

EFFECTS OF LONG-TERM CEREAL RYE WINTER COVER CROP ON SOIL QUALITY,  
SOIL N AVAILABILITY AND YIELDS ACROSS A NITROGEN GRADIENT IN A  
RAINFED MICHIGAN CORN SYSTEM UNDER CONVENTIONAL TILLAGE

By

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## ABSTRACT

### EFFECTS OF LONG-TERM CEREAL RYE WINTER COVER CROP ON SOIL QUALITY, SOIL N AVAILABILITY AND YIELDS ACROSS A NITROGEN GRADIENT IN A RAINFED MICHIGAN CORN SYSTEM UNDER CONVENTIONAL TILLAGE

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The objectives of this study were to (1) evaluate the 9-year cumulative effects of a winter rye cover crop on soil quality in a corn-corn-soy rotation, (2) measure N supply and soil N pools after cereal rye WCC incorporation and its impacts on corn N availability across an N fertilizer gradient (0, 34, 67, 101, 134, 168, or 202 kg ha<sup>-1</sup>) during the 2013 growing season, and (3) assess N response to corn yield under WCC and no cover crop (fallow) treatments from 2006 to 2013. This experiment was conducted at Michigan State University's W.K. Kellogg Biological Station using a split-split plot RCBD with a main plot effect of winter rye cover crop versus winter fallow, split into seven subplots, which were randomly assigned to a gradient of N fertilizer. Between 2006 and 2013, the annual aboveground cereal rye WCC accumulation ranged between 0.53 to 1.46 Mg ha<sup>-1</sup>. Cereal rye WCC did not affect soil structure (bulk density, water stable aggregates), soil-water relationships (total porosity, soil water retention), or soil biological activity (POXC, soil enzyme activity, litterbag decomposition rates). Our most interesting finding was the effect of WCC on enhanced POM-C content of the large POM fraction ( $p=0.05$ ), but no other measure of soil organic matter pools. Our results find evidence of synchrony of N mineralization to crop N demand after cereal rye WCC compared to fallow plots during the 2013 corn growing season. Over the 9-yr period of this study, WCC posed no negative impacts on corn yields compared to fallow treatment plots.

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To the explorers of the universe who far too often forget to look under their feet.



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## KEY TO ABBREVIATIONS

WCC: Winter cover crop

SOM: soil organic matter

SOC: soil organic carbon

C/N: Carbon-to-nitrogen ratio

BD: bulk density

TP: total porosity

CEC: cation exchange capacity

WSA: water stable aggregate

POM: Particulate organic matter

POM-C: Carbon in Particulate organic matter

POM-N: Nitrogen in Particulate organic matter

POXC: Permanganate oxidizable C

TAP: tyrosine amino acid peptidase

NAG: N-acetyl- $\beta$ -glucosaminidase

$\text{NO}_3^-$ -N: nitrate

$\text{NH}_4^+$ -N: ammonium

## LITERATURE REVIEW

### *Winter cover crops in corn systems*

Escalating global food and fuel demands have driven Midwestern U.S. farmers to grow consecutive years of corn (*Zea mays* L.) in the U.S. Corn Belt, a historically highly productive region. However, agricultural practices like that of increased tillage and excessive fertilizer applications used in corn production are key contributing factors to soil quality degradation (Tisdall and Oades, 1982; Tilman, 1999; Hatfield, 2013). In addition, farmland use practices from the U.S. Corn Belt have been identified as the cause of increased nitrate ( $\text{NO}_3^-$ ) contamination of surface water (Turner and Rabalais et al., 2003; Burkart and James, 1999). The inclusion of winter cover crops (WCC) is recognized as a promising management tool to mitigate several soil quality and nutrient challenges associated long-season row crops (Aronsson and Torstensson, 1998; Baggs et al., 2000; Andraski et al., 2000; McSwiney et al., 2010). Cover crops, grown in between periods of normal crop production and incorporated into the soil, provide valuable ecosystem services and nutrients for future crops (Shipley et al., 1992; Swinton et al., 2007).

Low N use efficiency of applied fertilized by crop contributes to elevated N concentrations in waterways from non-point-source for nutrient loading is a reoccurring problem in corn agroecosystems (Cassman, 2002; Turner and Rabalais et al., 2003). Inexpensive fertilizers have allowed farmers to excessively apply N to their fields as a form of “yield insurance.” Nutrient loading from agricultural lands into the Mississippi River has resulted in altered natural population dynamics in the Gulf of Mexico (Hatfield, 2013). Imbalances in aquatic nutrients have exacerbated occurrences of hypoxia and anoxia, leading to vast “dead

zones” of contaminated water. Boyer and others (2002) estimate that approximately 60% of U.S. coastal rivers and bays have been degraded by nutrient pollution from agricultural lands. The contamination of the Great Lakes, especially Lake Erie, directly affects the quality of tap water, which is a public health concern. Planting cereal rye as a WCC is one solution that can help reduce the leaching of nutrients into waterways (Sainju et al. 1998; Baggs et al. 2000; Vyn et al. 2000; Dabney et al, 2001).

Desirable attributes of WCC in corn systems include: quick establishment, cold tolerance, residual fertilizer N uptake, and normal growth of the subsequent crop. Although there are trade-offs between choosing one WCC over another, farmers have the ability to maximize cover crop ecosystem services by individualized tailoring of crop selection criteria for their agroecosystem conditions and needs. The short window of opportunity for WCC to be planted in SW Michigan after long-season row crops harvest is the predominant factor limiting WCC selection. Typically, corn harvest occurs between October 23 and November 17, but it can end as late as December 3, making WCC planting challenging for farmers (USDA NASS, 1997). The Midwest Cover Crop Council (MCCC) provides a tool to mitigate these challenges. Cover Crop Decision Tools (<http://www.mccc.msu.edu>), a web-based instrument that assists farmers in the selection of cover crops that can be included in crop rotations. This valuable tool shows farmers how to make decisions considering soil environmental conditions and crop rotation history. In Michigan, WCC options after corn harvest are limited to hairy vetch (*Vicia villosa* L.) and cereal rye (*Secale cereale* L.). Legume crops often are the preferred WCC due to their ability to fix N during the off-season, consequently providing N-credits to the subsequent crop (Waggoner, 1989; Stute and Posner, 1995). Non-legume WCCs, such as cereal rye, can be used as nutrient

management tools because they reduce nitrate ( $\text{NO}_3$ ) leaching and erosion (Dinnes et al., 2002; Ruffo et al., 2003; Strock et al., 2004). Cereal rye is the preferred WCC in the U.S. Midwest because of its winter-hardiness and its exceptional ability to scavenge residual N (Shipley et al., 1992; Ditsch et al., 1993; Bollero and Bullock, 1994).

Winter rye is a versatile cover crop that is known to be one of the hardiest of cereals and is tolerant to a wide range of soil types, nutrient availability and cold temperatures. The use of winter rye has been shown to reduce  $\text{NO}_3^-$  leaching potentials in a variety of soil types (Kuo et al., 1997). In Michigan, N losses via leaching are a concern in annual cropping systems due to the large amount of precipitation from November to April, when fields are left bare. Reduced system N losses can be a key to economic and sustainable corn yields (Warncke et al., 2004; Andraski and Bundy, 2005). Additionally, the deep extensive roots of winter rye improve soil structure and nutrient cycling, relative to other cover crops (Kuo et al., 1997; Rasse et al., 2000; Villamil et al., 2006). As a nutrient management tool, WCCs accumulate and immobilize N in their biomass which is later mineralized and made available for subsequent plant uptake after its incorporation (Kuo and Jellum, 2000; McSwiney et al., 2010).

Research has established the many benefits of using cereal rye as a N management tool, but U.S. farmer adoption rates are relatively low. Singer et al. (2007) estimated that only 18% of corn producers use cover crops in their rotations. Recently, national and local awareness of diminishing soil resources are incentivizing farmers to adopt WCC in their cropping rotations. The Environmental Quality Incentives Program (EQIP) and the Conservation Stewardship Program (CSP) compensate farmers in the Midwest region for planting cover crop (Acuna and Villamil, 2014). Additionally, the USDA-NRCS has been leading promotional and educational

efforts in the US Midwest. Locally, the Great Lakes Commission has contributed \$100,000 to promote soil conservation projects in the Paw Paw River Watershed to reduce sediment contamination of local waterways. These incentive programs are contributing to some the increase in adoption of cover crops in corn-producing fields and education on different types of WCC planting options.

