, when humus starts to dominate the decomposition process, decomposition rates generally increase with in increasing temperature if moisture
conditions are adequate (>30%) (Paul, 2006). Increasing the temperature in a boreal black spruce (*Picea mariana*) forest floor by 9 °C for three summers increased respiration and N mineralization rates, and decreased the mass on the forest floor, including the humus layer (Van Cleve et al., 1981).

### 2.4 Synchrony of nitrogen release and crop uptake

#### 2.4.1 Nitrogen release during decomposition

Nitrogen is considered as a macronutrient element. Nitrogen represents 79% of the earth’s atmosphere and even more is found in the soil as organic sediments. Unfortunately, atmospheric N\textsubscript{2} exists in a form that cannot be taken up and used by plants, as only oxidized (NO\textsubscript{3}⁻) or reduced (NH\textsubscript{4}⁺) forms of N can be used. The nitrogen cycle is the process by which nitrogen is converted between its various chemical forms (see Figure 2.2). This transformation can be carried out through both biological and physical processes. Rhizobium is one of those microbes that have a mutually beneficial relationship with legumes. Basically, legume roots provide nutrients and place for Rhizobia growth; Rhizobia then uptake nitrogen gas from the atmosphere and turn it into a plant usable form. During the fixation process, atmospheric N\textsubscript{2} is combined with hydrogen from methane to form anhydrous ammonia (NH\textsubscript{3}), the basic nitrogen fertilizer. These bacteria take free N from the soil air and synthesize it into plant useable forms. It is likely that N compounds produced within the bacterial cells are diffused out the cell wall and absorbed by the host plant.

When fresh plant materials or crop residues are added to the soil, microorganisms begin to decompose this material. There are two processes involved, immobilization and mineralization. Microbial populations increase soon after the addition of the fresh plant residue. If the plant material has a C/N ratio greater than 25, the microbial population will use available soil nitrogen to decompose the residue.
This process is referred to as immobilization of nitrogen. If the C/N ratio of the fresh plant material is less than 25, the microbial population will release additional available nitrogen.

Mineralization is the microbiological process that converts organic nitrogen to available forms. The pattern and timing of mineralization depends on the residue quality, particularly C/N ratio, soluble C, lignin, polyphenol content, soil type, temperature, soil moisture content and timing and method of incorporation (Swift, et al., 1979) (see Figure 2.3).

Ammonification occurs when organic matter is broken down into simpler amino compounds. Nitrogen is released in the form of ammonia through enzymatic digestion of bacteria and fungi, and then is dissolved in the soil solution as ammonium (NH$_4^+$). Plants can use NH$_4^+$, although most N uptake is in the nitrate (NO$_3^-$) form. Ammonification progresses best in well-drained, aerated soils but will occur under almost any condition because of the wide variety of organisms capable of accomplishing these changes.

Another mineralization process is nitrification. This is an oxidative process that converts ammonium (NH$_4^+$) to nitrate (NO$_3^-$). Two groups of bacteria, collectively called nitrobacter, are involved. _Nitrosomonas_ species are responsible for the conversion of NH$_4^+$ to nitrite (NO$_2^-$), and then nitrobacteria oxidize nitrite into nitrate. The second transformation follows the first so closely that little nitrite (toxic to plants) accumulates. In most soils the nitrite produced by ammonia oxidizers does not accumulate but is quickly oxidized to nitrate by the nitrite-oxidizing bacteria when they perform nitrite oxidation. The nitrite and nitrate are able to be taken up by plants. Nitrate is the end product of the nitrogen cycle. Organic matter, crop residue, manure, anhydrous ammonia, urea, and ammonia salts are all converted eventually to nitrate.
Plants can and do absorb ammonium ions but the majority of the total nitrogen is obtained from nitrate ions.

Except for nitrite and nitrate, ammonia oxidizers are also able to produce NO via nitrite reduction, which results in the production of N$_2$O, an important greenhouse gas that can escape to the atmosphere. Denitrification is the process of reducing soil nitrate to the N gases NO, N$_2$O, and N$_2$. A wide variety of mostly heterotrophic bacteria can denitrify, whereby they use NO$_3^-$ rather than oxygen as a terminal electron acceptor during respiration. Because nitrate is a less efficient electron acceptor than oxygen, most denitrifiers undertake denitrification only when O$_2$ is otherwise unavailable. In most soils this occurs mainly following rainfall as soil pores become water-saturated and the diffusion of O$_2$ to microsites is slowed. Typically denitrification starts to occur at water-filled pore space concentrations of 60% and higher (Galitz, 2009).

2.4.2 Crop nitrogen uptake curve

The amount of N taken up by the crop has a major impact on overall crop growth rate. Maximizing N recovery by the following crop is of paramount importance in organic systems. This requires synchrony of N release from incorporated plant material with crop N demand (Wagger, 1989). Swift (1987) originally proposed the concept of synchrony to describe the linking of nutrient demand with nutrient release from mineralization of organic matter.

Since N is not stable in soil and becomes less available for crop uptake over time, application timing is important. As Figure 2.4 shows, much of the N uptake occurs in a relatively short time period. If nitrogen is insufficient during this period, yield loss will occur. Applying nitrogen immediately before or during this period will result in
higher uptake by the crop and less nitrate lost to leaching or transformations to unavailable forms and ultimately in greater yields.

