

ABSTRACT

SHANGTAO LIANG. Short-term Effects of Legume Winter Cover Crop Management on Soil Microbial Activity and Particulate Organic Matter. (Under the direction of Dr. Wei Shi).

Legume cover crops have been increasingly used in organic farming systems because, as an organic C and N source, they can provide plant available N, raise soil organic matter, and thus improve soil fertility and quality. Despite benefit certainty, the magnitude of cover crop effects often varies with management practices. Rapid development of legume cover crop effects is particularly important for organic transition systems where soil quality needs to be enhanced in a short period. In this regard, however, it is still not clear which cover crop management practices are better. The objectives of this study were to (1) investigate the short-term impacts of cover crop species and termination methods on soil microbial properties and processes, and (2) evaluate if soil enzyme activity and particulate organic matter can be used as early indicators of cover crop management effectiveness.

Soil samples (0-15 cm depth) were taken from two newly-established study sites located in Kinston and Goldsboro, North Carolina, each having 12 treatments that were the combinations of three termination methods (disk, flail, and spray) and four cover types (no cover crop, Austrian winter pea [*Pisum sativum*], hairy vetch [*Vicia Villosa*], and crimson clover [*Trifolium incarnatum*]). Objective 1 was performed mainly on soil samples collected from the Kinston site, and measurements were made on soil microbial biomass, C and N mineralization, nitrification potential, and the activity of enzymes (exoglucanase, β -glucosidase, and β -glucosaminidase). Objective 2 was conducted with soil samples from the Goldsboro site and measurements included the C and N content of particulate organic matter obtained by soil density fractionation and the activity of soil enzymes (exoglucanase,

β -glucosidase, β -glucosaminidase, and peroxidase). Short-term cover crop effects were only shown on nitrification potential and the activity of β -glucosidase and β -glucosaminidase, with Austrian winter pea showing the most positive influences. Cover crops also modified the quality of soil particulate organic matter, with C-to-N ratio the lowest in Austrian winter pea. Flail method prevailed over disk and spray in improving soil microbial properties and processes. Microbial biomass C, C and N mineralization, and nitrification potential were greater in flail than in disk and spray by ~ 17%, 25%, 16% and 36%, respectively. However, flail led to ~25% lower particulate organic matter compared to disk method. Nonetheless, significant positive correlations were found between soil enzyme activities and soil process rates, i.e., C and N mineralization and nitrification, whereas negative relations existed between soil enzyme activities and particulate organic matter content.

The results indicated that the location and size of cover crop residues affected the rapidness of cover crop effects. Furthermore, cover crop species with low C-to-N ratio and low lignocellulose index, i.e., Austrian winter pea, was proved to be better for rapid development of cover crop effects than crimson clover and hairy vetch. Although soil particulate organic matter varied quantitatively and qualitatively with the species and termination method of cover crops, it should not be used to imply cover crop effects on soil C sequestration. Instead, changes in particulate organic matter reflected the degradability of cover crop residues. Overall, β -glucosidase and particulate organic matter can be used to indicate short-term effects of cover crop management on soil microbial properties and processes.

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Short-term Effects of Legume Winter Cover Crop Management on Soil Microbial Activity
and Particulate Organic Matter

by
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DEDICATION

To my parents, Xiaoju Peng and Guanghui Liang

BIOGRAPHY

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
Chapter 1: Transition to Organic Farming: Challenges, Management Strategies, and Indicators of Success	1
1. Challenges in organic transition stage	1
2. Legume cover crops	3
3. Soil microbial properties	7
4. Light- and heavy-fraction organic matter	10
5. Research objectives	12
6. References	14
Chapter 2: Soil Microbial Responses to Winter Legume Cover Crop Management during Organic Transition	27
Abstract	27
1. Introduction	28
2. Materials and methods	32
2.1. Study site and soil sampling	32
2.2. Cover crop sampling and chemical analysis	33
2.3. Soil microbial biomass, C and N mineralization, and nitrification potential	34
2.4. Soil enzyme activity assays	36
2.5. Data analysis	37
3. Results	38
3.1. Soil microbial properties as affected by the cover crop termination methods	38
3.2. Soil microbial properties as affected by the cover crop application	39
3.3. Soil microbial properties as affected by cover crop quality	39
3.4. Correlations of soil enzyme activities with soil C and N processes	40
4. Discussion	40

4.1. The impact of termination methods on soil microbial activity	41
4.2. The impact of cover crop species on soil microbial activity	42
4.3. Microbial indicators of soil quality	43
4.4. Practical implications	45
References	47
Tables and figures	55
Chapter 3: Soil Particulate Organic Matter as Related to Short-term Legume Winter Cover Crop Management	61
Abstract	61
1. Introduction	61
2. Materials and Methods	64
2.1. Study site and soil sampling	64
2.2. Soil density fractionation	65
2.3. Carbon and nitrogen measurement	66
2.4. Statistical data analysis	66
3. Results	67
3.1. Soil light fraction C and N as affected by cover crop species and termination methods.....	67
3.2. Soil light fraction C-to-N ratio	68
3.3. Relationship between soil light fraction C content and enzyme activities	68
4. Discussion	68
4.1. The impact of termination methods	69
4.2. The impact of cover crop species	71
4.3. Implications	72
References	73
Tables and figures	81
Chapter 4: General Conclusions and Future Research Direction	87

LIST OF TABLES

Chapter 2

Table 1. Yield and biochemistry of three legume cover crops in the Kinston and Goldsboro sites.	55
Table 2. Soil microbial biomass, mineralization, and nitrification potential following the application of different cover crops in the Kinston site.	56
Table 3. Soil enzyme activities following the application of different cover crops in the Kinston and Goldsboro sites.	57

Chapter 3

Table 1. Mass percentage and C, N contents of soil light fraction (LF) and heavy fraction (HF) in three cover crop treatments and no cover crop control.	81
Table 2. Carbon, N input and C mineralization to N mineralization ratio of different termination methods and cover crop species.	82

LIST OF FIGURES

Chapter 2

Fig. 1. Soil microbial properties as affected by three termination methods in the Kinston site. 58

Fig. 2. Soil enzyme activities as affected by three termination methods in the Kinston and Goldsboro sites. 59

Fig. 3. Principal component analysis (PCA) showing the separation of cover crop species and the contribution of microbial properties. 60

Chapter 3

Fig. 1. Mass percentage and C, N contents of soil light fraction (LF) and heavy fraction (HF) in three termination methods. 83

Fig. 2. Soil light fraction C-to-N ratio in three termination methods. 84

Fig. 3. Soil light fraction C-to-N ratio in cover crop treatments. 85

Fig. 4. Correlations between soil light fraction C content and extracellular enzyme activities. 86

Chapter 1: Transition to Organic Farming: Challenges, Management Strategies, and Indicators of Success

1. Challenges in making successful transition from conventional to organic farming

1.1. Organic farming benefits

Organic agriculture has been acknowledged as an environmentally sound production system, due to significant exclusions of synthetic fertilizers, pesticides, herbicides, growth regulators, and livestock feed additives (USDA, 1980). It aims to minimize environmental pollution, enhance ecological harmony, and maintain the health of soil, plants, animals, and human (USDA, 1995). Ever since the establishment of Organic Foods Production Act (OFPA) (USDA, 1990), which set a national standard for organic production, organic foods industry has experienced a dramatic growth in the United States. With increasing consumer demand, retail sales of the U.S. organic foods reached \$21.1 billion in 2008 from \$3.6 billion in 1997, and the organic farmland acreage was more than doubled from 1997 to 2005 (Dimitri and Oberholtzer, 2010). Farmers are willing to switch to organic farming for its economic profits as well as environmental benefits. It was reported that the number of organic growers was increasing by about 12% every year in the United States (Rigby and Cáceres, 2001).

1.2. Challenges facing organic transition

The market booming of organic foods industry has encouraged farmers to devote into organic farming. However, farmers often struggle for profits because yields of organic farming are generally lower than those of conventional farming (Dimitri and Oberholtzer, 2010). On average, yield reduction is about 20-25%, although variations exist due to different crops and N fertilization rates for the systems to be compared (De Ponti et al., 2012; Graziani et al., 2012; Maggio et al., 2008; Terho et al., 2003; Thorup-Kristensen et al., 2012). This yield gap has been attributed to the disuse of synthetic chemicals in organic farming. Without herbicide and pesticide applications, weeds and pests in organic farming may become more diverse and abundant compared to conventional farming. This can be an issue for weed and pest controls (Graziani et al., 2012; Terho et al., 2003), although some studies suggest that the diversity increase can be beneficial to weed and pest controls as it helps boost the competition among weeds as well as the population of predators and parasitoids (Birkhofer et al., 2008; Macfadyen et al., 2009). The deficiency of nutrients, especially nitrogen, is another issue for sustaining yields during the first few years of organic farming (Clark et al., 1999). This is because nutrients in soil organic matter as well as in organic amendments, such as manure, are slowly released and thus nutrient supplies can be far less than the nutrient requirements of high crop yields. As a consequence, soil organic matter in organic farming must reach and be maintained at a level, above which nutrient supplies via mineralization are comparable to those from synthetic fertilizers. Certainly, time is an important factor for soil organic matter accumulation.

A minimum of 3 years is required for organic transition from conventional farming systems; during the transition, no prohibited substances can be applied and no produce can be sold as organic goods (USDA, 1990). This is the period most difficult to organic growers. First, farmers' incomes are likely dropped due to low yield and non-certified organic produce. Second, farmers need to develop efficient management strategies to overcome soil nutrient deficiency and weed and pest outburst, which are often associated with organic farming following disuses of synthetic fertilizers, herbicides, and pesticides (Tu et al., 2006). Farmers may also need to find ways to rapidly increase soil organic matter content so that crop productivity can be sustained over time. Although soil organic matter can be built up via annual inputs of animal wastes and crop residues, this process usually takes a long time for impacts to be seen (Carey et al., 2009; Wang et al., 2011).

2. Legume cover crops for soil C and N management in organic transition

2.1. Cover crops for sustainable agriculture

Grasses, legumes, and forbs that are planted to offer seasonal cover for the arable soil, are referred to as cover crops (USDA, 2009). Based on the season for soil cover, they are further divided into summer and winter cover crops. The primary function of cover crops is for soil and water conservation. By reducing nutrient runoff, protecting water quality, and enhancing biodiversity, cover crops are of environmental and ecological significance. In addition, cover crops provide many other benefits, such as nutrient supply, source of soil organic matter, pest control, and weeds suppression. Given multifaceted advantages, cover

crops have been widely used as a good management practice for sustainable agriculture. The acreage of agricultural land managed with cover crops keeps increasing in the United States. In Maryland, for example, more than 414,000 acres of cropland has been planted with cover crops in 2010. In Michigan, 1.1 million acres of agricultural land has been planted to cover crops in 2011.

2.2. Cover crops for soil organic matter buildup and N supply

Via annual biomass input, cover cropping can enhance the concentration and also modify the biochemistry of soil organic matter. A 5-year field study conducted in Georgia, USA, showed significantly higher soil carbon concentration under three winter cover crop treatments (i.e. rye, hairy vetch, and crimson clover) than no cover crop control (Sainju et al., 2002). And such effects have been found to depend on cover crop species and tillage practices (Steenwerth and Belina, 2008). It is widely accepted that no-till, together with cover cropping is a good management practice for carbon storage, since it slows down soil organic matter turnover by reducing disturbance. No tillage practice was found to enrich soil organic C by $0.35 \text{ Mg C ha}^{-1}$ than conventional tillage under cover crop systems in a dryland rice production field (Metay et al., 2007). Besides total amount of organic C, the quality of soil organic matter was also reported to vary under different cover crop treatments (Ding et al., 2006). Compared to the long-term effect of cover crop on soil organic C, which can be reflected by quantity change in bulk soil, the short-term impact is more detectable via physical fractionation of soil organic matter (Nascente et al., 2013). Thus, the distribution of

organic C in soil fractions may be a better approach to evaluating short-term cover crop management. Legume plants can fix N₂ through rhizobium symbiosis, by which rhizobia provide N for host plants through N₂ reduction to ammonia, whereas rhizobia acquire organic carbon from the hosts (Atkins, 1984). Thus, legume cover crops, typically high in N content, are utilized as an efficient strategy to increase soil N supply, especially in N deficient soils. With similar N contents ~30-40 g N kg⁻¹ but yields varied with soil types, cropping systems and climate conditions, legume cover crops generally offer 150-350 kg N ha⁻¹ yr⁻¹ (Blevins et al., 1990; Decker et al., 1994; Holderbaum et al., 1990). However, due to asynchrony between N mineralization and plant N uptake, N loss via leaching, microbial N assimilation, and low plant N use efficiency, less than 40% of legume plant N was recovered by subsequent non-legume crops (Harris et al., 1994; Hesterman et al., 1987). Despite limited N recovery, legume cover cropping could still significantly increase corn yields (Fleming et al., 1981). And this N contribution made by hairy vetch in a no-till corn production system was calculated to be equal to 90 to 100 kg ha⁻¹ fertilizer N (Ebelhar et al., 1984).