An important obstacle in adoption of WCC by farmers is due to the lack of knowledge about best management practices to reduce potential negative effects on subsequent crops. A common concern with cereal rye WCC use is the risk associated decreased profitability from potential immobilization of N after residue incorporation on summer crop (Waggar 1989; Vyn et al., 2000; Thelen and Leep, 2002). Effective management strategies, particularly residue incorporation timing and method, in relation to environmental parameters (soil moisture and temperature), can help increase the synchrony of WCC services to crop N demand (Baggs et al., 2000). Raimbault et al. (1991) showed that a lag time of two weeks between residue incorporation and field crop planting can increase the ecosystem services of WCC as a green manure. Tollenaar et al. (1993) reported that a two-week delayed kill date increases winter wheat aboveground dry matter from 1.5 Mg ha<sup>-1</sup> to 4.0 Mg ha<sup>-1</sup>. This biomass increase also accounts for greater quantities of phytotoxic compounds that cause delays in corn development. Proper management can suppresses some allelopathic effects often exhibited by winter rye (Mwaja, 1994).

Typically, increasing biomass is a positive WCC attribute to increase SOM pools; however, in the instance of preceding corn, it is important to take factors of allelopathic effects and potential N immobilization into consideration. Krueger et al. (2011) reported that allowing

rye to grow until boot stage resulted in greater soil-N resource depletion, which negatively impacted subsequent corn. Ensuring that WCCs remain in a vegetative stage can maintain a lower C/N ratio in plant tissue. Incorporating WCC while still vegetative can alleviate a common challenge experienced with WCCs and N immobilization (Wagger et al. 1998; Kuo and Jellum 2002). The potential risks associated make it essential that farmers are planning how to properly manage spring biomass accumulations of cereal rye to maximize WCC benefits.

### *Soil quality*

The definitions and assessment of soil quality can be very ambiguous, and often related to concepts of soil productivity, health, and function, which are all related to the chemical, physical, and biological soil properties. The Soil Science Society of America's simplest definition of soil health is "the capacity of soil to function" (Karlen et al., 1997). Cover crops have the ability to improve soil quality by affecting a variety its chemical, physical, and biological properties; they supply organic residue and the products of decomposition, thereby changing soil organic carbon (SOC) pools (Kuo et al., 1997; Sainju et al., 2003). SOC has been recognized as a key indicator of soil quality (Karlen et al., 1997). SOC increases are directly related to the return of *fresh* organic material to soil in both short- and long-term experiments (Larson et al., 1972; Rasmussen et al., 1980; Reeves et al., 1997). WCC effectiveness in increasing or maintaining SOC depends on the quality and quantity of biomass, its N content, management history (crop rotation, incorporation method, seeding rate, fertilizer application), soil characteristics, and environmental conditions (temperature and moisture). Selection of a suitable WCC is important because the quality of organic residue derived from



may influence the quality of the soil.

### *Decomposition and nutrient cycling*

Winter rye immobilizes residual soil N over the wet fall and early spring months. Once biomass is incorporated into the soil in spring, microbial decomposition releases plant-available nutrients (Ranells and Waggoner, 1996; Vyn et al., 2000). Decomposition is one of the most important processes that accounts for C and N cycling in ecosystems. The cycling of nutrients between the soil, plants, and atmosphere is controlled by the degradation of SOM via microbes, which use these raw materials as a source of energy and maintaining cellular functions. Decomposition includes two important processes: mineralization and immobilization, which govern the release of nutrients from organic residue additions. SOM encompasses the plant and animal residues and all of its various decay products.

Mineralization refers to the oxidation of SOM to plant available forms. Immobilization is the opposite; plant available nutrients are taken up by soil microbes and are made temporarily unavailable to the crop. Organic N mineralization of residues encompasses two distinct microbial processes: ammonification and nitrification. Ammonification is the process of converting SOM-N to ammonia ( $\text{NH}_3$ ) by both aerobic and anaerobic bacteria. Nitrification, a process only carried out by *Nitrosomonas* and *Nitrobacter*, is the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ . Although plants can and do absorb  $\text{NH}_4^+$ , the majority of N is taken up as  $\text{NO}_3^-$ .

The fate of N from organic residue additions is determined by the N content of the biomass, reflected by the C/N ratio. Other important factors that control mineralization include soluble C, lignin, and polyphenol content in relation to C. Additionally, many uncontrollable

factors such as soil physical properties, temperature, moisture content and management of incorporation contribute to the rate of mineralization. To optimize organic residue addition benefits, it is paramount that the mineralization of nutrients be synchronized with corn N uptake. Synchronizing N mineralization with uptake would reduce soil nitrate in solution, thus are restricted from leaving the system via volatilization, leaching, runoff, and erosion. However, decomposition is a microbial driven process, which makes control N mineralization difficult in rainfed systems (Mikha et al., 2006).

Immobilization occurs when decomposers assimilate N from soil to continue the decomposition process. As a general rule, approximately 1/3 of applied organic residue C remains in the soil after months of decomposition, while the rest is lost to the atmosphere as CO<sub>2</sub>, a byproduct of microbial respiration. Mineralization is more likely with a narrow C/N ratio in WCC. C/N ratios below 24 have a net mineralization effect. Conversely, C/N ratios above 24 have a net immobilization effect. A C/N ratio maintained near 8 facilitates microbial soil mineral N immobilization for cellular respiration. Organic residue additions with C/N ratios > 8 induce competition for available soil N between microbes and the crop, impacting crop yields. Higher N fertilizer rates may be required to replace N immobilized during decomposition of residues with high C/N ratios to avoid yield losses (Tollenaar et al., 1993). Timing of incorporation of WCC is an important management strategy because it determines the C/N ratio of the residue, thereby determining release of nutrients from decomposition.

The dynamic and transient natures of soil N pools make it challenging to control synchrony between soil N supply and crop N demand. Brennan et al. (2013) reported that soil mineral N following cover crop incorporation varied considerably between years from weather

patterns and management practices (e.g. planting method, date, tillage). In rainfed systems, recent weather events such as record droughts and heavy rainfall exacerbates the challenge of predicting N synchrony. The soil N pool is composed of organic (SOM-N) and inorganic ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) N pools. Nitrogen in SOM is the amount of N the crop obtains from the net mineralization of SOM and incorporated crop residues (Cassman et al., 2002). Economic benefits of WCC are from increasing the organic N pools of soils by adding labile SOM pools with WCC residue.

It is essential that appropriate management practices be implemented to ensure positive WCC effects on yields and nutrient availability. Proper timing of fall planting can increase  $\text{NO}_3^-$  retention through crop stand establishment; winter rye scavenges residual soil N and maintains N in the system (Sainju and Singh, 2001). Additionally, proper tillage management aids in promoting microbial decomposition, necessary for N mineralization by (1) physically breaking down crop residues (increasing residue surface area) and (2) aerating the soil. Chemical killing of WCC can be used as a management strategy to control residue quality for the purpose of reducing immobilization and allelopathic effects. Adequate time between WCC incorporation and subsequent corn planting also reduces allelopathic effects (Tollenaar et al., 1993; Mwaja, 1994). Given that proper management practices are followed, farmers can successfully reduce the potential negative impacts of WCC. Teaching these techniques can increase adoption of WCC by farmers. In addition, it is important to understand how WCC can provide other ecosystems services, most notably improved soil quality. For example, the maintenance of soil quality can optimize microbial decomposition, which may result in more proper synchrony of N mineralization and crop N uptake.

## *Chemical soil quality indicators*

### *1. Soil pH*

Soil pH directly influences enzyme activity and is therefore important in many biological and chemical reactions. It is known to affect nutrient availability (e.g., chemical, adsorption, and precipitation), cation exchange capacity (CEC), plant root growth, soil microbial communities, and the fate and transport of heavy metals and pesticides (Haynes and Naidu, 1998; Brady and Weil, 1999). Sources of soil acidity related to management practices are from changes in SOC and use of ammonium-based fertilizer (Liebig et al., 2002). The benefits of increased SOC on soil pH are reflected in increased buffering capacity. There is limited research about cover crop effect on soil pH. One study by Eckert (1991) reported no changes in soil pH after 4-yr of winter rye WCC in a no-till system experiment.