In practice, the pathways by which plant-available forms of N are released from legume organic residues and taken up by a subsequent crop can be complex. While annual legume rotations often have a flush of N mineralization from residues, the rate of accumulation of inorganic N in soils does not normally match that caused by conventional fertilizer applications (Groffman, et al., 1987). The decomposition and mineralization of legume proteins in organic residues into inorganic forms is a microbial-mediated process with the breakdown of organic compounds being used to provide the soil microbes with a carbon source for respiration and growth. Much of the simple organic N released is rapidly assimilated (immobilized) by the soil microbial population. Inorganic N only accumulates in soil if the amounts of N released from the organic residues exceed the C-limited microbial requirement for N for growth (Crews and Peoples, 2005).

Since legume residues tend to have a relatively high N content and a low C/N ratio they are usually expected to result in net mineralization (Kumar and Goh, 1999). However, a range of other constituents (e.g. lignin, polyphenols, and soluble C and N compounds) also influence microbial activity and mineralization, and predictions based simply on the basis of the %N or C/N ratio of legume tissues can be misleading.

The relatively low recovery of legume residue N by subsequent crops, particularly in temperate regions, has led some to suggest that legumes are an inefficient source of N (Hesterman et al. 1987; Harris et al. 1994). However, in studies that compared yields of crops grown on legume vs. fertilizer sources of N, the yields achieved were often similar (Ladd and Amato, 1986; Janzen et al., 1990; Harris et al., 1994). Thus, studies that estimate uptake efficiencies of labeled N from recently
applied legume residues have a tendency to underestimate the overall N-supplying capacity of a legume-based system.
Figure 2.1 Established decomposition pattern. The + and – signs indicate positively and negatively related effects, respectively, to increased concentrations of nutrients and lignin (Berg and McClaugherty, 2007)
Figure 2.2 General Nitrogen Cycle (Taitt)

Diagram showing the nitrogen cycle with various processes including:
- Plant - N → N₂ Fixation → N₂
- Animal-N → Harnessed
- Soil organic N → N-Mineralization
- Sediment-N → N-Nitrification
- Fertilizer-N → NH₄⁺ → N₂O
- Precipitation-N → N₂O
- Groundwater-N → Denitrification
- Plant - N → N₂O → N₂
Figure 2.3 A conceptual diagram depicting the factors that control rates of nutrient release from organic matter. (Prescott, 2005a)
Figure 2.4 Generalized Nitrogen Uptake Pattern in Corn (Hergert, et al.)
3 General materials and method

3.1 Experiment site

This study was conducted in field W789, which has been organically certified since 2005. It is located at the University of Nebraska’s Agricultural Research and Development Center (ARDC) near Mead, Nebraska (see Figure 3.1). The climate is temperate continental with an average annual precipitation of 27.7 inches (703.6 mm), and a mean annual temperature of 54.1 °F (12.3 °C). The soil is Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalf). Prior to initiation of the study in 2011, the study site had a 6-year history of corn-soybean-wheat rotation cropping system under organic management. Winter wheat (*Triticum aestivum*) was no-till sown in October, 2010 following soybean harvest. On March 24, 2011, red clover (*Trifolium pratense*) at 100 lbs/acre (112kg/ha) and white clover (*Trifolium repens*) at 100 lbs/acre (112kg/ha) were randomly seeded into the wheat field (see Figure 3.2). Winter wheat was harvested in July, 2011, and then the soybean cover crop was sown.

Weather data were acquired by a Campbell Scientific data logger (Model CR10X, Campbell Scientific, Logan, Utah). Air temperature was monitored with the Temp Thermistor; precipitation was monitored with a RH Hema Cap; soil temperature and moisture were monitored with Type E Thermocouples and a CS616 Water content reflectometer, respectively (Campbell Scientific, Logan, Utah). Measurements were made every 60 seconds, and hourly averages calculated and recorded. Seven data logger systems were set up in the plots (see Figure 3.4).
3.2 Litter bag test

Litter bag test is a very common method to define litter decomposition. It is used for incubations in the field or in laboratory microcosms (Berg and Mc Claugherty, 2007). A known quantity of leaf litter is placed into a mesh bag, and the bag is then buried. Bags are extracted at periodic intervals, dried to constant weight and reweighed to determine the amount of mass lost. By incubating the leaves in situ, they are exposed to the normal fluctuations in temperature and moisture. The mesh bags allow smaller insects and microorganisms access to the leaves.

In this study nylon bags (Ankom, 10 x 20 cm) with a mesh size of 50 μm were filled with 3.80 to 4.20g of air dried plant samples. The litter bags were weighted empty, again after adding the plant material and the weights recorded. The bags were closed at the top by folding the top 5cm of the bag over and stapling two times. An aluminum tag with the bag ID was stapled with each bag. The litter bags were buried to a depth of 15cm in each plot. From each plot, 4 litter bags for each legume species were extracted according to the timeline (see Table 3.1). The litter bag study was a factorial design including three treatment factors: legume species, time of burial, and extraction time. Extracted litter bags were analyzed for mass left, nutrient content (C, N), and fiber construction in the Ecosystem Analytical Lab at UNL. Details will be discussed in Chapter 4.