2.3. Cover crop management practices

Good cover crop management often results in high subsequent main crop yields and, in the long term, better soil quality. Cover crop species, seeding rate and method, fertilization, tillage, termination strategy, and cost are the major considerations for managing cover crops. The selection of cover crop species should be made based upon better adaptation and growth potential under local soil and climate conditions (Power and Zachariassen, 1993). When

legume cover crops are applied as the N source for following crops, cover crop properties (e.g., N percentage, C-to-N ratio, lignin content, and biomass), which determine the plant available N, are often regarded as the primary criteria for choosing legume species (Salmeron et al., 2011). Besides, competitive ability to weeds (Den Hollander et al., 2007) and pathogen resistance are important criteria as well, especially for organic systems where weeds and pests are mainly controlled by natural means. Higher seeding rate could stimulate higher cover crop dry mass, increase N availability, reduce N leaching, and suppress weed invasion; however, excessive seeding can cause harsh competition and be costly (Boyd et al., 2009; Brennan and Boyd, 2012; Brennan et al., 2009). Generally, broadcast seeding performed better in increasing cover crop biomass than no-till drill, although the effect was depended on climate conditions (Fisher et al., 2011). While fertilization is an optional management practice for cover cropping, phosphorus, potassium, and sulfur are often applied for conventional cover cropping systems to help increase biological N-fixation efficiency of legumes based on soil test results (Engels et al., 1995; Scherer and Lange, 1996).

Termination of cover crop is the last step of management that needs to be considered before cash crop planting. Basically, a good termination strategy includes suitable termination time and efficient termination methods. And this process is important as it determines the subsequent effects of cover crop residue amendments on following crops. Different termination times mainly mean different time intervals between dried cover crop returning and cash crop planting. It is hard to determine the right termination time because of a tradeoff between N accumulation and mineralization of cover crops. A shorter period between cover

crop termination and cash crop planting means a longer period for cover crop growth and thus N fixation and accumulation. In contrast, a longer period may result in less N fixation and accumulation in cover crops, but more available soil N through longer period of mineralization (Vaughan and Evanylo, 1998; Waggoner, 1989). Termination method refers to any mechanical operation process of killing cover crops and returning them back to fields. A killing method using synthetic herbicides after rolling was reported to reach a termination rate up to 90%, which was 10% higher than using physical means alone (Kornecki et al., 2012). However, synthetic herbicides retained in soil may inhibit soil microbial activity, and their application is often restricted from organic farming systems. Termination methods also affect the size of cover crop residues and thus the surface area for microbes to attach and function. Cover crop residues can be chopped into small pieces or remain intact. After termination, cover crops can be placed either on the surface or incorporated into soil. Surface spreading is good for maintaining soil moisture and reducing temperature fluctuation (Acharya et al., 1998; Gicheru, 1994; Murungu et al., 2011; Ramakrishna et al., 2006; Sarkar et al., 2007; Sarkar and Singh, 2007; Varadan and Rao, 1983).

3. Soil microbial properties as indicators of successful agricultural management

3.1. Soil microbial community functions for sustainable agriculture

Active soil microbes are crucial to plant nutrient uptake, as they regulate nutrient supply to as well as nutrient use efficiency of plants. Via microbial decomposition, nutrients in crop residues and soil organic matter are converted into inorganic compounds and/or soluble

organic compounds that can be directly assimilated into plant and microbial biomass (Swift et al., 1979). Net N mineralization, which reflects soil N availability to plants, has been found to be positively related to the biomass of soil microbial community (Booth et al., 2005). However, soil microbes may also compete with plants for available nutrients, specifically when nutrients are limiting (McGuire and Treseder, 2010; Van Der Heijden et al., 2008).

Active microbes also dictate C sequestration in soil. On one hand, microbes degrade plant materials, leading to CO₂ evolution. On the other hand, C assimilated into microbial biomass may be stabilized in soil. The net gain of soil C is the balance between microbial degradation and C assimilation. This microbially-mediated soil C turnover depends on plant material biochemistry and also soil microbial community structure. Microbial community with fungal dominance has been proposed to be more favorable for soil C sequestration (Six et al., 2006). Soil microbes also contribute to soil C sequestration via facilitating the formation and stabilization of aggregates, which act as physical protection of soil organic matter (Tisdall and Oades, 1982).

Microbial involvement in the formation and stabilization of aggregates is imperative for achieving good soil structure for crop production. Fungal hyphae and microbial biofilms can bind soil particles together and form micro-aggregates (Rillig et al., 2010; Six et al., 2006). As a consequence, soil physical properties can be substantially modified, including stability against physical disruption, porosity, hydraulic conductivity, and water repellency (Hallett and Young, 1999; Young et al., 1998). It should be noted that population as well as microbial

diversity are equally important for functional microbial community (Van der Heijden and Wagg, 2013).

3.2. Microbial indicators of soil quality

Soil quality, also referred to as soil health is defined as “the capability of soil to function”. In practice, however, soil function of interest varies with different uses of soil, e.g., crop production, urban construction, waste and hazardous material remediation, and carbon sequestration. In agroecosystems, high quality and healthy soil must be able to sustain biological productivity as well as maintain environmental quality (Doran and Zeiss, 2000; Karlen et al., 1997). Actively involved in nutrient cycling, organic matter turnover and soil structure development, soil microbes have been highlighted as a crucial component of the evaluation system for soil quality (Bending et al., 2004; Jordan et al., 1995; Kennedy and Papendick, 1995).

As indicators of soil quality, microbial properties show high sensitivity to land use change and management practices, and therefore are superior to soil physical and chemical properties for assessing short-term impacts (Bending et al., 2000; Jackson et al., 2003). Several biological parameters are commonly used, including microbial biomass, microbially-mediated soil processes, such as mineralization and nitrification, and soil enzyme activities (Schloter et al., 2003). Due to its simplicity and rapidness, enzyme activity measurement has gained increasing popularity (Alkorta et al., 2003). However, its specificity adds challenge when choosing a suitable enzyme as the indicator of soil quality. This is

because several enzymes are often involved in one specific soil process and yet respond differently to management practices (Trasar-Cepeda et al., 2008a; Trasar-Cepeda et al., 2000). Hence, it is recommended to use a minimum set of microbial properties to assess soil quality under various management practices (Alkorta et al., 2003; Schloter et al., 2003; Trasar-Cepeda et al., 2008b; Trasar-Cepeda et al., 2000).

4. Light fraction organic matter as the robust measure of agricultural management

4.1. Fractionation of soil organic matter

Soil organic matter is the focal point when assessing soil quality and health, because of its role in maintaining soil structure, as well as the energy source for soil organisms and the sink of atmospheric CO₂. In general, soil organic matter is made up of a very large amount of humic substances and a small amount of “*labile organic matter*” comprised of fresh debris and readily degradable materials. Unlike humic substances that are fairly resistant to microbial degradation, labile organic matter turns over quickly, on time scales of months to a few years and therefore is able to respond to management and land use change timely. However, changes in labile organic matter cannot be easily detected on the basis of total soil organic matter due to dilution by humic substances (Haynes, 2005). Therefore, efforts have been made to separate labile and recalcitrant fractions of soil organic matter, according to differences in size or density. Size fractionation is a physical method that separates aggregates-occluded and mineral-associated organic matter based on the diameter and water stability of aggregates (Six et al., 2002). Alternatively, density fractionation separates light

fraction of soil organic matter from the heavy fraction using a high density liquid (1.5 g cm^{-3} – 1.7 g cm^{-3}), such as sodium iodide, sodium hexametaphosphate, or sodium polytungstate (Ford et al., 1969; Gregorich and Ellert, 1993; Strickland and Sollins, 1987; Tan et al., 2007). In this case, the light fraction is collected as floating materials and the rest as the heavy fraction. Although different cut-off values have been used to separate light and heavy fractions of soil organic matter, a density of 1.6 g cm^{-3} is proved to be the best in terms of the recoveries of soil mass and C and N concentrations (Cerli et al., 2012). Method modification has also been developed to minimize possible effects of chemical dispersion on soil biological activities (Allison and Jastrow, 2006).

4.2. Important measures of soil fractionation and their potential as early indicators

Following fractionation, several parameters are often measured, including the concentration of organic C and N, C-to-N ratio, mineralization, microbial activity and mass distribution between the light and heavy fraction. These measures have been used to examine the impacts of management practices on the quality of soil organic matter and the potential of soil C sequestration (Barrios et al., 1997; Bending and Turner, 2009; Blackwood and Paul, 2003; Boone, 1994; Compton and Boone, 2002; Sollins et al., 1984; Song et al., 2012). Generally, soil light fraction-C and -N are higher in continuous cropping systems than in crop rotation systems with the high frequency of fallow (Bremer et al., 1994; Janzen et al., 1992). Tillage practice reduces organic C content in both bulk soil and light fraction since intense physical disturbance exposes aggregate protected organic matter to decomposition

and thus stimulates organic matter turnover (Larney et al., 1997; Tan et al., 2007). Upon conversion from tillage to no till approaches, however, the increase in organic C is usually in the light fraction, suggesting that light fraction is most dynamic (Tan et al., 2007). This rapid response of light fraction also appears to other management practices, land use change and potential global warming (Song et al., 2012; Bending and Turner, 2009; Tan et al., 2007; Malhi et al., 2003; Conti et al., 1997). Bending and Turner (2009) showed that increases in light fraction C and N contents could be detected 112 days after soil amendment with plant residues. Nitrogen fertilization-enhanced soil C accumulation was found to be much greater in light fraction than in bulk soil. As the small and reactive pool of C and N, light fraction can be used to examine the early impacts of land use and management practices. Light fraction C content has been found to correlate well with soil respiration, as well as light fraction N content with soil N mineralization (Alvarez, 1998; Janzen et al., 1992). This indeed indicates that light fraction properties are sensitive indicators for early detecting management impacts. However, disagreement exists over this issue. Leifeld and Kögel-Knabner (2005) showed that total soil organic C rather than light fraction C was sensitive to land use change. Bending et al. (2000) also showed that light fraction C and N was insensitive to different cropping systems.

5. Research objectives

This study aims to address the question: If legume winter cover crop management can be an efficient strategy to improve soil N supply and help organic matter buildup during

organic transition. Although input legume winter cover cropping can improve soil quality over the long time via annual biomass contribution, its short-term impacts remain unclear. In this study, soil microbial activity and light fraction of soil organic matter are used to assess cover crop species and termination methods. I expected my results would help make informed management decisions regarding legume winter cover cropping. In addition, soil microbial properties proved to be sensitive in this study could be applicable to short-term evaluations for other agroecosystems.

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Chapter 2: Soil Microbial Responses to Winter Legume Cover Crop Management during Organic Transition

Abstract

Legume cover cropping has been widely used as an efficient strategy to improve soil quality and also to supply nutrients for following cash crops. Although this management practice is important to resolve N deficiency for the transition from conventional to organic production systems, it needs to be optimized, specifically pertaining to the choice and termination method of legume cover crops. This study aimed to use soil microbial properties and processes to evaluate the suitability of common legume cover crops and termination methods for organic transition in the southeastern US. Soil samples (0-15 cm depth) were taken from two newly-established study sites, each containing 12 treatments including combinations of three termination methods (disk, flail, and spray) and four cover types (no cover crop, Austrian winter pea [*Pisum sativum*], hairy vetch [*Vicia villosa*], and crimson clover [*Trifolium incarnatum*]). Compared to disk and spray, flail mowing significantly increased soil microbial biomass C by ~17%, C mineralization by ~25%, N mineralization by ~16%, and nitrification potential by ~36%. However, cover crops only stimulated nitrification potential compared to no cover crop treatment. Furthermore, the activities of soil enzymes (exoglucanase, β -glucosidase, and β -glucosaminidase) appeared to be more responsive to cover types than to termination methods. Among three cover crops, Austrian winter pea showed the greatest positive effects on nitrification potential, β -glucosidase, and

β -glucosaminidase. Microbial qCO_2 also differed with cover types, being lowest in Austrian winter pea. Our results indicated that legume species even with small variations in C-to-N ratio and lignin and cellulose contents could cause significant divergence in soil microbial properties and processes. Nitrification potential, representing the function of a small group of soil microbial community, was proved to be sensitive to both legume species and termination methods.