### *2. Phosphorus*

Phosphorus, an essential plant nutrient is used as an indicator of soil quality. Sufficient P is required for crop seed formation, root development, maturity, quality, and energy accumulation and release during cellular metabolism (Tisdale and Nelson, 1964; Brady and Weil, 1999; Schoenholtz et al., 2000). Soil pH exerts one of the strongest influences on soil P availability to plants, thus it is important that soil pH is maintained for optimal plant P uptake. Aune and Lal (1997) reported a positive relationship between crop yield and Bray-1 P availability. Because N fertilizers are often added to ensure yields, it is important to account for interactions between N fertilizer. Though studies have established the relationship of soil P to soil quality and crop growth, only a few studies have investigated influence of WCC in rotation on P

availability. Villamil et al. (2006) found that winter rye in a corn-soy rotation was effective in trapping soil P under no-till.

### *3. CEC and Available cations*

Cation exchange capacity (CEC) is the ability of a soil to hold and adsorb cations in exchangeable forms, corresponding to the overall negative charge in a soil (Brady and Weil, 1999). Soil pH and CEC are intimately related in that an increase in pH results in an increase in CEC. The use of N fertilizer can indirectly influence CEC by affecting soil pH. Soil cations commonly found in CEC include  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$ . Bivalent  $Ca^{2+}$  and  $Mg^{2+}$  cations improve soil structure through cationic bridging with negative sites in clay particles and SOC. Soil CEC's influence on crop nutrient availability has often been used as a soil chemical property indicator (Schoenholt et al., 2000). Ubiquitous carboxylic acid groups in SOC increase CEC by providing negative exchange sites for the adsorption of cations. In addition, edge and surface charges on clay particles (soil texture) can influence soil CEC. Soils with higher clay content have a higher CEC.

### *Physical soil quality indicators*

#### *1. Soil texture*

Although soil texture is not a soil quality indicator, it is still critical soil properties. Texture refers to the relative contribution of clay, silt and sand particles. As an example, coarse and fine-textured soils are known to have fundamentally different soil properties. Soil texture plays a significant role in soil aggregation, CEC, pH, ions ( $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ), soil moisture and

SOC decomposition rates. Understanding soil texture is important for proper management decision-making. For example, fine-textured soils with high SOM content have a higher buffering capacity than coarse-textured, low SOM soils, and thus require more lime to have an effect of soil pH and CEC.

## *2. Bulk density and total porosity*

Bulk density (BD) is a common indicator of soil compaction and is a measurement of the ratio of dry mass to the total volume of soil and. The total volume not taken up by soil particles (void space) is known as total porosity (TP), and is calculated by BD. Compacted soils can restrict water movement, inhibit plant root growth and limit plant nutrient availability; therefore, maintaining ideal BD, is key these essential soil processes and productivity. Although BD and TP are influenced by inherent factors such as soil texture, manageable factors such as tillage and SOM can affect BD and TP. Enhanced TP has been correlated with SOM content and root abundance (Lamande et al., 2003; Rasse et al., 2000). Studies have concluded that dense roots, formed by winter rye cover crops, can improve bulk density in compacted soil (Williams and Weil, 2004). Other studies suggest that the benefits of cumulative organic matter additions that increase SOC of WCC use ultimately influence BD (Kong et al., 2005; Blanco-Canqui et al, 2011; Steele et al., 2012). However, because organic material additions are small in proportion to the mineral fraction of soil, only minor influences on bulk density after residue additions are observed (Waggoner et al., 1998). Conversely, some studies found no effect of WCC on BD due to the inability of small root channels to change gross soil BD (Chen and Weil, 2011). Contradictory results in the literature are likely due to the fact that soil responses to agronomic

practices require many years of management practice for changes in soil structure to occur (Jokela et al., 2009). Additionally, plowed fields are likely to have more temporally variable BD measurements; therefore it is important to take sampling times and methods into consideration when comparing effects. For example, Steele et al. (2012) found that WCC improved BD only during the months post-rye planting but not during the corn-growing period, suggesting that there is some benefit to BD at certain times of the rotation.

### *3. Soil water retention*

Soil water retention (SWR) information is a practical indicator for the farmer because it calculates plant-available water, which is the soil water content between field capacity (defined at matric potential -33 kPa) and permanent wilting point (defined at matric potential -1500 kPa). An indicator of the soil's ability to retain water necessary to sustain plant water needs between rainfall events or periods of irrigation; it is an important pool of soil water, particularly in rainfed agroecosystems like those of Michigan. Previous studies have shown an inverse relationship between the soil water content and the tenacity with which the water is held in soils; this is a function of the sizes and volumes of the water-filled pores and of the amount of water adsorbed to the soil particles (Hudson, 1994). Management practices can change soil structure and influence water retention properties, thereby affecting aggregate stability and pore space. Other studies suggest that increasing SOC improves SWR by creating greater overall pore space (Colla et al., 2000; Dabney et al., 2001). A 5-yr study by Villamil et al. (2006) showed significant increases in the volume of water held between saturation (0 kPa) and field capacity (-33 kPa) in WCC compared to fallow plots under a corn-soy rotation in no-till. A 15-yr study in south-central

Kansas found the use of cover crops to have no significance on SWR or plant-available water under no-till-corn-soy rotation (Blanco-Canqui et al., 2011). Overall, there is little evidence to date that suggests a positive effect on SWR from long-term use of WCC, especially under conventional tillage.

#### *4. Distribution of water stable aggregates*

Soil structure relates to the arrangement of primary soil particles in groupings called aggregates, which create a pattern of pores that define the functions of soil. Soil structure favorable to plant growth in agricultural production is essential to increase soil fertility and agronomic productivity. The ecological importance of soil is often associated with the size and distribution of pore space created by soil structure (Horn et al., 1994). The development of soil structure is a complicated process that has biological, chemical, and physical components. The formation and maintenance of soil structure is primarily mediated by SOC; however, soil biota, ionic bridging, clay, and carbonates also play a role (Horn et al., 1994; Bronick and Lal, 2005).

Soil aggregates are often grouped into size classes; in general, macroaggregates are  $>250\text{ }\mu\text{m}$ , and microaggregates are  $<250\text{ }\mu\text{m}$  (Tisdall and Oades, 1982). The aggregate hierarchy theory suggests that microaggregates are the building blocks of soil structure because the actions of temporary and transient binding agents on microaggregates form macroaggregates (Tisdall and Oades, 1982; Elliott, 1986). Alternatively, macroaggregates can form around POM (Bronick and Lal, 2005). Microbial exudates from the decomposition of POM may be an import agent that cement microaggregates into more stable, low C/N macroaggregate (Mendes et al., 1999). This results in macroaggregates with a large concentration of low density POM, a more labile



SOC pool, and thus there are primary sources of nutrients that are more susceptible to loss from tillage (Elliott, 1986; Grandy and Robertson, 2006).

Some studies have found significant changes in soil aggregates with WCC. In a study that included WCC in rotation with corn-soy rotation under no-till, Villamil et al (2006), found water aggregate stability significantly increased with WCC use under soybean, but not when corn was in the rotation. This study concluded that the higher water aggregate stability was attributable to the greater root mass of WCC since no changes in SOC were detected. Steele et al. (2012) confirmed these results of temporary benefits in aggregate stability in a long-term no-till WCC study in the Costal Plains. Liu et al. (2005) demonstrated that incorporating cover crops into the soil rapidly increases the POM pool in soil, promoting soil aggregation. A 3-yr study by Sainju et al. (2003) found that non-legume cover crops had more intermediate-sized aggregates and macroaggregates (2 to 0.85 mm) than legume cover crops and no cover crop systems. This study also suggested that non-legume cover crops might increase microbial activities, and C sequestration, thus improving overall soil quality. Although there has been significant work regarding the influence of WCC on water stable aggregates, there is no general consensus. Mendes et al. (1999) and Schutter and Dick (2002) found no effect of WCC on the aggregate size distribution compared to fallow plots. This literature review suggests that there are more studies that are able to increase WSA with WCC use under no-till systems due to the transient soil benefits under conventional tillage regimes (Chan and Heenan, 1996). However, Hermawan and Bomke (1997) found residual effects of WCC on increased soil aggregation even after spring tillage operations.