3.3 Plant Materials

White clover and red clover were over seeded in March, 2011 onto a wheat crop. Soybean was sown in July, 2011, after wheat harvesting. On October 14, 10,000 g of whole soybean plants were cut from the 4 soybean plots. At the end of October, approximately 10,000 g of whole red clover plants and 5,000 g of whole white clover plants were cut randomly from the clover plots. On April 11, 2012 5,000g of whole
red clover plants and white clover plants were collected. All plant materials were air dried in the greenhouse to constant weight immediately after collection. After drying, ground samples were analyzed for total C, N and biomass fractions (soluble, hemicelluloses, cellulose and lignin) in the Ecosystem Analysis Lab (UNL). These samples represent the starting values before decomposition.

3.4 Data Analysis
The data collected were analyzed statistically using Analysis of Variance implemented in the SAS PROC GLIMMIX. Any results declared as statistically different are done so at a 5% level of significant.

Ecological models are used to describe litter decomposition patterns. The single exponential model was first proposed by Jenny et al. (1949), and elaborated by Olson (1963). It is often used for predictive purposes. A basic condition for applying this equation is to assume that the decomposition rate is constant, and that all material is decomposed. The formula may be written (Wider and Lang, 1982):

\[ M_t = M_0 e^{-kt} \]  

(3.1)

and is often used in the form

\[ \ln\left(\frac{M_t}{M_0}\right) = -kt \]  

(3.2)

In these and subsequent equations, \( M_0 \) is the initial mass, \( M_t \) the mass at a certain time, \( t \), and \( k \) the decay rate constant. Aber et al. (1990) suggested that this model works reasonably well for a variety of litters until 20% of initial mass is remaining. This simplified model is widely used, and it fit the early stage decomposition very well.

But in reality, some chemical components of litter cannot decompose completely, and the amounts of remaining after decomposition of these litter components approach a minimum level. Howard and Howard (1974) used an asymptotic non-linear model to describe such process:
\[ Mt = A + Br^t \]  

(3.3)

where \( M_t \) is the percentage of remaining litter mass, \( t \) is time in days, \( A \) and \( B \) are model parameters, and \( r \) is an expression for the decomposition rate. Based on this model, Berg and Ekbohm (1991) developed another non-linear model that they found more feasible to use:

\[ Lt = m(1 - e^{-\frac{kt}{m}}) \]  

(3.4)

In this equation, \( Lt \) is the percentage of accumulated mass loss, \( t \) is time in days, \( k \) is the decomposition rate at the beginning of the decay, and \( m \) represents the asymptotic level that the accumulated mass loss will ultimately reach.

In this study, I tested all three models discussed above. Details are given in Chapter 4.

3.5 Missing Data in this study

Because of the malfunction of the drying oven, the first batch of spring treatment samples and the 4th batch of fall treatment samples were burned and lost. After testing several samples, I found that both carbon content and fiber fractions were affected. So the plant nutrients and fiber fractions data from these two sampling times were lost. But there’s no difference for the mass changes, since mass loss data were obtained before the accident happen. The last set of soil sample was not able to be sent for testing because of time limitation.
Table 3.1 Litter bag extraction timeline in decomposition study

<table>
<thead>
<tr>
<th>Date</th>
<th>Fall Treatment</th>
<th>Spring Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Dec-11</td>
<td>Bury litter bags</td>
<td></td>
</tr>
<tr>
<td>3-Jan-12</td>
<td>1 Extraction</td>
<td></td>
</tr>
<tr>
<td>27-Mar-12</td>
<td>2 Extraction</td>
<td></td>
</tr>
<tr>
<td>26-Apr-12</td>
<td>3 Extraction</td>
<td></td>
</tr>
<tr>
<td>11-May-12</td>
<td></td>
<td>Bury litter bags</td>
</tr>
<tr>
<td>25-May-12</td>
<td>4 Extraction</td>
<td>1 Extraction</td>
</tr>
<tr>
<td>8-Jun-12</td>
<td></td>
<td>2 Extraction</td>
</tr>
<tr>
<td>22-Jun-12</td>
<td>5 Extraction</td>
<td>3 Extraction</td>
</tr>
<tr>
<td>19-Jul-12</td>
<td>6 Extraction</td>
<td>4 Extraction</td>
</tr>
<tr>
<td>17-Aug-12</td>
<td>7 Extraction</td>
<td>5 Extraction</td>
</tr>
<tr>
<td>18-Sep-12</td>
<td></td>
<td>6 Extraction</td>
</tr>
<tr>
<td>18-Oct-12</td>
<td>8 Extraction</td>
<td>7 Extraction</td>
</tr>
<tr>
<td>15-Nov-12</td>
<td></td>
<td>8 Extraction</td>
</tr>
</tbody>
</table>
Figure 3.1 Farm management timeline for the crop rotation on the organic fields at ARDC.
Figure 3.2 Google view of field W789
Figure 3.3 Plot map on Google view, plot area: clover plots= 450 x 30', C9 = 450 x 30', C1 - C8 = 100 x 30'
Figure 3.4 Weather stations on Google view
4 Decomposition Study of Three Cover Crops