1. Introduction

In recent decades, organic farming has received a considerable amount of public attention due to its benefits in natural resources and healthy foods. In the United States, certified organic farmland has steadily increased and organic product sales continue to grow. When farmers decide to switch to organic farming, a minimum of 3-year transition is required before the product can be certified as organically grown (USDA, 1990). During this period, farmers often face great challenges in maintaining soil fertility, controlling pests and weeds, and sustaining yields after the disuse of synthetic chemicals (Zinati, 2002). While cover cropping has showed promising results to tackle some of these issues (Katsvairo et al., 2007), its effectiveness varies with management practices, in part pertaining to the choice of species and termination method of cover crops.

Generally, cover crops are chosen based on their adaptation to local climate and a range of benefits they provide. Due to their ability to fix atmospheric N, legumes have been widely applied in agroecosystems (Blevins et al., 1990; Salmeron et al., 2011). Hairy vetch, for

example, could provide equivalent to 90 to 120 kg ha⁻¹ of fertilizer N to succeeding corn in a no-tillage system (Ebelhar et al., 1984). Legumes can also help to enrich surface soil organic matter (Cadisch et al., 1996). However, such benefits may highly depend on legume species. This is because the chemical quality of plant material, often measured as the C-to-N ratio and lignin-to-lignin plus cellulose ratio (i.e., the lignocellulose index), has been deemed as the major factor for determining the magnitude and rate of the decomposition of plant materials. Normally, plant materials with low ratios of C-to-N and lignin-to-lignin plus cellulose contain more readily-degradable compounds and thus will decompose faster than those with high ratios (Carreiro et al., 2000; Cotrufo et al., 1995; Gholz et al., 2000; Melillo et al., 1982). Compared to non-legume cover crops, legumes generally have higher N contents and therefore lower C-to-N ratios. Thus legumes can decompose more rapidly than non-legume cover crops (Murungu et al., 2011a).

The method of cover crop termination can also affect the decomposition of cover crops. Surface-applied cover crops killed by effective burndown herbicides or flail mowers may keep soil temperature and moisture more appropriate for microbial activity and residue decomposition than cover crops incorporated into the soil (Garcia et al., 2008; Havstad et al., 2010; Moore, 1986; Van Meeteren et al., 2007). By exposing more surface area to soil microbes, water and nutrients, chopped cover crops may decompose faster than the cover crops kept intact. This impact of residue size has been found to vary with the residue quality, i.e., C-to-N ratio and also the phase of decomposition (Angers and Recous, 1997; Bending and Turner, 1999). At the early phase, the decomposition rate is enhanced by small residue

size, regardless of residue quality. At the late phase, however, the relationship between the decomposition rate and residue size is residue quality-specific. For residues with low C-to-N ratios (<20), such as green rye and brussels sprout shoot, decomposition rates were positively related to residue size (Angers and Recous, 1997; Bending and Turner, 1999). For residues with high C-to-N ratios, such as wheat straw, decomposition rates were negatively related to residue size at the late phase (Angers and Recous, 1997). These observations suggested interactive impacts between residue quality and size on residue decomposition and nutrient cycling.

Active microbial community represents the imperative component of healthy and productive soil. Because of high sensitivity to environmental changes and a tight correlation with soil and ecosystem functions, soil microbial properties have been considered as the indicators of soil fertility and quality (Bending et al., 2004; Doran and Zeiss, 2000; Fauci and Dick, 1994; Gil-Sotres et al., 2005; Karlen et al., 1998). In comparison with soil physicochemical properties, microbial ones appear to be better, specifically for the early evaluation of management and land use change (Garcia et al., 2008; Raiesi, 2007; Sicardi et al., 2004; Zagal et al., 2009). In this regard, microbial biomass, C and N mineralization, and soil enzyme activity are often used. It has been reported, however, that the sensitivity of microbial properties can vary with management, soil type, and even parameters used (Bandick and Dick, 1999; Biederbeck et al., 1998; Garcia et al., 2008; Werner, 1997). For example, the potential rate of N mineralization was found to be more sensitive than C mineralization in evaluating the impacts of annual legume addition to a fallow-wheat system

(Biederbeck et al., 1998). Apparently, a set of microbial properties need to be used together to reliably evaluate management practices

In the southeastern USA, winter legume cover cropping has been adopted into many organic production systems, specifically during the transitional period. While organic growers can choose from several popular legume species (Austrian winter pea [*Pisum sativum*, AP], hairy vetch [*Vicia villosa*, HV], and crimson clover [*Trifolium incarnatum*, CC]) and termination methods (flail, disk, and spray) in the region, the guideline is solely based on aboveground biomass as well as ease and cost of termination. This study aimed to help improve the evaluation criteria by using soil microbial properties and processes. The short-term impacts of legume species and termination methods were, therefore, examined on soil microbial biomass C, C and N mineralization, nitrification potential, and the activities of exoglucanase, β -glucosidase, β -glucosaminidase, and peroxidase. We hypothesized that soil microbial properties would be enhanced following one-year application of legume cover crops. However, this enhancement might be independent of legume species because the C-to-N ratios of legume species usually vary within a very narrow range. Furthermore, termination methods would affect soil microbial properties via controls on soil environmental conditions and residue size. We expect that our results can help organic growers to make informed decisions on the management of winter legume cover crops.

2. Materials and methods

2.1. Study site and soil sampling

Soil samples were taken from two study sites that were newly established for examining the impacts of cover crop species and termination methods on improving soil organic matter as well as N fertility. The study site on sandy loam (siliceous, termic Aquic Hapludults) soil was established in 2010 at the Kinston Research Station, Kinston, NC, and contained 24 treatments, representing the combinations of four termination methods (flail, roll, disk, and spray) and six types of N application (no N, N at 150 kg ha⁻¹ as urea and ammonium nitrate, and four species of legume cover crops: Austrian winter pea [*Pisum sativum*], hairy vetch [*Vicia villosa*], crimson clover [*Trifolium incarnatum*] and balansa clover [*Trifolium balansae*]). The same termination methods were used at the study site of loamy sand (mixed, termic Typic Hapludult), established in 2011 at the Center for Environmental Farming Unit, Goldsboro, NC, but N application types were reduced to four, i.e., no N and three legume cover crops: Austrian winter pea, hairy vetch, and crimson clover. Treatments at both study sites were replicated six times and arranged in fields via a split-plot design, with termination methods as the main plots and N application types as the sub-plots. Both study sites had similar planting and termination/harvest schedules. Legume cover crops were planted in late fall, grew over winter, and then were terminated in the following spring. Thereafter, corn was planted in May and harvested in fall.

In the present study, three legume cover crops (Austrian winter pea, hairy vetch, and crimson clover) and three termination methods (flail, disk, and spray) were used for

investigating the effects of termination methods and N application types on soil microbial properties and processes. For flail treatment, cover crops were cut down and chopped using a 1.8-meter flail mower (John Deere Model 370, Moline IL). For spray, cover crops were terminated via a spray of certificated organic herbicide (Burn OutII, 4.4% final concentration clove oil, St. Gabriel Organics, Orange, VA) and then left on soil surface. For disk treatment, cover crops were terminated, chopped, and incorporated into ~15 cm soil depth by using a 3.65-meter disk (John Deere Model 225, Moline IL). Soil samples were collected from three randomly selected replicate plots, twice (i.e., one and 12 weeks after cover crop termination) at the Kinston site in 2011 and five times (i.e., one, two, four, ten, and 12 weeks after cover crop termination) at the Goldsboro site in 2012. Six soil cores (2.5 cm dia. × 15 cm depth) were taken randomly from each field plot and then combined to form a composite soil sample. We also collected soil samples from the no-N treatment to evaluate if cover crops had positive effects on soil microbial properties and processes. Soil samples were transported to the laboratory in a cooler, passed through a 2 mm-mesh sieve, and stored at 4 °C for later use.

2.2. Cover crop sampling and chemical analysis

Aboveground biomass was also sampled from each field plot immediately after cover crop termination at both study sites. Plant materials were then oven-dried at 60 °C, passed through 0.1 mm sieve, and analyzed for total C and N contents via a dry combustion method using a CHN Elemental Analyzer (Perkin Elmer 2400 CHN Elemental Analyzer, Norwalk,

CT). Plant materials were also analyzed for cellulose and lignin contents using a HPLC chromatograph (Dionex ICS 2500 system, Sunnyvale, CA) (Davis, 1998; Pettersen, 1991).

Cover crop yields and chemical properties are given in Table 1.

2.3. Soil microbial biomass, C and N mineralization, and nitrification potential

Soil samples at the Kinston site were measured for microbial biomass C, C and N mineralization, and nitrification potential. Microbial biomass C was determined by the chloroform fumigation extraction method (Vance et al., 1987). After 24 h fumigation, each soil sample (15 g moist weight) was made into slurry with 50 ml of 0.5 M K_2SO_4 , shaken for 30 min. at $\sim 200 \text{ rev. min}^{-1}$, and then filtered through a Whatman #42 filter paper.

Non-fumigated soil samples were also subjected to the same procedure of 0.5 M K_2SO_4 extraction. Total C in solution was measured using a total C analyzer (TOC-5000, Shimadzu, Kyoto, Japan). Microbial biomass C was calculated from the differences between fumigated and non-fumigated soil samples using an extraction coefficient of 0.38.

Soil C and N mineralization was determined via a two-month incubation at $23 \pm 2 \text{ }^\circ\text{C}$. A 1-L Mason jar was used as an incubation unit that contained six scintillation vials, each having 15g soil at 50% of water holding capacity. A scintillation vial containing 5 ml of 0.5 M NaOH was also placed into the Mason jar to trap CO_2 released from soil. Furthermore, 10 ml of distilled water was added into the Mason jar to minimize evaporation from soil samples. Periodically (i.e., 7, 14, 28, 42, and 60 d after the incubation), scintillation vials containing the base trap were removed from the capped Mason jars and the remaining NaOH

was titrated with 0.2 M HCl for the measurement of CO₂ released. Mason jars were left open for 30 min to allow air diffusion and then replaced with new base traps. Cumulative CO₂-C over the incubation was calculated and represented the potential mineralization of soil organic C.

A soil sample-containing scintillation vial was also removed from each of the Mason jar for the measurement of soil inorganic N. Soil samples were slurried with 1 KCl at 1:5 weight (g)-to-volume (ml) ratio, shaken for 30 min. at ~ 200 rev. min⁻¹, and filtered through Whatman #1 filter paper. Inorganic N in filtrates was measured using a Lachat flow-injection auto-analyzer (QuikChem 8000, Lachat Instruments, Mequon, WI). The difference of soil inorganic N at the end and beginning of the incubation represented the potential mineralization of soil organic N.

Nitrification potential was determined according to the shaken slurry method (Hart et al., 1994). A soil sample (~ 15 g moist weight) was added into a 250 ml Erlenmeyer flask and made slurry using a solution of 100 ml at pH 7.2, containing 1.5 mM of NH₄⁺ and 1mM of phosphate. Soil slurry was shaken at ~ 200 rev. min⁻¹ for 24 h and slurry samples of 10 ml were taken periodically (i.e., 2, 4, 19, and 21 h after the incubation). After centrifuge, soil slurry samples were measured for NO₃⁻-N as described above. The potential rate of nitrification was calculated from the slope of NO₃⁻-N regression against sampling time and expressed as mg N kg⁻¹ soil h⁻¹.

2.4. Soil enzyme activity assays

The activities of β -glucosidase, exoglucanase and β -glucosaminidase were determined by the colorimetric method, using 50 mM of p-nitrophenyl- β -D-glucopyranoside, 10 mM of p-nitrophenyl- β -D-cellobioside, and 10 mM of N-acetyl- β -D-glucosaminide as the substrate solution, respectively. Briefly, soil (~ 4 g moist weight) was homogenized in 10 ml of 50 mM acetate buffer at pH 5.0. Then, 0.8 ml of soil slurry and 0.2 ml of substrate solution were added into a 2-ml Eppendorf tube and incubated for 1-2 h at 37 °C. The reaction was terminated and allowed for color development by adding 0.5 M CaCl₂ and 0.1 M Tris buffer at pH 12. Finally, the reaction solution with the product, p-nitrophenol (pNP) was pipetted into a 96 well plate for the measurement of optical density at 410 nm using spectroscopy.