## *Biological soil quality indicators*

### *1. Labile SOC pools as POM and POXC*

The limitations of using SOM as an indicator of soil quality stems from its insensitivity to new management practices (Wander and Drinkwater, 2000). However, POM, POXC, and MBC have often been used as measures of SOM labile C pool. Labile C fractions reflect the most readily available energy sources for soil microbes, and thus play an important role in maintaining nutrient cycling in agricultural ecosystems. The differences of POM and POXC, as a measure of labile C, come from their methods. Particulate organic matter is partially decomposed organic residue that is measured by size or density fractionation (Cambardella and Elliott, 1992; Wander, 2004). The C and N concentrations in POM have been found to be elevated in organic fertilizer systems compared to synthetic fertilizer (Wander et al., 1994). Additionally, as described earlier, POM is thought to promote macroaggregate formation POXC is a chemical process where  $\text{KMnO}_4$  is used to oxidize SOC. Culman et al. (2012) observed greater sensitivity to changes in management in the POXC fraction than POM-C with some management practices. They concluded that POXC is closely related to smaller sized (0.053– 0.250 mm) and heavier ( $>1.7 \text{ g cm}^{-3}$ ) POM-C fractions. However, Plaza-Bonilla et al. (2014) found that POM-C reflected changes in SOC with greater sensitivity than POXC to N fertilizer management.

### *2. Litterbag decomposition*

Although litter bag experiments are prone to many limitations, they are a widely accepted method to study organic litter decomposition (Hector et al., 2000; Berg and McClaugherty, 2007). Studies are inconsistent on the effects of N fertilizer or N availability on litter decomposition

rates. In a meta-analysis, Knorr et al. (2005) concluded that N fertilizer can alter rates of litter decay, but the direction and degree of response are mediated by the rate of fertilizer and quality of litter.

### *3. Extracellular soil enzyme activity*

There is a wide array of extracellular enzymes in soil that carry out numerous chemical and biological transformations of SOM. Two commonly studied enzymes are tyrosine amino acid peptidase (TAP), which is a phenol oxidase that regulates the breakdown of aromatic compounds, and N-acetyl- $\beta$ -glucosaminidase (NAG), playing a role in N metabolism, as well as vital roles in SOM mineralization. Grandy et al. (2013) found N fertilization increased the activities of two hydrolase enzymes involved in simple carbohydrate metabolism ( $\beta$ -d-cellobiohydrolase and  $\beta$ -1,4-glucosidase) and periodic increases in one related to N metabolism ( $\beta$ -1,4-N-acetylglucosaminidase), but had no effects on enzymes regulating the breakdown of aromatic compounds (phenol oxidase). Understanding the role of these enzymes in relation to management practice is important since they mediate key functions on C and N mineralization, and thus SOC formation.

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# CHAPTER ONE: EFFECT OF LONG-TERM CEREAL RYE COVER CROP ON SOIL QUALITY ACROSS A NITROGEN GRADIENT IN A MICHIGAN CORN SYSTEM UNDER CONVENTIONAL TILLAGE

## ABSTRACT

The inclusion of winter cover crops (WCC) in crop rotation to abate problems associated with reduced soil quality is often promoted in the U.S. Corn Belt, however the evidence to support improvements in soil quality is often inconsistent. There is a need for evidence on the extent to which soil quality is enhanced by WCC under conventional tillage practices, and a corn-dominated rotation sequence. The objective of this field-based study in southwest Michigan was to evaluate the 9-year cumulative effects of a winter rye cover crop on soil quality in a corn-corn-soy rotation. Soil quality was assessed through measures of improved soil structure (bulk density, water stable aggregates), improved soil-water relationships (total porosity, soil water retention), increases in labile SOM pools (POM, POXC), and enhanced soil biological activity (soil enzyme activity, litterbag decomposition rates). This experiment was conducted from 2006-2013 at Michigan State University's W.K. Kellogg Biological Station using a split-split plot randomized complete block design with a main plot effect of winter rye cover crop versus winter fallow, split into seven subplots, which were randomly assigned to a gradient of N fertilizer (0, 34, 67, 101, 134, 168, or 202 kg ha<sup>-1</sup>). There were several lines of evidence that WCC modestly increased winter cover biomass (less than 2 Mg ha<sup>-1</sup>), but had no detectable impact on soil quality. WCC did not effect soil structure, soil-water relationships or soil biological activity. Presence of a WCC did enhance POM-C in the large POM fraction (p=0.05), but no other measure of soil organic matter pools. The effects of WCC in soil quality indicators

may be limited from the realistic practice of planting cereal rye WCC after harvest of corn or soybean, imposing a short growth window within which the WCC can accumulate biomass. Conventional tillage practices in this study likely further diminished the effects of WCC biomass on soil quality. Further research is needed to determine the long-term effects of WCC on soil quality indicators under conventional tillage in northern U.S. Corn Belt states.

## 1.1 Introduction

The inclusion of winter cover crops (WCC) in crop rotation to abate problems associated with reduced soil quality is often promoted in the U.S. Corn Belt. However, farmers are often limited in the choice of WCC that can be planted after corn harvest due to a narrow planting window in autumn, particularly in the upper Midwest. For example, corn harvest in the state of Michigan generally ranges from October 23 - November 17, but it can be as late as December 3 (USDA NASS, 1997). The very short window of opportunity for planting after a late harvest limits the options of available WCC that can be successfully planted and established before freezing. Winter rye (*Secale cereale* L.) is the most cold tolerant of winter cereal cover crops and is the most widely used WCC in the upper Midwest (Snapp et al., 2005). Previous studies show that winter rye can be an important tool for managing N to reduce  $\text{NO}_3^-$  leaching potential (Aronsson and Torstensson, 1998; Andraski et al., 2000; Baggs et al., 2000; McSwiney et al., 2010). In addition, winter rye produces large amounts of aboveground biomass compared, and develops extensive, deep roots that potentially could improve soil structure (Kuo et al., 1997; Villamil et al., 2006).

In the simplest of terms, soil quality is the capacity of soil to function (Karlen et al., 1997). Soil quality encompasses chemical, physical and biological properties, and it is important to investigate a range of properties to understand this complex attribute of soils. Soil organic carbon (SOC), though its interdependence on fundamental soil process has been recognized as a key indicator of soil quality (Reeves, 1997). Incorporating organic residue of WCC can increase labile SOC pools, which will ultimately lead to improved soil quality, which in turn can improve soil productivity (Rasmussen et al., 1980). The addition of fresh plant material to soil begins the

process of decomposition by microbial action. After incorporation, there are two pathways of decomposition: mineralization and immobilization. The process of decomposition and mineralization converts organic nutrients to plant-available forms. Immobilization converts inorganic forms of nutrients to organic forms in microbes as cellular components, making them temporarily unavailable for plant uptake. An immobilization effect from recalcitrant WCC tissues can be a useful tool in conserving nutrients in root zone in between growing seasons (McSwiney et al., 2010).

There is evidence that both supports and refutes the ability of WCC to improve soil quality. Changes in soil quality are heavily influenced by soil texture, which varies vastly throughout the landscape. Management practices such as planting dates, WCC biomass production, residue quality, rotation history, tillage practices, and incorporation methods can all influence WCC effects on soil quality. Considering that changes in soil quality parameters can be gradual, and may not become detectable for many years, contradicting research outcomes are often attributable to the differences in study duration. Blanco-Canqui et al. (2011) reported that the addition of cover crops improved near-surface soil physical and hydraulic properties and increasing the SOC concentration in south-central Kansas in no-till after 15 consecutive yrs of cover crops use. In another long-term no-till study, Villamil et al. (2006) assessed the effects of WCC on several soil physical and chemical properties, and concluded that, compared with fallow plots, cropping rotations with WCC provided substantial improvements in soil productivity. Villamil and others (2006) reported that WCC increased SOM, increase in proportions of water stable aggregates, reduced bulk density and penetration resistance, increased total porosity, plant-available water, and soil water retention, and was also more

effective in trapping soil P compared to fallow plots.