4.1 Introduction

Cover crops are critical to organic farming systems since they are helpful to building soil health and preventing soil erosion. With the increase use of legume cover crops in crop rotations, there is need for a better understanding of their role in nitrogen cycling. There has been concern of cover crops tying up too much N and the timing of its release to the next crop (Vyn et al., 1999). Proper management of plant residues as a source of nutrients may increase plant productivity and reduce dependency on mineral fertilizers, especially N fertilizer. One of the most well-established patterns in ecosystem ecology is that litter decay rates are correlated with the initial ratios of C/N, lignin/N, or lignin/cellulose in litter (e.g., Melillo et al., 1982; Aerts, 1997; Hobbie, 2008). These chemical traits are strong predictors of litter decay, accounting for over 73% of the variation in litter decomposition rates worldwide (Zhang et al. 2008). Climate conditions, such soil temperature and moisture, also can affect litter decomposition by changing soil microbe activities. Information on cover crops nitrogen release patterns after incorporation is important for crop management decisions. While many studies have been conducted considering cover crop decomposition pattern, the influence of cover crop kill timing on its decomposition process hasn’t been fully understood.

Decomposition study was carried out in order to get a comprehensive understanding of the different decomposition patterns of three cover crops and the effect of cover crop management on nutrient release. Two management options were considered, one incorporated the cover crop in the fall (1-Sep-2011); the other incorporated the cover crop in the spring (1-May-2012). Three cover crops (white
clover, red clover, and soybean) were compared. We hypothesize that: 1) the three target crops will have different performances according to their chemical properties, and 2) most mass will be lost in the earliest stage of decomposition.

4.2 Materials and Method

4.2.1 Site Statement
This field study was conducted at UNL’s Agricultural Research and Development Center (ARDC) near Mead, Nebraska. Site information was given in Chapter 3.

4.2.2 Litter Bags Tests Experiment Design
The litter bag study was a factorial design including two treatment factors: legume species and time of burial (see Table 4.1). Litter bags of the three legume species were buried on December 1, 2011 and on May 11, 2012. Considering that temperature in first 8 weeks following fall burial was very low and soil was frozen, the nine extraction times for fall treatment were 0, 4, 16, 20, 24, 28, 32, 36, 44 weeks after burial. For spring treatment samples were dug up every four weeks beginning on the day of burial. The extraction timeline is shown in Table 3.1. We randomly chose four plots for each species and in each plot each extraction time had 4 samples, in case some bags were lost (see Figure 4.1). Thus, there were a total of 384 bags buried in fall and 256 buried in the spring.

4.2.3 Plant Properties Analysis

4.2.3.1 Mass loss
Extracted bags were oven-dried at 55 °C for three to five days. Plant litter then was carefully removed from the sample bag to a clean weighted bag. The clean bags with plant materials were dried for another two to three days to constant weights.
Mass left was weight of bags with plant material minus the weight of empty bag. The percentage of mass left was calculated using:

\[
\text{Mass left} \% = \frac{\text{Mass left}}{\text{Initial mass}} \times 100\%
\] (3.1)

4.2.3.2 Nutrient content

Plant material was removed from litter bags into clean nylon bags, ground by in a Wyle mill and stored in the wrap bags. Following the extraction and processing, plant samples were analyzed in the Ecosystem Analysis Lab (UNL). Total C and N concentrations were measured with COSTECH Analytical Elemental Combustion System 4010 (ESC 4010). Biomass fractions were determined after extraction of structure biomass according to the method of Blaschke et al. (2002). Residue quality was estimated with the Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY), which is a common technique used to determine forage digestibility (Goering and Van Soest, 1970, Van Soest, et al., 1991). This technique utilizes a series of heated extractions to determine the amount of soluble, hemicellulose, cellulose and lignin fractions within each sample. These classifications do not represent strictly identical chemical compounds, but rather groups of similar compounds with similar resistance to decomposition. The extractions were done in the following order: NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), and then ADL (Acid Detergent Lignin). The data for tissue fractions analysis were presented as the four fractions (soluble, hemicellulose, cellulose and lignin) totaling 100% of the plant tissue carbon quality.

4.2.3.3 Degree Days

Soil temperature was monitored with a Type E Thermocouples. Daily soil temperature data was collected every month. Decomposition degree days were calculated using following equation:
DDD = \sum (\text{Daily Average Temperature} - \text{Minimum Temperature}) \quad (4.1)

the minimum temperature is 0°C.

4.2.4 Statistical method
Analysis of variance (ANOVA) (see Table 4.2) was conducted to tests main effects of legume, time of burial, and time of extraction and interactions between the treatments. To assess differences between the remaining amounts of different nutrients, the sampling time was considered as main factor in addition to legume type.

As concluded in Chapter 3, three empirical models that have been used to describe the decomposition of litter. Organic matter is regarded as one “unified” material in all of these models (see Table 4.3). All three models were tested using the PROC NLIN procedure in SAS.

4.3 Results and discussion
4.3.1 Decomposition Rate of Cover Crops
From Figure 4.2 and Figure 4.3 we can see a rapid decrease of litter mass at the beginning of four weeks of decomposition for both spring and fall treatment. The percentages of clover mass left trended to constant after an initial period of rapid decomposition. For fall incorporation (see Figure 4.2), cover crop species has a significant effect on mass loss (P-value<0.0001). White clover had the most mass loss (79.38% mass lost after 36 weeks), while red clover only had 71.97% of mass loss during the same time. Clovers lost mass more rapidly than soybean at the early stage (first 4 weeks) of decomposition (P-value<0.0001). After four weeks, the mass loss of clovers trended to constant, while the litter of soybean still had a significant decrease at the 16th week (P-value=0.02). Soybean has a longer rapid decomposition period than clovers. For spring incorporated (see Figure 4.3), the effect of cover crop species is not significant (P-value=0.12), but there’s an interaction effect of extraction time
and plant species (P-value<0.0001). The decomposition patterns are similar to the fall treatment: for both white and red clovers, mass was lost most rapidly in the first four weeks and then there were no significant changes.