The activity of peroxidase was determined using 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate by the method of Johnsen and Jacobsen (2008). The 0.2 ml of soil slurry and 0.4 ml TMB were added into a 2 ml Eppendorf tube and incubated for 20 min. at room temperature in dark. Reaction was terminated by adding 1 ml of 0.3 M H₂SO₄ and then optical density was measured at 450 nm.

Two negative controls (i.e., substrate alone and soil slurry alone) were included in the assay of each soil enzyme activity. Soil hydrolytic enzyme activity was expressed as μmol of pNP released g^{-1} soil h^{-1} . Soil peroxidase activity was calculated based upon an extinction coefficient (TMB = $0.059 \mu\text{M}^{-1} \text{cm}^{-1}$) and expressed as μmol of substrate conversion g^{-1} soil h^{-1} .

2.5. Data analysis

ANOVA with a split-plot design was used to assess the significant impacts of cover crop species, termination methods, and their interactions on soil microbial properties and processes. Replication effect was considered random because three field replicates were randomly chosen from total six field replicates. Therefore, a model PROC MIXED was used (SAS 9.2, SAS Institute Inc., Cary, NC, 2008) with least significant difference (LSD) for the comparison of mean values at $P < 0.05$, unless stated otherwise. A linear regression was also performed for examining the relationship of soil enzyme activity with soil C and N mineralization as well as nitrification potential at $\alpha = 0.05$.

As shown in Table 1, three cover crops differed significantly in biomass production and quality. To better compare the impacts of different species of cover crops, microbial properties were further normalized by cover crop biomass, i.e., $(A-B)/C$, where A and B represent a microbial property in field plots with and without a cover crop, respectively, and C is the cover crop biomass. This normalization was performed only when a microbial property was significantly greater in cover crop plots than in no cover crop plots. Normalized data were then subjected to principal component analysis (PCA) (Canoco software, Microcomputer Power Inc., Ithaca, NY) to address the question if cover crop species could be distinguished based upon their effects on soil microbial properties.

3. Results

3.1. Soil microbial properties as affected by the cover crop termination methods

Soil microbial biomass C varied significantly with the termination methods, being ~ 17% greater with the flail than with the disk and spray (Fig. 1). Differences in soil C and N mineralization were also marginally significant among the termination methods. In comparison to the disk, flail mowing enhanced soil C mineralization by 30% and N mineralization by 19%. Furthermore, flail significantly improved soil nitrification potential by ~ 36% compared to the disk and spray.

Termination methods also influenced soil enzyme activities; however, the effects appeared to be study site-dependent (Fig. 2). In Kinston, while exoglucanase activity was similar among the termination methods, β -glucosaminidase activity was significantly higher in the flail than in the disk and spray. The activity of β -glucosidase was also marginally higher in the flail than in the disk. In the Goldsboro site, exoglucanase activity differed significantly among the termination methods, being greater with the flail and disk than with the spray. The activity of β -glucosidase was also greater in the disk than in the spray. Yet, soil peroxidase activity was lower in the disk than in the spray. Furthermore, there were no differences in β -glucosaminidase activity among the termination methods. Despite varying effects of the disk and spray treatments between the two study sites, the flail treatment showed consistent positive influences on enzyme activities.

It should be noted that these effects were independent of sampling time and cover crop application. There were no interactions between termination methods and sampling time or

cover crop application. Except soil microbial biomass C and C mineralization, however, other microbial properties significantly changed with sampling time. Nitrogen mineralization and nitrification potential increased with sampling time, whereas soil enzyme activities significantly decreased over time.

3.2. Soil microbial properties as affected by the cover crop application

Application of cover crops did not increase soil microbial biomass C nor soil C and N mineralization (Table 2). However, C mineralization per unit of microbial biomass C differed significantly between cover crop treatments, with Austrian winter pea the lowest. Application of Austrian winter pea and hairy vetch also enhanced soil nitrification potential compared to the no cover crop treatment.

Generally, application of cover crops enhanced the activities of soil hydrolytic enzymes, i.e., exoglucanase, β -glucosidase and β -glucosaminidase (Table 3). The effects appeared to be independent of sampling time and were more pronounced in the Goldsboro site than in the Kinston site. Application of cover crops, however, did not affect soil peroxidase activity.

3.3. Soil microbial properties as affected by cover crop quality

Impacts on soil microbial properties varied with cover crop species (Fig. 3). In the Kinston site, hairy vetch was found to be different from Austrian winter pea and crimson clover as shown by non-overlapping PCA scores (Fig. 3). Apparently, Austrian winter pea and crimson clover affected the activities of β -glucosidase and β -glucosaminidase more

positively than hairy vetch. Furthermore, Austrian winter pea enhanced nitrification potential more than crimson clover and hairy vetch. In the Goldsboro site, Austrian winter pea also tended to have more positive effects on the activities of β -glucosidase and β -glucosaminidase, although three cover crops were not separated completely (Fig. 3). Furthermore, Austrian winter pea played more positive roles in stimulating exoglucanase activity than hairy vetch.

3.4. Correlations of soil enzyme activities with soil C and N processes

Of three enzyme examined in the Kinston site, only β -glucosidase activity was significantly correlated with C mineralization (Pearson's $r = 0.253$, $P < 0.05$), N mineralization (Pearson's $r = 0.355$, $P < 0.01$) and nitrification potential (Pearson's $r = 0.356$, $P < 0.01$). There were no significant correlations between soil exoglucanase activity and soil C and N processes. While showing no correlations with C and N mineralization, β -glucosaminidase activity was significantly correlated with soil nitrification potential (Pearson's $r = 0.383$, $P < 0.001$).

4. Discussion

Cover crop species and termination method have been deemed as the critical factors determining the efficacy of cover crop management in improving soil fertility and quality. Usually, yield is a more important consideration when it comes to the choice of cover crop species. This work, however, demonstrated that the biochemistry of cover crops should not be ignored. Despite significantly lower yield by up to 40%, Austrian winter peas enhanced

soil microbial activity to the level comparable to, if not greater than, that made by crimson clover and hairy vetch. Our results rejected the hypothesis that legume species would not impact soil microbial activity. Instead, this work supported that legume species, even with small variations in C-to-N ratio and lignin and cellulose contents, could cause substantial divergence in soil microbial activity. Furthermore, this work confirmed that flail was a better termination method than disk and spray for facilitating soil microbial activity.

4.1. The impact of termination methods on soil microbial activity

The results that flail operation was better than disk and spray in promoting microbial growth and activity were likely due to the termination method-associated changes in soil properties. One major difference between disk, flail and spray is the location where cover crops were placed following growth termination. Residue locations can be important to soil microbial activity if they change soil properties. Compared to soil incorporation, residues left on soil surface are believed to improve water infiltration, reduce soil evaporation, and therefore help maintain soil moisture. A number of studies have consistently shown that surface placement of crop residues as mulch can improve soil water conservation (Acharya et al., 1998; Gicheru, 1994; Murungu et al., 2011b; Ramakrishna et al., 2006). Zhang et al. (2007) reported that mulching on drylands raised soil water storage by up to 8% and decreased soil evaporation by up to 13%. Residues left on soil surface can also insulate soil from temperature changes. Mulching may easily cool down soil temperature by several degrees in hot summer, keep soil warm in cold winter, and reduce diurnal soil temperature

fluctuations (Ramakrishna et al., 2006; Sarkar et al., 2007; Sarkar and Singh, 2007; Varadan and Rao, 1983). While both water conservation and stable temperature could cause soil microbes to be more active, the positive effects of flail were perhaps associated more with water conservation. This is because soil temperatures during the experimental period (May to August) were generally within the optimal range for microbes (Grayston et al., 2001).

Unlike flail operation, spray kept plant materials intact, rather than chopped to small pieces. It has been shown that microbial activity and the rate of residue decomposition were inversely related to residue size (Angers and Recous, 1997; Bending and Turner, 1999; Tarafdar et al., 2001). This could in part explain why microbial growth and activity were lower in spray than flail. In addition, herbicides used with spray might adversely affect soil microbes, but such effects were likely short (Gaston et al., 2001; Zablotowicz et al., 2007).

4.2. The impact of cover crop species on soil microbial activity

Compared to no cover crop treatment, cover crops generally stimulated soil microbial activity. It should be noted that yields of three cover crops were significantly different, with Austrian winter pea the lowest. Yet, soil microbial properties under Austrian winter pea were comparable to, if not greater than, those under crimson clover and hairy vetch. This suggested that positive effects were attributed not only to the amount of cover crop input but also to the chemical compositions. Using normalized data that minimized the confounding effects of cover crop quantity, PCA analysis further confirmed that the quality of legume species made differences in soil microbial activity.

Indicators of residue biochemistry, e.g., C-to-N ratio and lignocellulose index, can be used as quantitative variables for modeling soil and microbial processes (Vigil and Kissel, 1991; Whittinghill et al., 2012). They can also be used as qualitative parameters when comparing residue impacts on soil microbial properties and processes. A threshold of C-to-N ratio, for example, has been widely applied for estimating whether soil amendment of crop residues leads to net N mineralization. Generally, residues with low C-to-N ratio and/or low lignocellulose index are superior to those with high values for microbial decomposition (Carreiro et al., 2000; Cotrufo et al., 1995; Gholz et al., 2000; Melillo et al., 1982). Reliable evaluations require residues with large variations in chemical compositions. Despite minor differences in C-to-N ratio and lignocellulose index, however, the effects of legume species on soil microbial activity can be reliably predicted when the two biochemical parameters were taken into consideration. Austrian winter pea with lower C-to-N ratio as well as lower lignocellulose index showed more positive effects on soil microbial activity than the other two legume species.

4.3. Microbial indicators of soil quality

Our study showed that not all microbial properties and processes were sensitive to short-term (i.e., one year) management of legume cover crops. While soil enzyme activities were more responsive to legume species than to termination methods, microbial biomass and respiration appeared to be more sensitive to termination strategies. Among the measured microbial parameters, only nitrification potential responded well to both termination methods

and legume species.

Microbial biomass was expected to increase following soil amendment of residues. A laboratory study showed that about 20% of ryegrass-C could be incorporated into newly-formed microbial biomass (Shi et al., 2006). Because the C-to-N ratio of ryegrass was close to legume species used in this study, it is reasonable to assume that legumes could increase soil microbial biomass equivalent to 20% of their C. This would be roughly 45 μg microbial biomass C g^{-1} soil, i.e., 30% increase compared to no cover crop treatment, provided that legume dry mass was 10% of yield, legume C was 45% of dry mass, and soil mass was 2,000 metric ton ha^{-1} . However, this increase is often temporary and cannot be sustained by indigenous soil organic matter (Shi et al., 2006). No detectable biomass change in our study indicates that the sampling time was not right for connecting microbial biomass response and cover crop amendments. Due to soil and climate complexity, it is difficult to predict the suitable time to capture microbial biomass response. This suggests that microbial biomass is not a reliable indicator for the short-term (i.e., one year) evaluation of soil fertility and quality. Similarly, no detectable change in potential C mineralization implied that it was not sensitive to reflect any potential change of the quantity or quality of soil organic matter. Or perhaps one-year soil amendment of legume cover crops could not essentially improve the quantity and quality of soil organic matter. It should be noted that the inference of this study is not contradictory to the longstanding concept that microbial biomass and C mineralization are sensitive to organic amendments. In fact, it only adds limitations to and thus helps broaden the general knowledge. It appears that the increase in microbial biomass and C

mineralization can be sustained after several years of the application of organic materials (Kallenbach and Grandy, 2011; Tu et al., 2006).

Unlike microbial biomass and C mineralization, soil enzyme activity changed significantly with legume cover crops, indicating its sensitivity to the short-term amendment of organic materials. This is likely because the pool size of soil enzymes was small enough to allow the detection of soil enzymes could be easily detected. The sharp contrast between soil enzyme activity, microbial biomass, and C mineralization appears to support that microbial parameters with low background or for describing a small functional group could be more sensitive for evaluating the impacts of short-term organic amendments. Indeed, nitrification potential, a parameter for indicating the population size of nitrifiers, which normally accounts for < 0.1% of total soil microbial population, responded most sensitively to short-term amendment of legume cover crops in our study.