Timing of soil quality measurements relative to agronomic operations also has an influence on soil properties, and thus on response to management. For example, Stelle et al. (2012) evaluated the long-term effects of WCC on soil physical properties after 13 years in Maryland, USA under no-till. Their results suggested that benefits to soil physical properties were detectable, but only for a couple months post-rye planting. Compared with winter fallow, improved bulk density, air permeability, water infiltration rate and hydraulic conductivity were observed in the surface soil layer under WCC. However, they observed no differences in soil labile C or total organic carbon (TOC) between WCC and fallow soils.

Despite the evidence that WCC can improve soil quality, there is limited knowledge of cover crop impact within a realistic rotation sequence. As corn has become one of the most profitable field crops in recent years, larger production areas are being allocated to corn; this has consequences for WCC planting times as corn is the latest harvested field crop (often in November). A corn-corn-soybean rotation, sequence has a very limited window for cover crop integration, severely limiting the amount of biomass that can be produced. Termination of the WCC may be necessary before the exponential growth phase can occur within this rotation sequence, and information is completely lacking regarding the biomass accumulation potential and impact on soil properties.

There are other challenges to cover crop adoption, and indeed uptake rates in Midwest field crops remain under 20% (Singer et al., 2007). Barriers to adoption include the potential risk of yield penalties in subsequent corn crops from asynchrony in N mineralization or allelopathic

effects when WCC is mismanaged (Crandall et al., 2005; Krueger et al., 2012). This requires close attention and data from multi-year studies to evaluate yield under different weather conditions. Interactions of weather with yield could include rainfall and temperature impact on N mineralization patterns, and in deficient years, cover crop water use has a negative impact on a cash crop (Smith et al., 2007).

In addition, previous research has focused generally on WCC in no-till systems (Villamil et al., 2006; BlancoCanqui et al., 2011; Stelle et al., 2012). While no-till management is growing, many farmers still use conventional tilling methods. Because reduction in tillage disturbance also has the potential to increase SOC sequestration, infiltration, and aggregate stability, it can confound efforts to understand the effect of WCC on soil quality (Angers et al., 1997; Six et al., 1999; Yang and Wander, 1999). There is a need to find evidence that show improvements in soil quality with WCC under conventional tillage practices. Lastly, because changes in soil quality indicators are gradual and may take many years of management practices to detect, it is likely that evidence of benefits of WCC use is more detectable in long-term studies.

We report here on a study established in 2006 to address corn yield response to N fertilizer rate and WCC. In the ninth and final year of the study, the cumulative effects of WCC on physical, chemical, and biological soil quality indicators in a corn-corn-soy rotation under conventional tillage practices were quantified. The objective of the study was to evaluate the effects of a winter rye cover crop on soil quality useful to agroecological farm management. Our main selection criteria for soil quality indicators were based on its varying degrees to sensitivity to management-induced changes. We hypothesized that under conditions conducive to cereal rye cover crop growth, detectable changes in soil quality would occur including with improved soil



structure (bulk density, water stable aggregates), improved soil-water relationships (total porosity, soil water retention), increases in labile SOM pools (POM, POXC), and enhanced soil biological activity (soil enzyme activity, litterbag decomposition).

## 1.2 Materials & Methods

### *1.2.1 Site and soil description*

This experiment was conducted at Michigan State University's W.K. Kellogg Biological Station (KBS) located in SW Michigan (42°24' N, 85°24' W, elevation 288 m), in the eastern portion of the U.S. Corn Belt. The soil at KBS is a well-drained sandy loam dominated by Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) soil series with A, E and B soil horizons (Crum & Collins, 1995). Mean annual temperature is 10.1°C with a total annual precipitation of 1,005 mm, about half of which falls as snow. More information about the KBS Long Term Ecological Research Station can be found online at <http://lter.kbs.msu.edu/datasets>.

### *1.2.2 Experimental design*

In fall of 2005, this study was established as a split-split plot randomized complete block design that included 14 treatments and 4 replications, with a total of 56 experimental subplots. The main plot consisted of two winter management systems: winter cover crop (WCC) or no cover crop (fallow). Main plots were divided into seven subplots, which were randomly assigned nitrogen (N) fertilizer rates of 0, 34, 67, 101, 134, 168, or 202 kg N ha<sup>-1</sup>. The size of each subplot was 9 x 9 m and contained six rows of crop. The subplot treatments remained the same for the 9-year duration of the experiment under a corn-corn-soybean rotation; full cropping history can be found in Table 1.1.

### 1.2.3 Agronomy

From 2006 to 2013, all experimental plots received the same agronomic management under a corn-corn-soy rotation (Table 1.2). After crop harvest in the fall, cereal rye (*Secale cereale* L.) was drilled at 134 kg seed ha<sup>-1</sup> as winter cover crop on plots receiving cover treatment. Planting date of cereal rye generally occurred in November. In early May, plots were sprayed with glyphosate (0.46 AI ha<sup>-1</sup>) to chemically kill cover crop and weeds. Two weeks after killing, biomass was incorporated with a chisel plow at an approximate 20 cm depth. The soil bed was prepared with a soil finisher (cultivator) and corn (*Zea mays* L.) or soybean (*Glycine max* L.) was planted.

### 1.2.4 Cover crop measurements

Biomass samples of cereal rye and weeds in WCC plots and weeds in fallow were measured prior to chemical killing by collecting above ground plants. Biomass samples were collected in 2007, 2009, and 2013. In 2013, weeds found in all subplots consisted mostly of Shepard's purse (*Capsella bursa-pastoris* L.) and annual bluegrass (*Poa annua* L.). Aboveground biomass was harvested from two 0.5 × 0.5 m quadrats per subplot. Plant samples were oven-dried at 60 °C to constant mass and data are reported as dry biomass weight (Mg ha<sup>-1</sup>). A subsample was then ground to pass a 1-mm screen in a Christy-Turner 8-inch (20.3 cm) Lab Mill (Ipswich, Suffolk, UK) and analyzed for total C and N using a Costech Elemental Combustion Analyzer (ECS4010, Valencia, CA). Additionally, winter rye biomass data were supplemented with growing degree day (GDD) data obtained from [www.climate.geo.msu.edu](http://www.climate.geo.msu.edu).

### *1.2.5 Soil quality indicators*

Data for soil quality indicators were collected only during the final year of the study in 2013. A variety of soil quality indicators were measured in this study to represent the numerous physical, chemical and biological processes that encompass soil function. Soil quality indicators that were assessed in this study and the rationale for selecting indicator can be found in Table 1.3.

#### *1.2.5.a Soil texture*

Although management practices do not influence soil texture, it is still a significant variable in soil quality. Soil samples were collected on 29 April 2013 with soil probes (2 cm dia.) and eight corers were composited from the 0 to 20 cm depth from each of the 56 subplots. Samples were sieved through a 6-mm sieve and subsamples were sent to MSU Soil and Plant Nutrient Laboratory (East Lansing, MI) for particle size analysis (soil texture) determined as percent clay, silt and sand.

#### *1.2.5.b Soil pH, total C, extractable P, K, and secondary micronutrients*

Soil samples collected on 29 April 2013 were also sent to A&L Great Lakes Laboratory (Fort Wayne, IN) for analysis. Soil pH was determined in a 1:1 soil to water slurry. Soils were extracted using the Mehlich III method to determine available P, reported as Bray P-1 (Bray and Kurtz, 1945; Mehlich, 1984). Exchangeable cations potassium, magnesium, calcium were correlated and reported as a 1N ammonium acetate ( $\text{NH}_4 \text{C}_2\text{H}_3\text{O}_2$ ) extraction (McIntosh, 1969). Results used to calculate CEC and percent base of saturation of soil cations. Soil samples

collected on 29 April 2013 were also analyzed for total C. Dry samples of whole soil were pulverized in a Shatterbox grinder and analyzed for total C and N using a Costech Elemental Combustion Analyzer. Total C data is reported as  $\text{kg C ha}^{-1}$  soil.