Decomposition constant values of cover crops in each treatment are given in Table 4.4. In model $Mt=A+Br^t$ and $Lt = m \left(1 - e^{-\frac{Lt}{m}}\right)$, higher values correspond to faster rates of decomposition, while lower values correspond to slower decay. We can see from Table 4.4 that cover crops incorporated in spring have larger decomposition constants, and decomposed faster. The decomposition constant is primarily controlled by climate and litter quality, which will be discussed in next section.

Results from the non-linear regression analysis illustrated that the single exponential model didn’t fit data from this study (see Figure 4.4). The asymptotic models are more accurate to describe the decomposition process in this study (see Figure 4.5 and Figure 4.6). As described in Chapter 3, the main difference between the single exponential model and the asymptotic models is whether or not to take the residue into consideration. In this study, the mass losses of plant litter under all treatments trended to constants, which illustrate that the decomposition processes reached a stage at which decomposition almost stops. This stage can be described as a limit value for the decomposition process. The limit values (A and m) in the two asymptotic models were calculated (see Table 4.4). The order of mass loss limit values is: SW>SR>FW>FS>FR. Plant litters incorporated in spring took a shorter time to reach the limit values than plant litters incorporated in fall. It may be attributed to the warmer temperature in spring.
4.3.2 Nutrient Change Pattern during Litter Decomposition

According to Berg (1991) the limit value is also negatively related to initial litter N concentrations. The initial nitrogen contents of cover crops are given in Table 4.5. They are FS>SR>SW>FW>FR, which is not consistent with the order of mass loss limit value. It is probably because of the drought in 2012 that disturbed the normal decomposition pattern.

Figure 4.7 shows that the Nitrogen contents change for fall and spring incorporation, plant species have a significant effect on N concentrations (P-value=0.01 and P-value=0.04). Nitrogen concentration of soybean changes the most among the three crops. White clover in fall treatment decreased significantly in the first 20 weeks, and decreased rapidly in the first 8 weeks for the spring treatment. There were no significant changes in nitrogen concentrations for red clover in either spring or fall treatment.

Nitrogen contents of the three cover crops increased in the first four weeks, but the difference is not significant for clovers in spring incorporation (P-value=0.53 and P-value=0.25) (see Figure 4.7). N concentrations then decreased or trended to constant. For fall incorporation, the nitrogen concentrations of red clover and white clover at the last extraction time are greater than initial concentrations (P-value=0.0001 and P-value=0.042). Nitrogen concentrations increasing in decomposing litter is widely known (Berg and McClougherty, 2007). Former studies showed that when the increase in N concentration is related to time since incubation, the result is a curve with an asymptotic appearance. The increasing N concentration can be attributed to litter mass loss, resulting in a linear increase, possibly until the limit value for decomposition is reached (Aber and Melillo 1982; Berg et al. 1997).

The C/N ratio is accepted as a general index of quality (Seneviratne, 2000):
mineralization rates tend to decrease with increasing C/N ratio. The generally faster decay of N-rich litter suggests that litter decay rates would increase if their N content were increased, or would decrease if the N content declined. From Figure 4.8, C/N ratios of cover crops decreased rapidly during the first month of decomposition, except for soybean. This indicates a higher mineralization rate and a faster decomposition rate, which is consistent with the result from the decomposition study. For spring treatment, there’s no significant difference between clovers, while for fall treatment, the white clover has a lower C/N ratio.

Substrate quality can vary in the process of decomposition (see Figure 4.9 and Figure 4.10). The overall trends of soluble substrate content of cover crops decreased, and the lignin concentration increased. Berg and co-workers (2007) have shown that in the initial stages (0 to 3 months) of leaf breakdown, small soluble carbon molecules, like starches and amino acids, are lost first leaving behind the more recalcitrant molecules like lignin. Decomposition during this first phase is rapid because these molecules are easy to break down and energy rich. The second stage of decomposition (the breakdown of lignin) is much slower because lignin consists of very large and complex molecules. This rapid initial breakdown followed by a longer period of slow decomposition results in a mass loss curve that resembles an exponential decay curve (see Figure 2.1).

4.3.3 Temperature Effect on Decomposition

Temperature has been known to have impacts on plant residue decomposition rate in soil (Aerts, 1997). Honeycutt et al. (1988) used the concept “heat unit”, which combines effects of temperature and time, to describe carbon mineralization and predict net nitrogen mineralization. Heat units were used in other laboratory studies. For example, Miller (1974) use heat units to predict sewage sludge carbon
mineralization at temperature treatments reflecting both diurnal and seasonal variations; Andren and Pausian (1987) also found degree days to be useful in modeling field decomposition of barley straw.