4.4. Practical implications

Given that microorganisms are small and dynamic entities in soil, microbial parameters are often used as indicators to evaluate agricultural management practices and land use change. However, the sensitivities of microbial parameters differed and were operation-dependent. Our study infers that a microbial parameter imparted by a small functional group, e.g., nitrifiers, were suitable to evaluate not only the impacts of legume cover crop species but also cover crop termination methods. Although soil enzyme activities have been considered sensitive to various management practices and land-use changes

(Bandick and Dick, 1999; Garcia et al., 2008; Knight and Dick, 2004; Sinsabaugh et al., 1992; Trap et al., 2012; Trasar-Cepeda et al., 2008; Turner et al., 2002), they are unreliable for evaluating termination methods. Our results suggest that soil enzyme activities should not be used for evaluating management practices that slightly modify soil temperature and moisture.

The significant finding of this study is that legume species even with small variations in chemical composition, i.e., C-to-N ratio and lignin and cellulose contents, caused substantial changes in soil microbial properties. With lower values in both C-to-N ratio and lignocellulose index, Austrian winter pea prevailed over hairy vetch and crimson clover in promoting microbial activity and nutrient availability. Our study suggests that the chemical composition should be taken into account when selecting legume species for cover crop management if enhanced microbial activity is the goal.

Austrian winter pea and flail operation appeared to be a good combination for managing legume cover crops. However, caution should be taken regarding the long-term use of Austrian winter pea. As shown in our study sites, yields of Austrian winter pea were up to 40% lower than those of crimson clover and hairy vetch. The low yield can be a significant disadvantage of Austrian winter pea from the perspective of soil C sequestration. Because C mineralization per unit of microbial biomass was significantly lower in Austrian winter pea than in crimson clover and hairy vetch, it can be argued that a higher fraction of Austrian winter pea-C could be translocated into microbial biomass and ultimately into stable soil C. Nonetheless, Austrian winter can be a good choice for short-term cover crop management.

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Table 1. Yield and biochemistry of three legume cover crops in the Kinston and Goldsboro sites[§]

	Kinston		Goldsboro		LCI
	Yield (kg ha ⁻¹)	C/N	Yield (kg ha ⁻¹)	C/N	
Austrian winter pea	5803 b	13.40 b	6476 b	13.73 b	0.44 (0.01)
Hairy vetch	6808 a	12.52 b	8670 a	13.29 b	0.48 (0.00)
Crimson clover	7507 a	17.96 a	9636 a	15.40 a	0.40 (0.00)

[§] Statistical analysis was performed on cover crop yield and C-to-N ratio since both were measured from each field plot/treatment.

Different letters in a column indicate significant differences at $P < 0.05$. Lignin and cellulose contents were measured for composite samples of cover crops collected from two field plots of each treatment. Therefore, LCI (lignin-cellulose index, i.e., lignin-to-lignin plus cellulose ratio) were not subjected to statistical analysis and expressed as means (standard errors).

Table 2. Soil microbial biomass, mineralization, and nitrification potential following the application of different cover crops in the Kinston site[§].

	MBC	Cmin	Nmin	Cmin:MBC	Nitrification potential
	$\mu\text{g C g}^{-1} \text{ soil}$	$\mu\text{g C or N g}^{-1} \text{ soil}$			$\mu\text{g N g}^{-1} \text{ soil d}^{-1}$
Austrian winter pea	154.9 a	369.4 a	19.5 a	1.71 <i>b</i>	5.14 a
Hairy vetch	147.3 a	400.6 a	18.8 a	1.93 <i>ab</i>	4.87 a
Crimson clover	152.8 a	471.9 a	16.6 a	2.22 <i>ab</i>	4.18 ab
No cover crop	143.2 a	470.4 a	16.6 a	2.37 <i>a</i>	3.38 b

[§]Different letters in a column indicate significant differences at $P < 0.05$. Different italic letters are also used to indicate significant differences at $P < 0.1$. MBC, Cmin, Nmin are short for microbial biomass C, C mineralization, and N mineralization, respectively.

Table 3. Soil enzyme activities following the application of different cover crops in the Kinston and Goldsboro sites[§]

	Exoglucanase	β -Glucosidase	β -Glucosaminidase	Peroxidase
	————— $\mu\text{mol h}^{-1} \text{g}^{-1} \text{soil}$ —————			
<u>Kinston site</u>				
Austrian winter pea	0.240 a	0.854 <i>ab</i>	0.378 ab	
Hairy vetch	0.222 a	0.849 <i>ab</i>	0.335 bc	
Crimson clover	0.237 a	0.900 <i>a</i>	0.399 a	
No cover crop	0.223 a	0.807 <i>b</i>	0.309 c	
<u>Goldsboro site</u>				
Austrian winter pea	0.226 a	1.379 a	0.403 a	0.581 a
Hairy vetch	0.218 ab	1.332 a	0.400 a	0.640 a
Crimson clover	0.226 a	1.402 a	0.412 a	0.632 a
No cover crop	0.202 b	1.191 b	0.366 b	0.622 a

[§]Different letters in a column indicate significant differences at $P < 0.05$. Different italic letters are also used to indicate significant differences at $P < 0.1$.

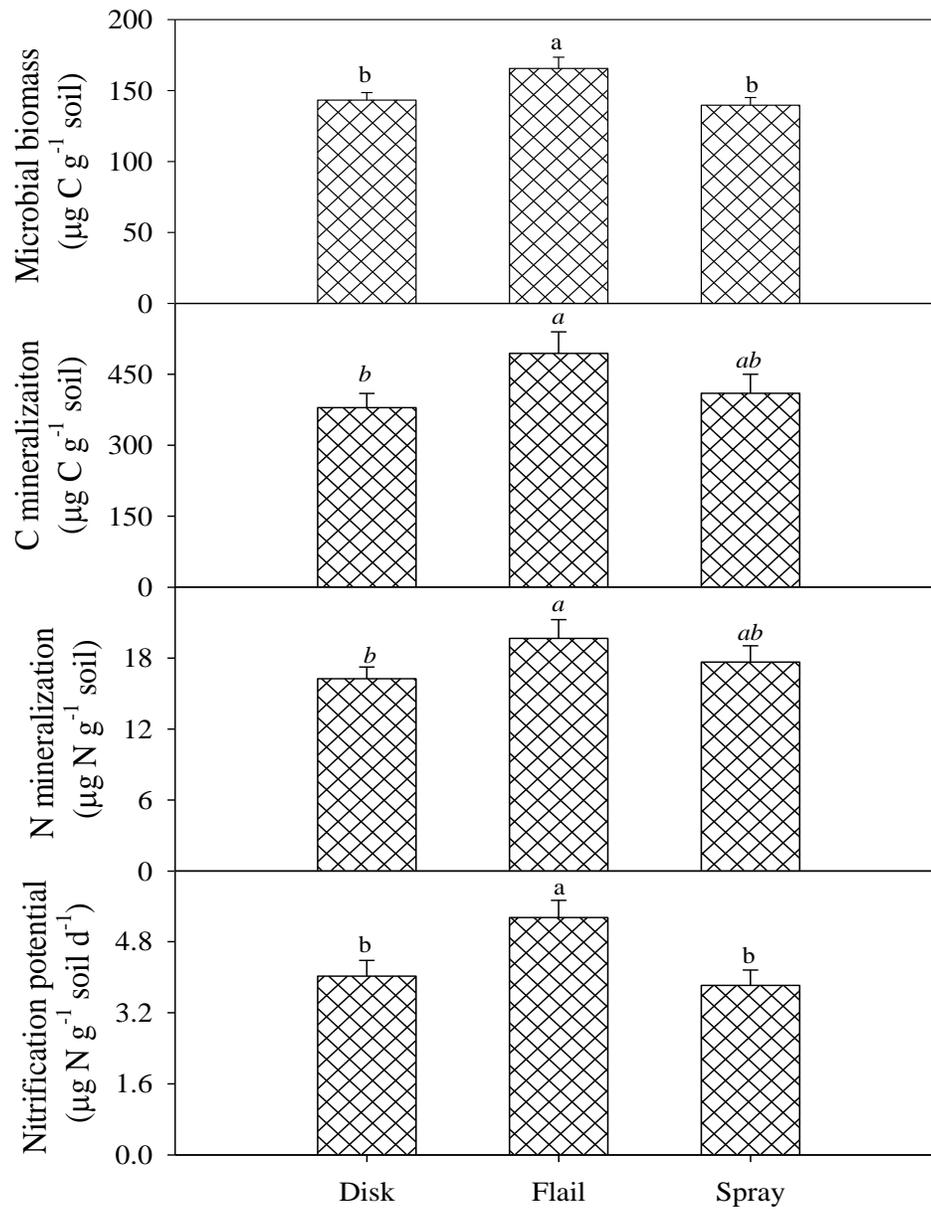


Fig. 1. Soil microbial properties as affected by three termination methods in the Kinston site.

Different letters indicate significant differences of means at $P < 0.05$. Different italic letters

indicate significant differences of means at $P < 0.1$.

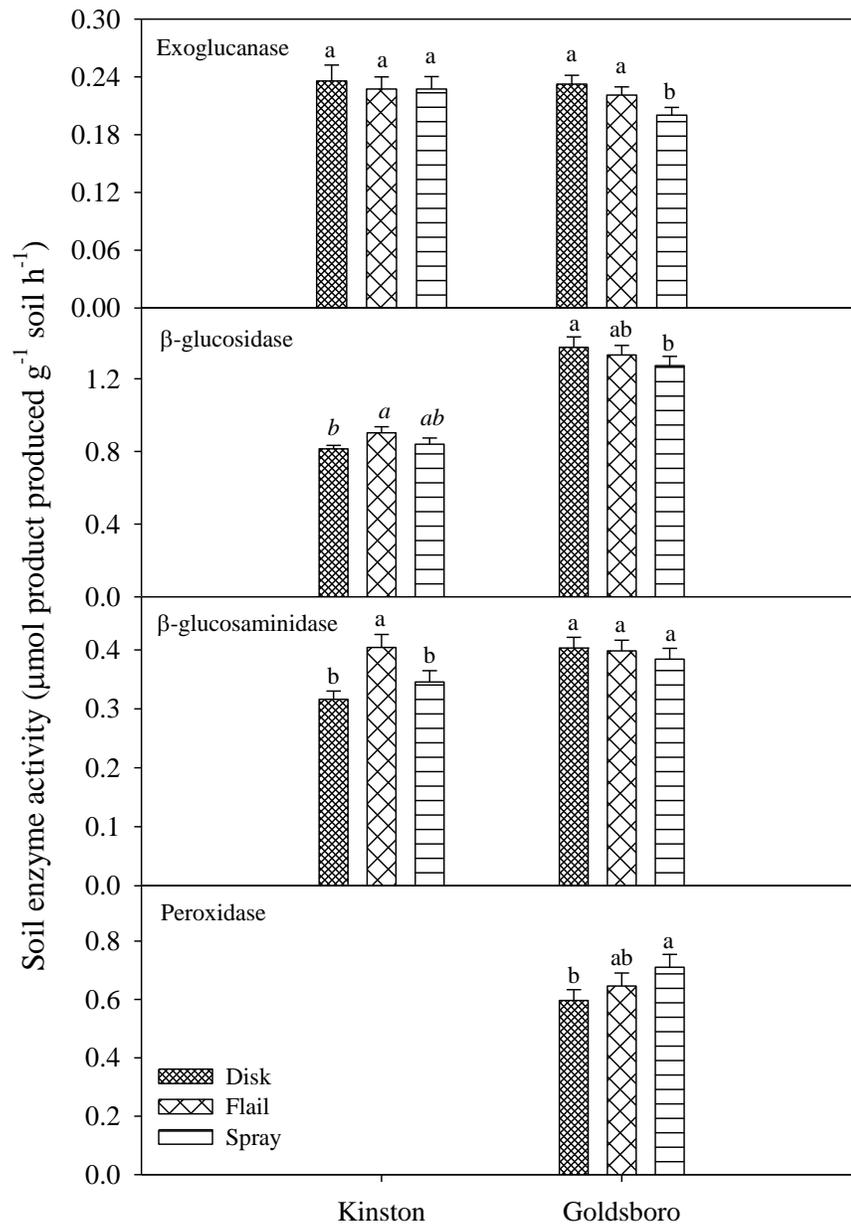


Fig. 2. Soil enzyme activities as affected by three termination methods in the Kinston and Goldsboro sites. Different letters indicate significant differences of means at $P < 0.05$. Different italic letters indicate significant differences of means at $P < 0.1$.