#### *1.2.5.c Bulk density & total porosity*

After corn harvest in 2013, soil cores were collected to 1-m depth. Blocks 1 and 2 were sampled on 19 November 2013 and blocks 3 and 4 were sampled on 22 November 2013. Three intact cores (7.6 cm dia.) were taken with a hydraulic probe (Geoprobe Model 540MT, Geoprobe Systems; Salina, Kansas) from all 56 subplots for a total of 168 soil cores. Cores were stored at 5 °C until analysis. Bulk density was measured in specific depth increments of 0-20, 20-40, 40-70 and 70-100 cm. Bulk density was determined using subsamples of oven dry soil mass and volume of core increments as listed above and is reported as  $\text{g cm}^{-3}$ . Total porosity was calculated as a function of bulk density, where particle density was assumed to be  $2.65 \text{ g cm}^{-3}$ .

#### *1.2.5.d Soil water retention*

To measure soil water retention, one subsample was taken from the 0 to 20 cm increment of the soil cores used for bulk density. Each core was treated as a replicate. The subsample was collected by driving a 5 cm (4.84 cm dia.) aluminum core into the larger intact soil core collected from hydraulic probe. To avoid compacting soil in aluminum core, the outer pipe was cut away to carefully remove soil within the aluminum core. A subsample was collected to measure gravimetric soil moisture content. A Whatman No. 1 filter paper (GE Healthcare Bio-Sciences, Pittsburg, PA) and a piece of cheesecloth were attached to one end of the aluminum soil core

with a rubber band. Excess cheesecloth was removed and the sample was weighed. The entire soil core assembly was wrapped in aluminum foil and stored at 5 °C until soil water retention analysis.

Soil cores were placed in a plastic tub lined with cheesecloth and filled with enough deionized water to saturate them from the bottom up. Initial saturation weight was recorded after soil cores had been saturated in deionized water for 24 h. The cores were then placed on a metal grating to allow water to drain freely. The top surfaces of all cores were wrapped with plastic to prevent evaporation. Each core was weighed after draining for 24 h. Cores were then saturated again and then placed on a ceramic plate inside pressure chamber apparatus (1600 Pressure Plate Extractor, Soil Moisture Equipment Corp., Goleta, CA) to measure desaturation.

Pressure plates were soaked in distilled water overnight before being used in pressure chamber apparatus. The 100-kPa ceramic plates that allowed only water to flow were covered with deionized water to 1-mm depth before saturated soil cores were loaded onto plate. Samples made good contact with the ceramic plate to ensure hydraulic conductivity. One to two plates were placed inside each chamber and each plate was pressurized for 4 to 5 d to force water to flow out until the force exerted by the air pressure and the force by which the water was held by the soil reached equilibrium. Equilibrium of soil cores was confirmed when water ceased to drain from the pressure chamber. The following pressures were used to create water retention curves: 0, -5, -10, -33, -100 kPa. Soil cores were removed from the pressure chambers and were weighed at each pressure increase. Volumetric water content ( $\text{cm}^3 \text{ cm}^{-3}$ ) was determined for each replicate by soil mass.

#### *1.2.5.e Water-stable aggregates (WSA)*

On 29 October 2013, five soil cores were collected from WCC and fallow plots that received 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer to measure water-stable soil aggregates. PVC pipes (7.6 cm dia.) were driven into the soil with a mallet to a depth of 10 cm. Cores were collected in-row between corn plants to avoid tractor track compaction sites in the furrow. Bulk soil was weighed, sieved through a 6-mm sieve, weighed again, and composited. Any material remaining on the 6-mm sieves was discarded. A subsample of 25 g was oven-dried at 105 °C to constant mass to measure gravimetric soil moisture content. A second subsample was air-dried on a watch glass overnight in a fume hood. Air-dried soil aggregates larger than 2 mm and smaller than 4 mm were collected with sieves. Two-100 g replicates of these aggregates were placed in a single layer on a 2-mm sieve over a cool mist humidifier for 24 h to slowly rewet them. Soil was rewetted to reach field capacity to maximize stability in subsequent wet-sieving procedure.

The 2-mm sieve containing the aggregates were placed on top of a nest of 1 mm, 250 µm and 106 µm sieves. The set of nested sieves was then submerged in distilled water with the water level adjusted so that aggregates on the 2-mm sieve were just submerged. The set of sieves was attached to an oscillator that moved the sieves up and down in distilled water at 35 cycles per min, with amplitude of 3 cm for a period of 10 min. Soil remaining on each sieve was collected into pre-weighed containers, and gravimetric soil moisture was calculated for each aggregate class size.

Water stable aggregate data are reported as oven-dry weight of aggregates remaining on the 2 mm, 1 mm, 250 µm and 106 µm sieves as a percentage of the sample weight at the start of the wet-sieving process. Aggregate-associated C content of each class size was measured by

pulverizing dry samples in a Shatterbox grinder (SPEX SamplePrep, Metuchen, NJ) and analyzing the powder for total C and N using a Costech Elemental Combustion Analyzer.

#### *1.2.5.f Particulate organic matter (POM)*

Eight soil cores were collected in all subplots with soil probes (2 cm dia.) and composited from the 0 to 20 cm depth on 29 April 2013 and were analyzed for POM based on modified methods by Cambardella and Elliott (1993) and Schulte (1988). A subsample of approximately 100 g of fresh soil was sieved through a 6-mm screen was air-dried, air-dried, and half of it was oven-dried at 60 °C to constant mass to determine gravimetric soil water content. Thirty milliliters of 5% sodium hexametaphosphate (HMP) was added to 10 g of air-dried soil in 50-mL polypropylene centrifuge vial and shaken for 6-8 h at 120 rpm. Mixture was poured through 210 µm and 56 µm sieves nested on top of each other over a catch pan. Deionized water was used to collect the large-size POM fraction from the 210 µm and the small-size POM fraction from the 56 µm sieves into pre-weighed aluminum tins. The POM samples were oven dried at 60 °C to constant mass and weighed. Dry samples were pulverized in a Shatterbox grinder and then each size fraction was analyzed for C and N using a Costech Elemental Combustion Analyzer. Particulate organic matter is reported as g POM kg<sup>-1</sup>soil. Contents of C and N in each POM fractions are reported as g C kg<sup>-1</sup> POM, g N kg<sup>-1</sup> POM, and C/N ratio.

#### *1.2.5.g Permanganate oxidizable C (POXC)*

POXC has also been called the following active C, chemically labile organic matter, readily oxidizable C, or labile C. POXC was measured on soils collected at two different times,



during corn grain fill (August) and after corn harvest (November) in 2013. Eight soil cores from 0 to 20 cm depth were collected with soil probe (2 cm dia.) and composited on 30 August 2013 during corn grain fill from WCC and fallow subplots receiving 0, 101 and 202 kg N ha<sup>-1</sup> (total 24 plots). Surface soils to a depth of 20 cm collected on 19 and 22 November 2013 from all WCC and fallow subplots (total 56) were also used to measure POXC. Soil POXC was measured based on methods by Culman et al. (2012).

Two replicates of 2.5 g of air-dried samples were weighed into 50-mL polypropylene vials. To each vial, 18 mL of deionized water and 2 mL of 0.2 M KMnO<sub>4</sub> was added to tubes with soil and were shaken on oscillator for exactly 2 min at 240 opm. Tubes were removed from oscillator and were allowed to settle for 10 min in darkness. After 10 min, 0.5 mL of supernatant were transferred into a second 50-mL centrifuge tube with 49.5 mL deionized water. A 200-μL aliquot of each sample was loaded into a 96-well plate with internal standards, deionized water blanks, four standard stock solutions (0.00005, 0.0001, 0.00015, and 0.0002 mol L<sup>-1</sup> KMnO<sub>4</sub>), and two laboratory reference samples (soil standard and solution standard). Sample absorbance was read with a SpectraMax M5 using Softmax Pro Software (Molecular Devices, Sunnyvale, CA) at 550 nm. Data are reported as μg C kg<sup>-1</sup>soil.