Soil temperature was low (around 2°C) when the litter bags were placed in the field in the fall of 2011 and the degree days accumulated slowly at the beginning couple of months (see Figure 4.12). Beginning on day 75 after burial soil temperature increased and degree day accumulated linearly till day 250. There was a loss of 47 to 82% of litter during the first two sampling periods when soil temperatures were low and little biological activity is expected (see Figure 4.11 and Figure 4.13). The mass loss was likely due to a physical process where soluble material was leached by water. From initial fiber analysis, cover crops had 65 to 70% of soluble materials (see Table 4.5 Initial nutrients contents), which agrees with the amount of mass loss during this time period.

**4.4 Conclusion**

There is an interaction effect of cover crop species and incorporation timing on the decomposition rates. Decomposition rates of the five treatments follow the order: SW > SR > WF > FR > FS. Mass loss, nutrient content, and litter quality were all changed, mostly in the first 0-3 months, which indicates that the early stage of cover crops incorporation is critical to cover crop management. To describe the decomposition process, the asymptotic models were most appropriate. However, the models didn’t perfectly fit the data; it may be due to the lack of data in the beginning couple weeks, and the drought of 2012.
Table 4.1 Treatment design for litter bag study

<table>
<thead>
<tr>
<th>Burial Season</th>
<th>Plants</th>
<th>Treatment</th>
<th>Plot</th>
<th>Repetition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>Red Clover</td>
<td>FR</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fall</td>
<td>White Clover</td>
<td>FW</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fall</td>
<td>Soybean</td>
<td>FS</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Spring</td>
<td>Red Clover</td>
<td>SR</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Spring</td>
<td>White Clover</td>
<td>SW</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 4.2 ANOVA analysis of treatment and incorporation time as it affects the mass left percentage

<table>
<thead>
<tr>
<th>Class Level Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
</tr>
<tr>
<td>Season</td>
</tr>
<tr>
<td>Plant</td>
</tr>
<tr>
<td>Time</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
</tbody>
</table>

Number of Observations Read 719  
Number of Observations Used 656

<table>
<thead>
<tr>
<th>Type III Tests of Fixed Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Time</td>
</tr>
<tr>
<td>Time*Treatment</td>
</tr>
</tbody>
</table>
Table 4.3 Models used to describe the decomposition of litter

<table>
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<th>Formula</th>
<th>Comments</th>
<th>Characteristic</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_t = M_0e^{-kt}$</td>
<td>Single exponential</td>
<td>Leaves no residue</td>
<td>(Olson, 1963)</td>
</tr>
<tr>
<td>$Mt = A + Br^t$</td>
<td>Asymptotic</td>
<td>leaves a residue</td>
<td>(Howard and Howard, 1974)</td>
</tr>
<tr>
<td>$Lt = m(1 - e^{-kt/m})$</td>
<td>Asymptotic</td>
<td>leaves a residue</td>
<td>(Berg and Ekbohm, 1991)</td>
</tr>
</tbody>
</table>
Table 4.4 A comparison of decomposition parameters estimated in different models. $k$, $\Upsilon$, and $\beta$ are decompose constants in model $M_t = M_0 e^{-kt}$, $M_t = A + Br$, and $Lt = m(1 - e^{-kt/m})$. $A$ and $m$ are limit values in model $M_t = A + Br$ and $Lt = m(1 - e^{-kt/m})$.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FR</th>
<th>FW</th>
<th>FS</th>
<th>SR</th>
<th>SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decomposition Constants</td>
<td>$k$</td>
<td>27.00</td>
<td>39.00</td>
<td>15.00</td>
<td>64.00</td>
</tr>
<tr>
<td></td>
<td>$\Upsilon$</td>
<td>0.66</td>
<td>0.60</td>
<td>0.80</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>0.05</td>
<td>0.09</td>
<td>0.06</td>
<td>0.41</td>
</tr>
<tr>
<td>Limit Values</td>
<td>$A$</td>
<td>33.41</td>
<td>22.61</td>
<td>26.34</td>
<td>19.29</td>
</tr>
<tr>
<td></td>
<td>$m$</td>
<td>66.60</td>
<td>77.39</td>
<td>73.50</td>
<td>80.71</td>
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</tbody>
</table>
Table 4.5 Initial nutrients contents