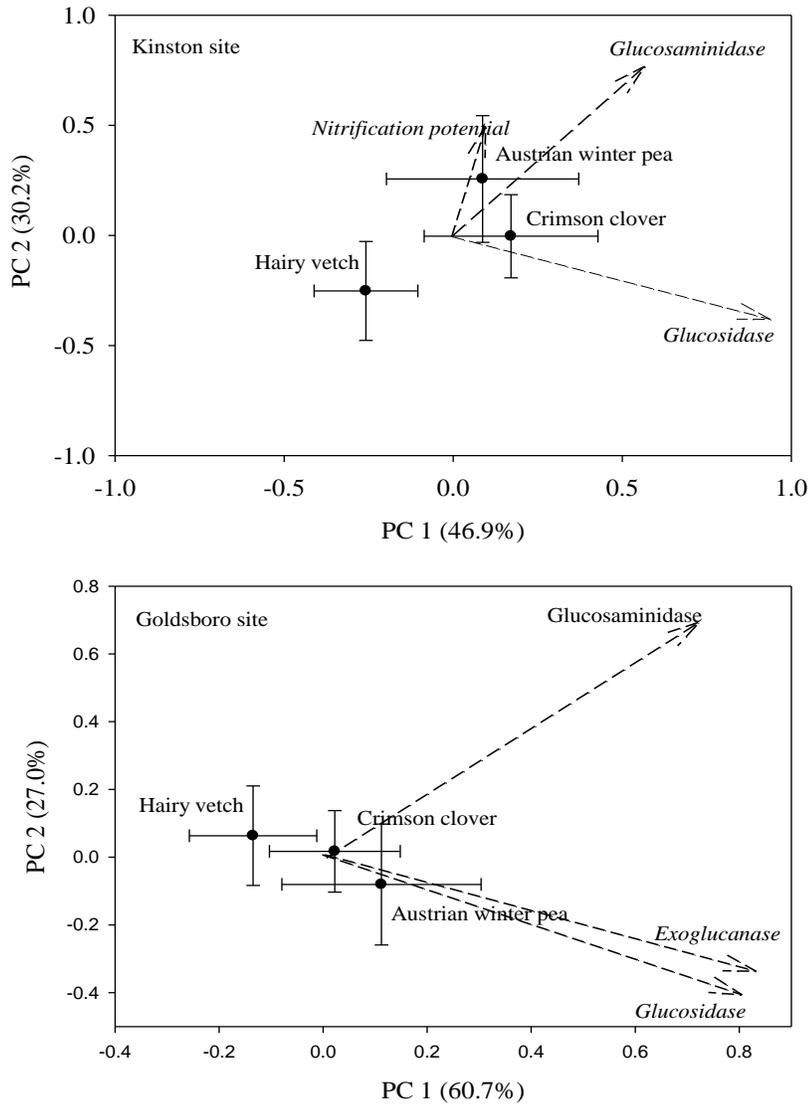


Fig. 3. Principal component analysis (PCA) showing the separation of cover crop species and the contribution of microbial properties. The loading scores along the first and second principal components were the mean values over sampling times, termination methods, and field replicates. Bars represent standard errors (n=18 for Kinston site and n=45 for Goldsboro site).

Chapter 3: Soil Particulate Organic Matter as Related to Short-term Legume Winter Cover Crop Management

Abstract

This study aimed to examine the responses of soil light and heavy fractions to termination methods (disk, flail, and spray) and cover types (no cover crop, Austrian winter pea [*Pisum sativum*], hairy vetch [*Vicia villosa*], and crimson clover [*Trifolium incarnatum*]) during the first year of cover crop management. Responses appeared only in soil light fraction that accounted for < 1% of total soil mass as well as soil C and N contents. The quantities of mass, C, and N of soil light fraction varied with termination methods, being greatest for disk. In contrast, cover types did not affect the quantity, but rather the quality of soil light fraction. Compared to crimson clover, Austrian winter pea produced soil light fraction with the greater C-to-N ratio. I found that soil light fraction mass was inversely related to the activities of exoglucanase, β -glucosidase, β -glucosaminidase, and peroxidase. Negative relations also existed between light fraction C-to-N ratio and the ratio of soil C- to N-mineralization. Together, these data suggested that soil light fraction could well manifest soil microbial properties and processes during a short period of cover crop management.

1. Introduction

Soil organic matter plays an important role in sustainable agriculture, which aims to maintain crop productivity for growing food demand and meanwhile to minimize adverse

impacts on environment (Lichtfouse et al., 2009; Tilman et al., 2011). Increased content of soil organic matter often leads to the increased crop yield (Bauer and Black, 1994; Diaz-Zorita et al., 1999) due to its positive impacts on mineralization-associated nutrient availabilities (Benbi and Chand, 2007) and aggregation-associated soil structure (Franzluebbers, 2002; Six et al., 2000). As for environmental benefits, soil organic matter may potentially reduce the mobility of pesticides, herbicides, and other contaminants through sorption and desorption processes (Ahmad et al., 2001; Kulshrestha et al., 2004). Soil organic matter can also help maintain the functional diversity of the soil microbial community, a driver of soil biological processes and an imperative component of ecosystem service (Bending et al., 2002; Cookson et al., 2008). Furthermore, soil organic matter is a large sink of carbon in the context of mitigating global warming and climate change (Lal, 2004; Six et al., 2002).

In agroecosystems, soil organic matter buildup is mainly achieved through crop residue recycling and/or the long-term application of cover crops, green manures, animal wastes, biosolids, and in some cases municipal and industrial organic by-products. As one of the important management practices, cover cropping can not only improve the quantity and quality of soil organic matter (Ding et al., 2006; Sainju et al., 2002), but also show advantages in reducing soil erosion, controlling pests and weeds, maintaining soil moisture condition, and stabilizing soil surface temperature (Acharya et al., 1998; Gicheru, 1994; Murungu et al., 2011; Ramakrishna et al., 2006; Sarkar et al., 2007; Sarkar and Singh, 2007; Varadan and Rao, 1983). Legume cover crops that capture N through biological fixation and

typically contain high N in leaf tissue, also provide additional N for subsequent cash crops (Blevins et al., 1990; Decker et al., 1994; Holderbaum et al., 1990). Generally, the impacts of cover crops on soil organic matter buildup rely on cover crop biomass and also the chemical composition of cover crops (Kuo et al., 1997; Martens, 2000; Sainju et al., 2006; Vieira et al., 2009).

Methods of cover crop termination, i.e., killing and then reapplying cover crops back into the fields, can substantially influence on the transformation of C from cover crops to soil organic matter. Through processes such as tillage, incorporation, and chopping, termination methods determine the placement and size of cover crop residues and therefore microbial decomposition of cover crops. Termination methods may also affect the degradation of existing organic matter in soil due to its impacts on soil physical structure and thus the accessibility of organic matter to soil microbes. Nevertheless, the net gain of soil C following the long-term application of cover crops can be significant. After 10 years of legume cover crops, for example, soil organic C was found to be enhanced by 24% in a rain-fed field with initially low soil organic C content (Venkateswarlu et al., 2007). Yet, the magnitude of this increase depends on management practices. In general, increase in soil organic C following the application of cover crops is greater in no-till than the tilled systems (Olson et al., 2010; Vieira et al., 2009).

Soil organic matter represents all organic materials derived from plants and animals in different stages of decomposition and degrees of association with soil mineral particles. It can be separated into chemically and physically distinct components, based upon their

differences in size and density (Six et al., 2002; Strickland and Sollins, 1987; Cerli et al., 2012). By flotation on a denser solution, for example, light fraction of soil organic matter, which is mainly comprised of partially decomposed roots and plant litter and therefore regarded as highly labile, can be removed from the rest of the mineral soil, i.e., heavy fraction. In general, management practices exert more influence on light fraction than on the heavy fraction of soil organic matter (Bremer et al., 1994; He et al., 2008; Janzen et al., 1992; Roscoe and Buurman, 2003; Song et al., 2012; Tan et al., 2007). Therefore, light fraction can be potentially used as an early indicator for management-induced changes in soil organic matter (Biederbeck et al., 1994).

This study aimed to examine if the quantity and quality of organic matter in soil light fraction could rapidly respond to legume winter cover crop management. The outcomes of this study may be some recommendations for growers that hope to improve soil organic matter content and soil biological functions in the short term.

2. Materials and Methods

2.1. Study site and soil sampling

The study site was established in 2011 on a loamy sand field (mixed, termic Typic Hapludult) at the Center for Environmental Farming Unit, Goldsboro, NC. Four termination methods (flail, roll, disk, and spray) and four cover crop treatments (Austrian winter pea [*Pisum sativum*], hairy vetch [*Vicia villosa*], crimson clover [*Trifolium incarnatum*] and no cover crop) were combined and replicated six times in the field. Treatments were arranged

via a split-plot design, with termination methods as the main plots and cover crop species as the sub-plots. Legume cover crops were planted in late fall, grew over winter, and then were terminated in the following spring. Thereafter, corn was planted in May and harvested in fall.

In this study, four legume cover crop treatments (Austrian winter pea, hairy vetch, crimson clover, and no cover crop) and three termination methods (flail, disk, and spray) were used for investigating the effects of termination methods and cover crop species on soil density fraction properties. For flail-mowed treatment, cover crops were cut down and chopped using a 1.8-meter flail mower (John Deere Model 370, Moline IL). The spray treatment terminated cover crops using a spray of certificated organic herbicide (BurnOutII, 4.4% final concentration clove oil, St. Gabriel Organics, Orange, VA) and then left on soil surface. For disk treatment, cover crops were chopped using a flail mower and incorporated into ~15 cm soil depth by using a 3.65-meter disk (John Deere Model 225, Moline IL). Soil samples were collected from three replicate plots five times (i.e., one, two, four, ten, and 12 weeks after cover crop termination) in 2012, but only the first and last week samples were analyzed in this experiment. Six soil cores (2.5 cm dia. × 15 cm depth) were taken randomly from each field plot and then combined to form a composite soil sample. Soil samples were transported to the laboratory in a cooler, passed through a 2 mm-mesh sieve, and stored at 4 °C for later use.

2.2. Soil density fractionation

Soil samples were separated into light and heavy fractions according to a modified

protocol of Strickland and Sollins (1987). Thirty grams of fresh soil was dispersed on 200 ml NaI solution (density = 1.6 g cm^{-3}) in a 250 ml Erlenmeyer flask. Soil slurry was shaken for 16 h at 150 rpm, and then allowed to settle for 48 h, generating two layers with light fraction materials floating on the surface. Top 3 cm of solution was aspirated using a vacuum suction system, and then filtered through Whatman #1 filter paper. Light fraction retained on filter paper was washed three times by distilled water to remove NaI. Soil heavy fraction remained at the bottom of Erlenmeyer flask was re-suspended, completely transferred into a 250 ml centrifuge bottle, and then centrifuged at 11,000 rpm for 1 min. After decanting NaI solution, soil heavy fraction was washed three times with 50 ml distilled water by dispersion and centrifugation. Both light and heavy fractions were oven dried at $60 \text{ }^{\circ}\text{C}$ for 48 h prior to the measurement of mass.

2.3. Carbon and N contents in soil light and heavy fractions

Soil light and heavy fractions were ground to pass 1 mm sieved and then analyzed for total C and N contents via a dry combustion method using a CHN Elemental Analyzer (Perkin Elmer 2400 CHN Elemental Analyzer, Norwalk, CT). Carbon and N contents in light and heavy fraction on the basis of soil mass were calculated from C and N concentrations in individual fraction and mass percentage of each fraction.

2.4. Statistical data analysis

ANOVA with a split-plot design was used to assess the significant impacts of cover crop

species, termination methods, and their interactions on C, N, and C:N ratio of light and heavy fractions. Replication effect was considered random because three field replicates were randomly chosen from total six field replicates. Therefore, a model PROC MIXED was used (SAS 9.2, SAS Institute Inc., Cary, NC, 2008) with least significant difference (LSD) for the comparison of mean values at $P < 0.05$, unless stated otherwise. A linear regression was also performed for examining the relationship between soil enzyme activity reported in Chapter two and light fraction carbon content at $\alpha = 0.05$.

3. Results

3.1. Soil light and heavy fraction-C and N as affected by species and termination methods of cover crops

Termination methods significantly affected the mass as well as C and N contents of soil light fraction, being greatest in disk treatment (Fig. 1). Compared to flail and spray, disk increased light fraction mass by ~ 25% and light fraction N content by ~ 23% (Fig. 1A and 1C). Although no significant difference was observed in light fraction C content between disk and flail, disk method did result in higher C content than spray (Fig. 1B). However, soil heavy fraction responded insensitively to termination methods (Fig. 1), showing similar mass and C and N contents.