#### *1.2.5.h Soil microbial activity by litter bag decomposition*

To investigate the effects of cover crop history and its influence of decomposition rate of biomass, litterbags were buried 10 cm deep in WCC and fallow subplots that were receiving 0 or 202 kg N ha<sup>-1</sup> treatment (total 16). Four litterbags (12 × 18 cm) made with 2-mm mesh screen were filled with approximately 12 g of air-dried winter rye biomass was uniformly cut to 2- to 4-

cm size pieces. Samples of winter rye cover crop from approximately early reproductive stage of plants were collected in spring of 2013 from the Great Lakes Bioenergy Research Center (GLBRC) site located at KBS. Plant subsamples were ground to pass a 1-mm screen in a Christy-Turner 8-inch (20.3 cm) Lab Mill and analyzed for total C and N using a Costech Elemental Combustion Analyzer.

All litter bags were buried on 19 Jun 2013. One litterbag from each subplot was randomly collected on the following dates: 25 Jul 2013 (after N fertilizer application), 27 Aug 2013 (corn tasseling), 22 Nov 2013 (post harvest), and 16 Apr 2014 (when the ground was no longer frozen). The area where the litterbags were buried was weeded to avoid plant root growth into the bags. Roots and soil attached to the litterbags were removed after collection and air-dried for 1 wk, and weighed. Decomposition rate was assessed in terms of percent mass remaining in litter bag. Mass remaining (%) was calculated on an air-dried basis as  $((\text{initial mass} - \text{final mass}) / \text{initial mass}) \times 100$ .

#### *1.2.5.i Soil extracellular enzyme activity*

Extracellular enzyme activity in soils was measured on soils collected during corn grain fill from the 0 to 20 cm depth from eight composite samples. Samples were taken from WCC and fallow subplots receiving 0, 101 and 202 kg N ha<sup>-1</sup> treatments (total 24 subplots). Two enzymes, tyrosine amino acid peptidase (TAP) and N-acetyl-β-glucosaminidase (NAG), were measured with a fluorescent enzyme assay as described by Grandy et al. (2007). Fresh soil was sieved through a 2-mm screen and weighed 1 g of soil into specimen cup. Samples were stored in -4 °C

until processing. Stock solution of 50 mM sodium acetate ( $\text{C}_2\text{H}_3\text{NaO}_2$ ) buffer was prepped and adjusted to the average soil pH of the soil samples using 0.1 M hydrochloric acid (HCl).

To prepare soil slurry, 125 mL of the stock  $\text{C}_2\text{H}_3\text{NaO}_2$  buffer was added to soil in specimen cup. Slurry was homogenized for 30 s and 200  $\mu\text{L}$  was loaded into 96-well microplates (USA Scientific, FLUOTRAC black immunology plates, Ocala, FL) for fallow plates with standards and substrates. The molarity of standards and substrates for each enzyme was consistent with previous studies. For NAG plates, 50  $\mu\text{L}$  of 4-methylumbelliferone (MUB) was used and 50  $\mu\text{L}$  of 7-amino-4-methylcoumarin (MC) was used for TAP. Plating setup was followed exactly as described in Grandy et al. (2007). After substrate was added, all plates were stored in 15 °C incubator. Plates measuring NAG and TAP activity were incubated for 2 h and 5 h, respectively. After incubation, hydrolytic enzyme reactions were stopped with 1M NaOH. Both TAP and NAG activity was measured by a fluorometric analyzer (Fluoroskan Ascent, Thermo Scientific, Hudson, NH). Soil enzyme activity is reported as  $\text{nmol h}^{-1}\text{g}^{-1}\text{soil}$ .

#### *1.2.6 Statistical analysis*

Experimental design was a split-split plot randomized complete block design with a main treatment of winter cover crop and split-treatment of N fertilizer rate. All statistical analysis was conducted in SAS 9.3 (SAS Institute, 2002). For all analysis used, block and all interactions were considered in the model as a random effect. Cover crop biomass was analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects and year as a repeated measures effect. Percent C, percent N and C/N ratio of cover crop biomass was analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. Sand content, clay

content, soil pH, CEC, Bray-P,  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  analyzed were analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. Bulk density and porosity were analyzed using the GLIMMIX procedure with cover crop use, N fertilizer rate, and depth were fixed effects in the three-way ANOVA model. Soil water retention model parameters were estimated for each water potential measured using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. WSA size distribution proportions were analyzed using the GLIMMIX procedure with WCC, N fertilizer rate, and size class as fixed effects with repetition as a repeated measures effect. Percent C, percent N and C/N ratios of WSA were analyzed separated by size class using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. POM content, percent C, percent N, and C/N ratio in POM of both small and large size fraction POM were analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. Mass remaining in litterbag after burial was analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects with weeks buried as a repeated measure variable. Soil enzymes TAP and NAG were analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. Residuals of all variables were assessed for normality using the UNIVARIATE procedure in SAS 9.3 (SAS Institute, 2002). F-values for all effects and included with indication of effects that were significant. Significant effects are discussed at the  $\alpha = 0.01$ ,  $\alpha = 0.05$  and  $\alpha = 0.10$  probability levels. The covariance structure type selected was compound symmetry and based on BIC criteria. Least square means for fixed effects were separated using appropriate standard errors with output from the PDIFF option of the LSMEANS statement in the GLIMMIX procedure in SAS 9.3. Pearson correlations were calculated with linear regression fits between variables that were tested using the ‘psych’

(Procedures for Psychological, Psychometric, and Personality Research) package in R version 1.4.8 (Revelle, 2014). Graphs were created using R version 3.1.1 (R Core Team, 2014).

### 1.3 Results

#### 1.3.1 *Weather data*

Monthly average temperature data are shown in Table 1.4. Temperature in November is important to consider for WCC establishment and early seedling growth. The average daily temperatures in November of 2006 and 2009 were higher than the 30-yr average, whereas 2012 was lower than the 30-yr average. Average temperatures in 2012 were 32% lower than the 30-yr average (3.2 vs. 5.0).

An integrated measure of temperature effects on WCC growth was provided by growing degree days (GDD) for 2009-2010 and 2012-2013 spring biomass data. The GDD data was calculated using a  $T_{base}$  5 C (41 F) from [www.enviroweather.msu.edu](http://www.enviroweather.msu.edu), which is greater than the 3.3 C that is expected for cereal rye growth (Rector et al., 2009). Both 2010 and 2013 had lower GDD accumulated in early spring (March and April) compared to the 6-yr (2008 - 2013) average. In 2010, 128 GDD were accumulated in November of the previous year, and 264 GDD were accumulated in the early spring, which was 17% less than the 6-yr average (264 vs. 317). A total of 394 GDD were available for cereal rye growth in the 2009-2010 winter. In 2012, 72 GDD were accumulated in November. A warm winter allowed 34 GDD to accumulate in December, but only 180 GDD were accumulated in early spring in 2013, which is 43% below the 6-yr average at this site.

#### 1.3.2 *WCC Biomass*

Biomass accumulation in WCC and Fallow was measured in 2007, 2010 and 2013 for three N levels (Figure 1.1) A three-way ANOVA revealed effects of WCC ( $F=54.46$ ,  $p = 0.01$ ),

N fertilizer rate ( $F=4.85$ ,  $p=0.01$ ), year ( $F=157.32$ ,  $p=0.01$ ), and  $WCC \times \text{year}$  ( $F=29.95$ ,  $p=0.01$ ) on the amount of WCC biomass accumulated when collected in April of each year. For the years monitored, higher WCC plot biomass was observed vs. fallow biomass, ( $F=54.46$ ,  $p = 0.01$ ). Biomass accumulations in WCC plots include both winter rye and weeds, while fallow plots contained only weeds. Weeds consisted mostly of annual bluegrass (*Poa annua* L.) and Shepard's purse (*Capsella bursapastoris* L.) (data not shown). In 2007 and 2010 the average biomass across N rates in WCC plots was higher than in fallow plots (Fig. 1.1). In 2013, biomass accumulation in WCC and fallow plots did not differ and averaged  $0.56 \text{ Mg ha}^{-1}$  overall (Fig. 1.1).

The amount of biomass accumulated was also impacted by the N fertilizer rate ( $F=4.85$ ,  $p=0.01$ ). More biomass accumulated in plots with higher N fertilizer applied (Fig. 1.1). In 2013, there were no effects of WCC treatment on C/N ratio (ranged from 24 to 28) of vegetation collected from WCC and fallow plots (data not shown). Effects of N fertilizer on biomass C/N ratio did occur ( $F=3.23$ ,  $p=0.01$ ) and an interaction of  $WCC \times \text{N fertilizer rate}$  was observed ( $F=1.86$ ,  $p=0.10$ ) (data not shown).