<table>
<thead>
<tr>
<th></th>
<th>% Carbon</th>
<th>% Nitrogen</th>
<th>C/N</th>
<th>Soluble</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>42.36</td>
<td>3.86</td>
<td>10.97</td>
<td>68.49</td>
<td>4.07</td>
</tr>
<tr>
<td>SW</td>
<td>41.07</td>
<td>3.56</td>
<td>11.54</td>
<td>70.25</td>
<td>3.35</td>
</tr>
<tr>
<td>FR</td>
<td>42.14</td>
<td>1.92</td>
<td>22.10</td>
<td>79.53</td>
<td>3.75</td>
</tr>
<tr>
<td>FW</td>
<td>42.18</td>
<td>2.40</td>
<td>17.55</td>
<td>66.58</td>
<td>5.55</td>
</tr>
<tr>
<td>FS</td>
<td>44.58</td>
<td>4.15</td>
<td>10.74</td>
<td>65.14</td>
<td>4.57</td>
</tr>
</tbody>
</table>
Figure 4.1 Sampling plots on Google view
Figure 4.2 Remaining mass of red clover, white clover and soybean during the decomposition time (weeks) for fall incorporation. Data were expressed as percentage of the initial mater before burial. Bar indicates standard error.
Figure 4.3 Remaining mass of red clover and white clover during the decomposition time (weeks) for spring incorporation. Data are expressed as percentage of the initial material before burial. Bar indicates standard error.
Figure 4.4 Application of the single exponential model for different treatments. Plots a, b, c, d, and e show the changes of mass left for treatments FR, FW, SR, SW, and FS respectively. On each plot, Y-axis stand for mass left in percentage. X-axis stand for extraction time (weeks).
Figure 4.5 Application of the asymptotic exponential model \((M_t = A + Br^t)\) for different treatments. Plots a, b, c, d, and e show the changes of mass left for treatments FR, FW, SR, SW, and FS respectively. On each plot, Y-axis stand for mass left in percentage. X-axis stand for extraction time (weeks).
Figure 4.6 Application of the asymptotic exponential model \(Lt = m(1 - e^{-\frac{k_t}{m}})\) for different treatments. Plots a, b, c, d, and e show the changes of mass loss for treatments FR, FW, SR, SW, and FS respectively. On each plot, Y-axis stands for mass loss in percentage. X-axis stand for extraction time (weeks).
Figure 4.7 Nitrogen contents change after incorporated into the soil.

Nitrogen contents of red clover, white clover and soybean during the decomposition time (weeks) for fall treatment. Axis-Y stands for LS-Means of nitrogen concentration, the unit is N%. Axis-X stands for extraction time, the unit is week. Bar indicates standard error.

Nitrogen contents of red clover and white clover during the decomposition time (weeks) for spring treatment. Axis-Y stands for LS-Means of nitrogen concentration, the unit is N%. Axis-X stands for extraction time, the unit is week. Bar indicates standard error.
Figure 4.8 C/N ratio change after incorporation

C/N ratios of red clover, white clover and soybean after being incorporated in fall. Axis-Y stands for C/N ratio, axis-X stands for extraction time, and the unit is week. Bar indicates error.

C/N ratios of red clover, white clover after being incorporated in spring. Axis-Y stands for C/N ratio, axis-X stands for extraction time, and the unit is week. Bar indicates standard error.
Figure 4.9 Soluble substrates content changes after incorporation

Soluble substrates content of red clover, white clover, and soybean after being incorporated in fall. Axis-Y stands for soluble substrates content, the unit is soluble substrates%. Axis-X stands for extraction time, and the unit is week. Bar indicates standard error.

Soluble substrates content of red clover and white clover after being incorporated in spring. Axis-Y stands for soluble substrates content, the unit is soluble substrates%. Axis-X stands for extraction time, and the unit is week. Bar indicates standard error.
Figure 4.10 Lignin Content Changes after Incorporation

Lignin contents of red clover, white clover, and soybean after being incorporated in fall. Axis-Y stands for lignin content, the unit is lignin%. Axis-X stands for extraction time, and the unit is week. Bar indicates standard error.

Lignin contents of red clover and white clover after being incorporated in fall. Axis-Y stands for lignin content, the unit is lignin%. Axis-X stands for extraction time, and the unit is week. Bar indicates standard error.
Figure 4.11 Percentage of mass left as a function of time. Axis-Y stands for percentage of mass left, the unit is mass left%. Axis-X stands for extraction time, and the unit is week. Bar indicates standard error.
Figure 4.12 Degree days as a function of time for decomposition study. Axis-X stands for days after burial, axis-Y stands for accumulated degree days, the unit is °C.
Figure 4.13 Percentage of mass left as a function of degree day.
Nitrogen Release and Crop Uptake

5.1 Introduction

The major soil nutrient limiting crop production in upper Midwest corn-based cropping systems is nitrogen. Plant available forms of N are released through mineralization into the soil after application of fresh or composted animal manure and/or chemical fertilizer (Loecke, et al., 2012). Legumes in rotation can provide significant quantities of N to succeeding non-leguminous grain crops (Wheatley, et al., 1992). Possible causes of changes in N needs due to the inclusion of legumes in the corn rotation included: 1) contribution of legume N; 2) recycling of mineralized soil N; 3) interruption of pest cycles; 4) improved soil physical properties; 5) soil moisture effects; and 6) growth promoting substances introduced by legumes (Baldock, et al., 1981).

Nitrate is unique in the nitrogen cycle because it is soluble and can be moved through and away from the root zone by percolating water. However, if it does not move out of the root zone, it remains available for plant uptake. Ideally, corn uptake of N should be synchronized in space and time with availability of soil inorganic N in order to insure maximum crop productivity and minimal loss of N to the environment. That is, the extent to which the rates of N supply to crops match rates of crop demand for N. Nitrogen has the potential to accumulate in soils and is then susceptible to various loss pathways when crop demand for N and N supply do not synchronize (Peoples, et al., 1995).

In order to get a better understanding of the synchrony of cover crops nitrogen release with following corn nitrogen uptake. Soil tests were carried out at the same time as plant nutrient test. Soil nitrate-N were analyzed by WARD Laboratories (Kearney, NE) as an indicator of plant usable N. Our hypothesis was that cover crops
incorporated in spring provide a better synchrony between nitrogen release and corn nitrogen uptake.