Cover crop species was shown to have no effect on mass and C and N contents of both light and heavy fractions (Table 1). Also, there were no differences in light and heavy fractions between field plots with and without cover crops.

3.2. Soil light fraction C-to-N ratio as affected by the species and termination methods of cover crops

Soil light fraction C-to-N ratios were similar among three termination methods, averaging 18.6 (Fig. 2), but varied with cover crop species (Fig. 3). The lowest ratio was associated with the application of crimson clover, whereas the ratio was similar between Austrian winter pea and hairy vetch.

3.3. Relationships of soil light fraction C with the activities of soil enzymes

Soil light fraction C was found to be negatively correlated with the activities of exoglucanase (Pearson's $r = -0.411$, $p < 0.001$), β -glucosidase (Pearson's $r = -0.404$, $p < 0.001$) and peroxidase (Pearson's $r = -0.427$, $p < 0.001$) (Fig. 4). Soil light fraction C was also inversely related to the glucosaminidase activity (Pearson's $r = -0.293$, $p < 0.05$), but the relation was less strong than to other three enzymes.

4. Discussion

Soil organic matter accumulation is a slow process during which newly-formed organic matter is physically and biochemically protected from microbial degradation via aggregation, association with clay and silt particles, and formation of recalcitrant organic compounds (Six et al., 2002). Normally, it takes at least several years for detectable changes in the entire pool of soil organic matter (Angers et al., 1995, 1997; Tan et al., 2007; Xu et al., 2011). However, the light fraction of soil organic matter has been proved to be responsive to short-term

management and land use change. This study also confirms that one year application and management of legume cover crops could alter the quality and quantity of soil light fraction, but had little effect on soil heavy fraction.

4.1. Termination methods affecting the quantity of soil light fraction

It is well known that soil light fraction represents a small, but dynamic portion of soil organic matter. In this study, it only accounted for < 1% of total soil organic matter, in terms of mass as well as C and N contents. Yet, its quantity was affected significantly by different cover crop termination methods. Compared to flail and spray, disk method that led to lower rates of soil C and N mineralization, improved the mass, C, and N of soil light fraction. Certainly, this quantity change was the net result of several processes, including soil input of crop residues, newly-derived soil light fraction, and degradation of “old” soil light fraction. Because a termination method did not affect crop growth and yield, its effects on soil light fraction were mainly through the production and consumption of soil light fraction.

A major process shaping the quantity of soil light fraction is the rate of microbial degradation. Small soil light fraction is often associated with rapid microbial degradation of crop residues and fast turnover of “old” soil labile organic matter. Because disk incorporated chopped cover crop residues into 6-inch deep soil, enhancing contacts between residues and microbial decomposers, it might be unprofitable for the formation of soil light fraction. Coppens et al. (2006) showed that about 18% and 55% of residue C were mineralized during a 9-week incubation for surface and incorporated application of residues, respectively.

Similarly, others also showed that surface applied residues decomposed more slowly than residues incorporated into soil (Aulakh et al., 1991; Breland, 1994). In addition, disk could disrupt soil aggregates, releasing “occluded” organic matter and improving microbial accessibility for degradation of “old” soil light fraction. As a result, the lower amount of soil light fraction was expected in disk but not in flail and spray. However, I found that disk was superior to flail and spray for improving soil light fraction. This indicates that termination methods affected soil light fraction via controls other than soil structure and physical contacts. As shown in Chapter 2, organic C and N mineralization was greater in flail than in disk, indicating the reverse impacts of termination methods on soil light fraction and organic matter degradation. In fact, negative correlations existed between soil enzyme activities and light fraction C content. Although flail was inferior to disk in producing suitable residue size and surface for microbial contact, it appeared to be better in optimizing soil temperature and moisture. Thus, my data suggest that termination methods affected soil light fraction mainly through controls on soil temperature and moisture.

However, cautions should be taken in extrapolating the results of this study to other soil systems. In my study site, soil had coarse texture and thus formation and maintenance of stable aggregates might be problematic. Even though a management practice can potentially impair soil structure, its effect won't show on poorly-structured soil. In consequence, termination method-induced soil temperature and moisture became dominant factors influencing organic matter degradation and soil light fraction.

4.2. Cover crop species affecting the quality of soil light fraction

Interestingly, there were no differences in the quantity of soil light fraction between field plots with and without cover crops, although on average $\sim 0.18 \text{ mg C g}^{-1}$ soil and $0.013 \text{ mg N g}^{-1}$ soil in residues were applied (Table 2). The residue C and N inputs could even be doubled if root biomass was accounted for, assuming that shoot-to-root ratio was close to one. Thus, the question is why soil light fraction did not manifest crop residue input.

Crop residues represent the predominant source of soil organic matter. In a laboratory experiment, soil light fraction C could be increased by 147%, 112 days following soil amendment of ryegrass (C-to-N ratio = 15) at 5.2 mg C g^{-1} soil (Bending and Turner, 2009). Annual return of residues at $2000 \text{ kg C ha}^{-1}$ (i.e., roughly 1 mg C g^{-1} soil assuming that one hectare contains $2 \times 10^6 \text{ kg soil}$) has been considered to be minimal for maintaining and/or improving soil organic matter (Larsen et al., 1972; Rasmussen et al., 1980). Apparently, C input in legumes was about 50 fold lower than the threshold necessary for the maintenance of soil organic matter. Kuo et al. (1997) also reported that sum of above- and below-ground C inputs in Austrian winter pea and hairy vetch was lower than $2000 \text{ kg C ha}^{-1}$. They demonstrated that due to significantly lower biomass, Austrian winter pea and hairy vetch were less suited as winter cover crops for building soil organic matter than non-legume species, such as cereal rye and annual ryegrass. Even with slightly $> 2000 \text{ kg C ha}^{-1}$, annual return of hairy vetch and crimson clover for 6 yrs still could not maintain soil organic matter, leading to 1% reduction; in contrast, rye increased soil organic matter by 3-4% (Sainju et al., 2006). Because cover crop decomposition is often rapid, with a half life of a month or two, it

is not surprising that the magnitude of C input from crop residues determine residue effect on soil organic matter (Kuo et al., 1997).

Despite no effect on the concentration of soil light fraction, legume species modified its quality, as shown by different C-to-N ratios. This suggests that soil light fraction could be replenished rapidly, but the newly-formed light fraction was equivalent to the degradation of “old” soil light fraction. Given that partially-decomposed residues were deposited to soil light fraction, following the partial removal of residue C as CO_2 and residue N as NH_4^+ and/or NO_3^- , the higher rate of residue C- relative to N- mineralization would lead to the lower C-to-N ratio of soil light fraction. Indeed, crimson clover with the highest relative rate of C- versus N-mineralization (Table 2) gave the lowest C-to-N ratio of soil light fraction.

4.3. Implications for using soil light fraction to evaluate legume cover crop management

While soil light fraction has been considered as an early indicator evaluating the efficacy of management practices and land use change on soil C accumulation, it appeared to be inappropriate for legume cover crops. In this study, the quantity and quality of soil light fraction were well and negatively associated with soil microbial activities, indicating that soil light fraction could be a good and simple index for soil microbial properties and processes. However, there exists some uncertainty because relations between microbial activity and soil light fraction can vary with management practices and environmental conditions (Henry et al., 2005; Qin et al., 2010).

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Table 1. Mass percentage and C, N contents of soil light fraction (LF) and heavy fraction (HF) in three cover crop treatments and no cover crop control[§]

	Mass portion (%)		C content (g C Kg ⁻¹ soil)		N content (g N Kg ⁻¹ soil)	
	LF	HF	LF	HF	LF	HF
Austrian winter pea	0.28	96.33	0.54	9.28	0.029	0.94
Hairy vetch	0.32	94.60	0.64	9.01	0.035	0.92
Crimson clover	0.30	98.00	0.58	9.71	0.033	0.97
No cover crop	0.31	95.04	0.57	9.15	0.031	0.91

[§]There are no significant differences between values within any column.

Table 2. Carbon and N inputs of cover crop residues, and soil C- to N-mineralization ratio for different termination methods and cover crop species[§]

	C input (mg C g ⁻¹ soil)	N input (mg N g ⁻¹ soil)	C min : N min
<u>Termination methods</u>			
Disk	0.18 a [#]	0.013 a	23.3 a [‡]
Flail	0.17 a	0.012 a	25.0 a
Spray	0.17 a	0.012 a	23.2 a
<u>Cover crop species</u>			
Austrian winter pea	0.14 c	0.010 b	18.9 b
Hairy vetch	0.18 b	0.014 a	21.3 b
Crimson clover	0.21 a	0.014 a	28.4 a

[§]Different letters in a column indicate significant differences at $P < 0.05$.

[#]C and N inputs were calculated from cover crop biomass yield and C-to-N ratios shown in Table 1, Chapter two.

[‡]The ratio of soil C- to N- mineralization was calculated from the results shown in Table 2 and Figure 1, Chapter two.

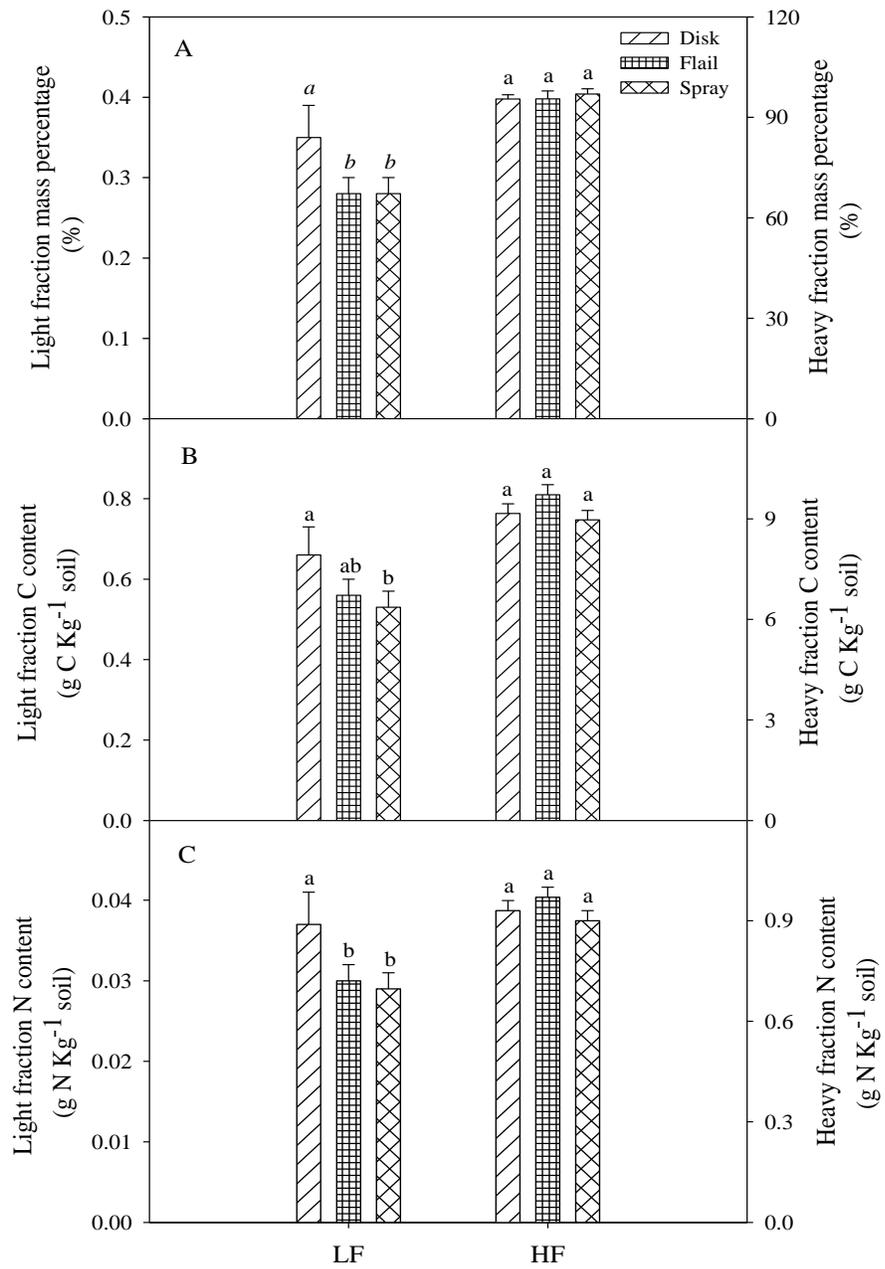


Fig. 1. Mass percentage and C, N contents of soil light fraction (LF) and heavy fraction (HF) in three termination methods. Different letters indicate significant differences of means at $P < 0.05$. Different italic letters indicate significant differences of means at $P < 0.1$.

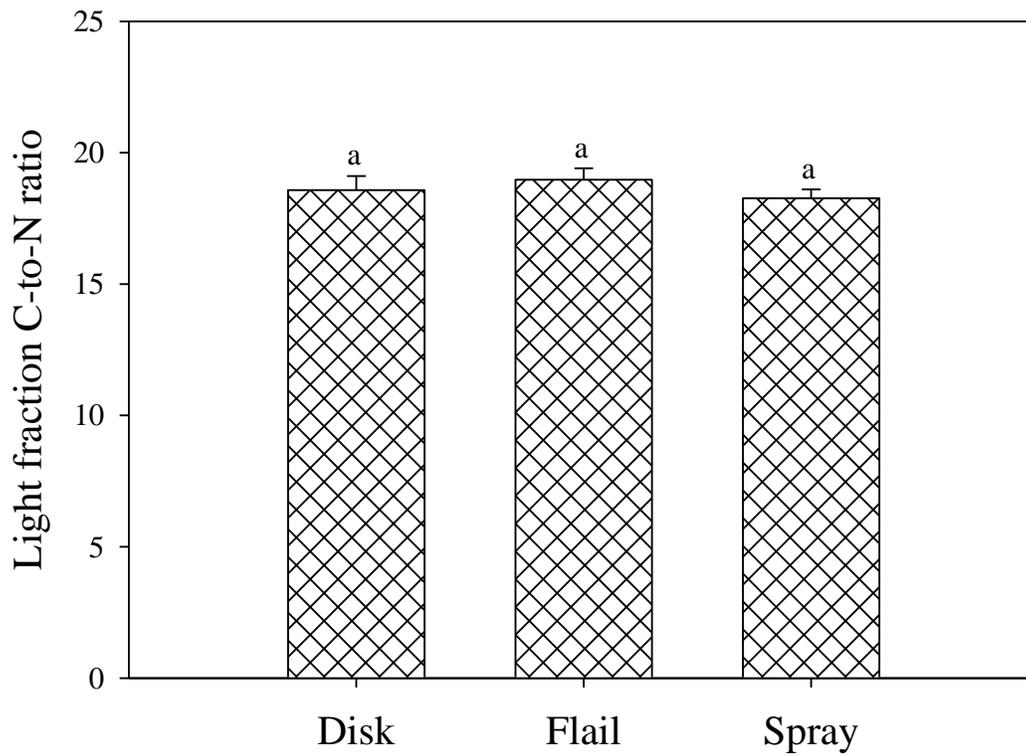


Fig. 2. Soil light fraction C-to-N ratio in three termination methods. Different letters indicate significant differences of means at $P < 0.05$.

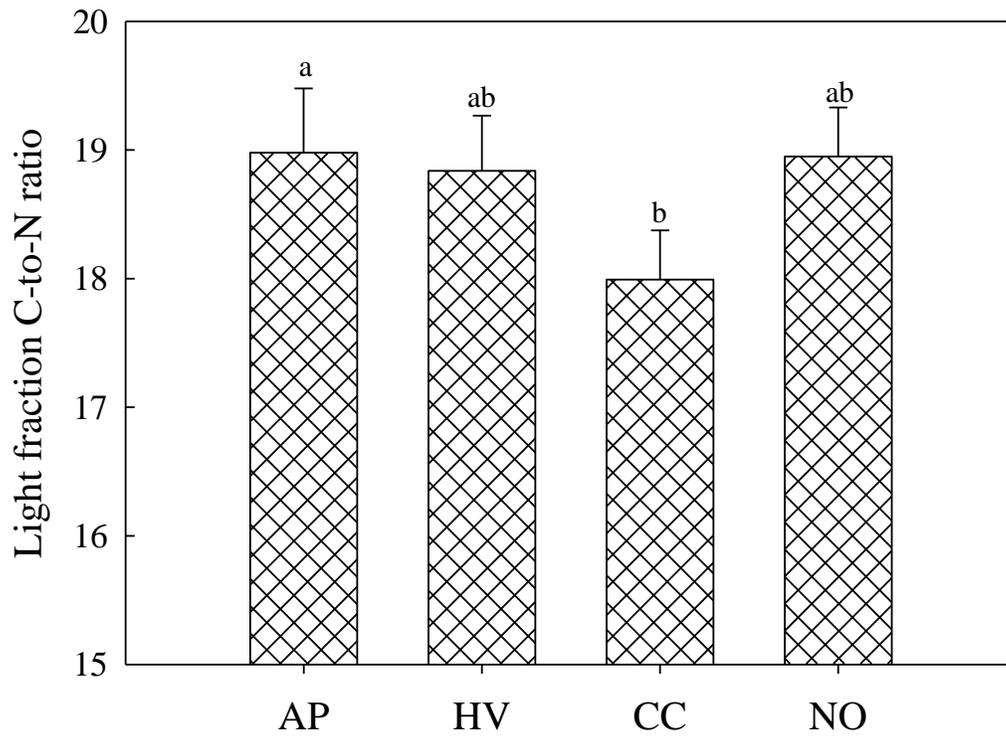


Fig. 3. Soil light fraction C-to-N ratio in three cover crop species. AP, Austrian winter pea; HV, hairy vetch; CC, crimson clover; NO, no cover crop. Different italic letters indicate significant differences of means at $P < 0.1$.

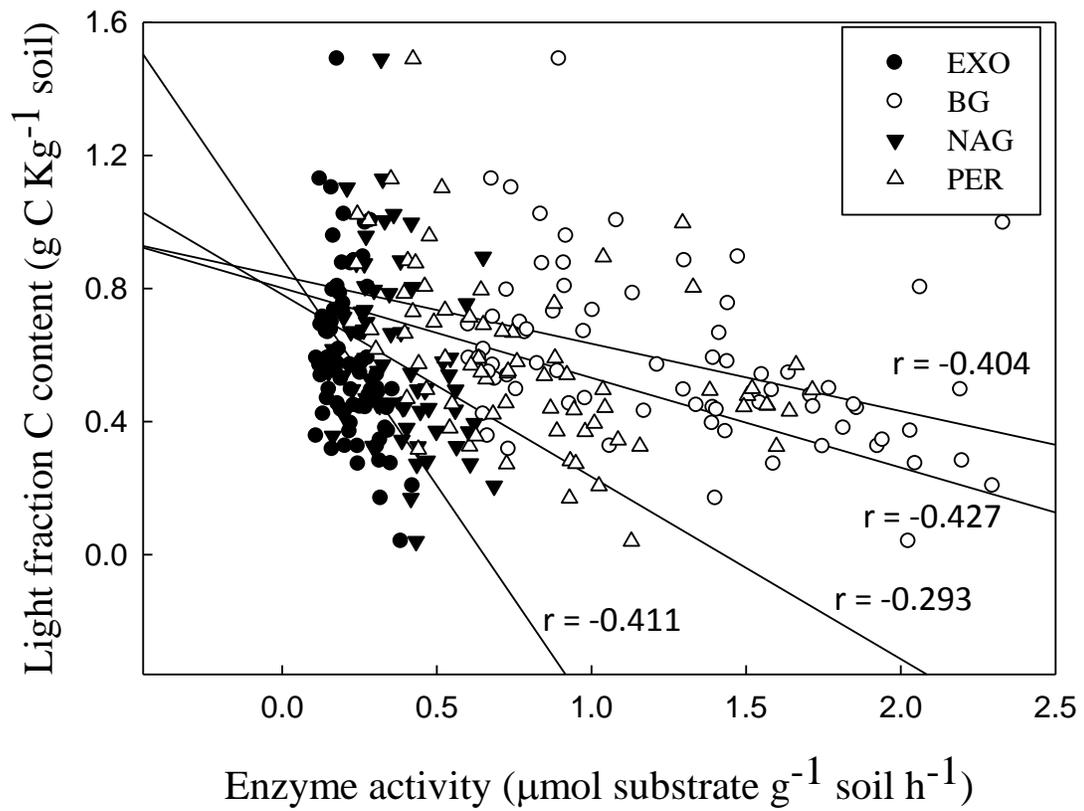


Fig. 4. Correlations between soil light fraction C content and extracellular enzyme activities.

EXO, exoglucanase; BG, beta-glucosidase; NAG, beta-glucosaminidase; PER, peroxidase.

The lowercase r represents Pearson's correlation coefficient when $n = 72$, $\alpha = 0.05$.

Chapter 4: General Conclusions and Future Research Direction

Over decades of “green revolution” that aims to maximize crop yields by applying synthetic N fertilizers, pesticides, and herbicides, environmental issues have emerged and become the key barrier for sustainable agriculture. Now, we are facing an extreme challenge of reconciling conflicting demands of agricultural productivity and natural resource conservation. This leads to a renewed interest in organic farming that excludes synthetic chemicals, but uses green manure, compost, crop rotation, and biological pest control for keeping the health of soil and ecosystem. Transition to organic farming often requires the rapid development of soil fertility and quality. Two questions that I addressed in the present study were: (1) which cover crop management practices could lead to rapid development of soil fertility and quality? and (2) what biological properties could respond rapidly to cover crop management practices?

Newly-established field plots were used to examine the effects of species and termination methods of legume cover crops on soil microbial activities and soil particulate organic matter. Termination methods could modify soil microbial activities and particulate soil organic matter via controls on soil moisture, temperature, as well as microbial accessibility to cover crop residues. Flail was found to be better than disk and spray in improving soil microbial activities, as shown by greater microbial biomass C, C and N mineralization, and nitrification potential. Unlike termination methods, cover crop species directly affected the quantity and quality of C and energy source for soil microbial community. With relatively low C-to-N ratio and lignincellulose index, Austrian winter pea produced the most positive effects on soil microbial

activities. I found that changes in soil microbial properties and processes were negatively associated with soil particulate organic matter. Disk that led to lower microbial activities raised the amount of soil particulate organic matter. Also, crimson clover that led to a higher ratio of C- to N-mineralization reduced the C-to-N ratio of particulate organic matter. Hence, changes in the quantity and quality of soil particulate organic matter further confirmed that cover crop effects determined by soil microbial activities. My results suggested that termination methods that made cover crop residues chopped and surface applied would be more effective in promoting soil microbial properties and processes. Also, legumes that had low C-to-N ratio and lignocellulose index could be better in rapidly stimulating soil microbial properties and processes.

As a small and dynamic pool of soil organic C and N, particulate organic matter changed significantly with cover crop species and termination methods. However, its change should not be used to assess the impacts of cover crop species and termination methods on soil C sequestration. This is because, first, soil particulate organic matter only accounted for a small portion of soil organic matter. Thus, any detectable change in particulate organic matter might have little impacts on the entire pool of soil organic matter. Second, soil microbial biomass turnover also contributed to soil C sequestration. In this study, however, the impacts of termination methods on microbial biomass versus on particulate organic matter were opposite. In the case of cover crop management over one growing season, soil particulate organic matter would not provide holistic, but rather one-sided story on soil organic matter accumulation.

It is generally believed that crop residues can be quickly decomposed into soil particulate organic matter. Compared to the mineral-associated organic fraction that has the mean residence time of hundred to thousand years, particulate organic matter turns over more rapidly, with the mean residence time of a few to ten plus years. While soil particulate organic matter does not contribute long-term soil C sequestration, it may serve as a useful indicator for assessing management practices on soil quality as well as short-term C dynamics. My biggest question is what insights soil particulate organic matter can shed in terms of soil quality. Perhaps, this question can be answered via a meta-analysis, given bulk publications on soil particulate organic matter.

This study also leads to an intriguing question: how early can the effects of cover crop management on soil C sequestration be detected? Because only a small fraction of crop residue-C may be converted into soil organic C in a short period, cutting-edge techniques, such as stable isotope labeling and analysis, will be most suitable for detecting changes in soil organic matter. The origin of newly-formed soil organic matter can also be determined using solid-state ^{13}C nuclear magnetic resonance (NMR) in that this technique differentiates organic matter derived from plant material versus from microbial biomass. With the use of new and cutting-edge technologies, we can gain the information necessary for making sound management decisions.