5.2 Materials and Method

5.2.1 Soil sampling
Soil samples were taken while extracting litter bags using a soil sampling probe. For fall treatment, 8 batches of soil samples were taken on 27 February, 26 March, 23 April, 25 May, 22 June, 19 July, 17 August, and 18 October. For spring treatment, 7 batches of samples and soil samples were taken on 25 May, 8 June, 22 June, 19 July, 17 August, 18 September, 18 October, and 15 November. Three sampling points were chosen randomly for each plot and a 15cm soil sample was taken by using a soil sampling tube. Samples were then combined, air dried and sent to WARD Laboratory for N content determination by the FIA method (Ward 2011).

5.2.2 Soil properties test
The nitrate soil test measures the amount of nitrate left in the soil and available for the next crop. Nitrate-nitrogen is extracted out of the soil by water saturated with a calcium solution. Nitrate is very soluble so it can be extracted with water. Calcium is added to increase soluble calcium to flocculate soil clays, so a clear filtrate can be obtained. Nitrate is analyzed in the filtrate by the cadmium reduction procedure with a flow injection analyzer (FIA). Nitrate is quantitatively reduced to nitrite when passed through a copperized cadmium column. The nitrate is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm wavelength (Ward, 2011).
5.2.3 Data analysis
Soil data were analyzed using ANOVA to examine statistical differences between treatments. The treatment structure was 3*2 *8 (3 cover crops, 2 incorporated times, and 8 sampling times). The total number of experimental units was 48.

5.3 Results and discussion
From SAS analysis, plant species had no significant impact on soil nitrogen concentration (P-value=0.15), while incorporation season had a significant effect on soil nitrogen concentration (P-value<0.0001). At the beginning of the 2012 growing season (May 14, 2012), soil nitrate-N concentrations to 15 cm were higher for spring than fall incorporated plots of both white clover and red clover. Higher soil Nitrate-N concentrations at the beginning of the growing season following white clover and red clover were expected due to their N contributions as legumes and subsequent N availability to the succeeding corn crop. As the growing season progressed, soil nitrate-N concentrations following all previous crops declined and coincided with rapid corn growth uptake (Figure 5.1 and Figure 2.4). At the last sampling date, soil nitrate-N concentrations increased likely due to the combination of reduced corn uptake and continued soil N mineralization (Figure 5.2). At the end of growing season Nitrate-N was greater in the spring incorporated plots in the fall incorporated plots (Figure 5.2).

5.4 Conclusion
In conclusion, cover crops killed in spring had a better synchrony with corn uptake curve. This conclusion is consistent with former studies.

During years with normal to dry weather patterns, the best time to kill cover crops that has been reported is usually two weeks before planting cash crops
(depending on weather forecasts). Biomass yield and nitrogen production by legume cover crops may not be at their maximum levels at this point. However, in most seasons, sufficient rainfall for adequate crop emergence will occur during the two-week preplant period or within the week immediately following planting. In wet years or when a rainy period is forecast, the cover crop can be killed immediately before soil preparation and planting of spring crops.
Figure 5.1 Soil nitrate-N concentration after incorporate through the first 10 months of 2012 for different treatment.
Figure 5.2 Soil nitrate-N concentration after fall and spring incorporation.
6 General Discussion

6.1 Cover Crops Replacement for Chemical Fertilizer

The question of whether a fertilizer- or a legume-based approach has a higher potential of achieving synchrony between crop N demands and nutrient supply and/or is less susceptible to losses is not straightforward. Unfortunately there are only a limited number of studies where legume and fertilizer sources of N have been directly compared. These investigations have generally used $^{15}$N-labeled inputs which allow direct measurement of plant uptake and soil retention of the applied N, and provide indirect information about losses based on the amount of the applied $^{15}$N not recovered in either the plant or soil.

The period of potential greatest asynchrony and therefore periods of greatest risk of N loss in fertilized systems occurs after fertilization early in the growing season when levels of soil available N far exceed the crop’s capacity to utilize it. In order to exemplify asynchrony, Groffman et al. (1987) compared soil mineral N concentrations in a Georgia soil following fertilization with either a single application of ammonium nitrate or incorporation of a clover cover crop (Figure 6.1). Levels of soil available N from legume mineralization also increased early in the growing season, but substantial amounts of N remain either immobilized or in undecomposed residues.

6.2 Limitation of this study

There are a few shortcomings of this study that should be mentioned.

First, there’s no control experiment on filed without planting cover crops. So it is difficult to draw the conclusion that nitrogen change in soil was due to cover crops effects.
Second, soil samples in the soil nitrate test were taken in 15cm. While actually, the nitrogen release from cover crops has an impact on deep soil. 30cm soil should also be considered.

Third is the lack of study on corn nitrogen uptake. I simply used a generally corn nitrogen uptake curve in study. However, a study on corn nitrogen concentration at different growth stages will show a clearer synchrony relationship between cover crops nitrogen release and corn nitrogen uptake.
Figure 6.1 Comparison of soil mineral N content in fertilizer system with cover crop system. Soil mineral N (NH$_4$-N plus NO$_3$-N) over 0-21 cm from fertilizer (square symbols, solid lines) or clover residues (diamond symbols, dashed lines) under (a) conventional and (b) no-tillage systems. The symbol* indicates significance at p = 0.05. (Crews and Peoples, 2005)
7 Reference


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