### 1.3.3 Soil chemical properties and soil texture

Results from soil texture and chemical properties are in Table 1.5. The soil at the experimental site contains an average of 16.6% clay and 39.8% sand. Nine years of WCC use did not influence soil pH, CEC, Bray-1 P,  $K^+$ ,  $Ca^{+2}$ , or  $Mg^{+2}$  content (Table 1.5). Increasing N application rate, however, was associated with a decrease in soil pH, CEC, Bray P,  $K^+$ ,  $Ca^{+2}$ , and  $Mg^{+2}$  content.

#### *1.3.4 Bulk density (BD) and total porosity (TP)*

Results from the effects WCC on BD and TP are presented in Figures 1.2 and 1.3, respectively. The use of WCC and N fertilizer from 2006 - 2013 did not affect BD or TP (Table 1.6). BD and TP both differed with depth ( $p = 0.01$ ). BD was highest at the soil surface (0 - 20 cm) layer with an average of  $1.63 (0.01) \text{ g cm}^{-3}$ . The average TP in this layer was 38.4%. The 20 - 40, 40 - 70, and 70 - 100 cm depth increments had average BD of 1.57 (0.01), 1.56 (0.01), and 1.49 (0.02)  $\text{g cm}^{-3}$ , respectively (Fig. 1.2). TP values were 40.1%, 41.2% and 43.8% from the 20 - 40, 40 - 70, and 70 - 100 cm depth increments, respectively (Fig. 1.3)

#### *1.3.5 Soil water retention*

The average soil volumetric water content at 0, -5, -10, -33 and -100 kPa water potentials were used to generate water retention curves that reflect the forces associated with holding water in soil (Figure 1.4, Table 1.7). Nine years of cereal rye WCC use had no detectable effect on soil water retention (Table 1.7). An effect of N fertilizer application was observed at -5, -10, -33 and -100 kPa water potentials ( $p = 0.10$ , Table 1.7). Soil water retention tended to be slightly higher in subplots with lower N fertilizer rates. Plots with zero N fertilizers had an average of  $0.33 \text{ cm}^3 \text{ cm}^{-3}$  volumetric water content while plots receiving  $202 \text{ kg N ha}^{-1}$  averaged  $0.31 \text{ cm}^3 \text{ cm}^{-3}$ .

Pearson correlation analysis detected only weak associations among bulk density, clay content, microaggregate, and macroaggregate content. A trend was observed for microaggregate content to be negatively associated with volumetric water content at 0, -5 and -10 kPa ( $r^2 = -0.48$ , -0.29 and -0.28; all  $p = \text{NS}$ ), all other correlations were 0.25 or less (data not shown).



### 1.3.6 Water stable aggregates

The use of WCC had no effect on the distribution of proportion of soils present in the WSA size classes measured (Figure 1.5, Table 1.8). However, there were differences in the proportion of soils in each size class ( $p=0.01$ ). On average, 22.8 (0.2)% of the soil was in the 2 - 4 mm (macroaggregate) WSA size class. Only 4.2 (0.1)% of the soil consisted of 1 - 2 mm sized aggregates. 22.3 (0.1)% of the soil were WSA sized 0.250 - 1 mm and 12.2 (0.1)% of the soil fell in the 0.106 - 0.250 mm (microaggregate) WSA size class (Fig. 1.5). There was no clear trend in the relative distribution of soil present in each WSA size class, where an interaction of N fertilizer rate  $\times$  size class was observed ( $p=0.10$ , fig. 1.6). Nitrogen fertilizer rate influenced size class distribution, with slightly lower proportion of macroaggregates being observed for zero N (20.2%) versus for the highest N rate, 202 kg N ha<sup>-1</sup> (25.3%), fig. 1.6.

There was no effect of the 9-yr use of WCC on the percent of C and N in any given WSA size class (Figure 1.6, Table 1.7). The interaction between N fertilizer rate  $\times$  size class influenced both percent C ( $p=0.01$ ) and percent N ( $p = 0.01$ ). In 202 kg ha<sup>-1</sup> N fertilizer plots, microaggregates contained a high percent C compared to zero fertilizer plots (1.04 vs. 0.93), fig. 1.6. In contrast, macroaggregates from 202 kg ha<sup>-1</sup> N fertilizer plots contained a slightly lower percent C than zero fertilizer plots (0.95 vs. 1.01), fig. 1.6.

The C/N ratio of WSA depended on size class ( $p = 0.01$ , Table 1.8, Fig. 1.6). Macroaggregates contained lower C/N ratios than microaggregates (10 vs. 12), fig. 1.6. Microaggregates in plots receiving the 202 kg ha<sup>-1</sup> N fertilizer rate had a higher C/N ratio than those in plots receiving zero N fertilizer (12 vs. 11), fig. 1.6.

### 1.3.7 Soil organic carbon pools: POM & POXC

This study evaluated POM in two size fractions: large (210 - 2000  $\mu\text{m}$ ) and small (53 - 210  $\mu\text{m}$ ). Overall, soils contained 65% more large fraction POM than small fraction (Table 1.9). The use of WCC for 9 years did not affect the amount of POM content in WCC vs. fallow plots for either size fraction (Table 1.10).

In the large POM fraction, there were effects of WCC on POM-C ( $p=0.05$ ), table 1.10. In addition, in the large POM fraction, there were effects of N fertilizer rate on POM-C ( $p=0.01$ ), POM-N ( $p=0.01$ ), and the C/N ratio ( $p=0.01$ ), table 1.10. Nitrogen enrichment, realized through lower C/N ratios in large fraction POM pools was observed with higher N fertilizer rates. Treatment plots receiving zero and 101 kg N ha<sup>-1</sup> had C/N ratios of 23 and 21, respectively (Figure 1.7). Plots with the highest N fertilizer (202 kg N ha<sup>-1</sup>) had an average POM C/N ratio of 4.

In the small POM fraction, the ANOVA analysis detected effects from interaction of WCC  $\times$  N fertilizer rate on POM-C ( $p=0.01$ ), POM-N ( $p=0.05$ ), and C/N ratio ( $p=0.10$ ), table 1.10. However, there were no clear trends of WCC and N fertilize rate effects on POM-C, POM-N, and ratio in small POM fraction (Figure 1.7). Plots receiving zero and 101 kg N ha<sup>-1</sup> fertilizer had higher POM-N in WCC plots compared to fallow plots in the small POM fraction pool, fig. 1.7. Interestingly, plots receiving 202 kg N ha<sup>-1</sup> fertilizer had lower POM-N in WCC compared to the fallow plots. Overall, high N applications increased the C/N ratio in small sized POM fractions (Figure 1.7).

POXC values from the two sampling dates are reported in Table 1.11. There were no effects of WCC on POXC (Table 1.11), whereas N fertilizer generally had a suppressive effect.

There were no clear trends of N fertilizer effect on POXC at grain fill. In this time period, 101 kg N ha<sup>-1</sup> fertilizer plots were associated with the highest POXC while zero and 202 kg N ha<sup>-1</sup> were associated with lower POXC values (Table 1.11). At the post harvest sampling in November 2013, N fertilizer rate negatively influenced POXC; highest values of 383 µg C (kg soil)<sup>-1</sup> in zero fertilizer plots, and lowest of 336 µg C (kg soil)<sup>-1</sup> was in 202 kg N ha<sup>-1</sup> plots, Table 1.11.

### *1.3.8 Litterbag Decomposition and soil enzymes*

The historic use WCC and N fertilizer did not affect the decomposition rate of winter rye litter (Figure 1.8). The amount of biomass lost reflected the number of weeks it had been buried (F=20.32, p=0.01). The average C/N ratio of the initial winter rye material in litterbag was 37. After 5 weeks of burial, all litterbags collected contained an average of 71.5 (1.1)% of their original mass (Figure 1.8). After 43 weeks of burial, an average of 36.7 (5.3)% of the initial mass remained in litterbags (Figure 1.8). There was a numeric difference; slightly higher biomass remained in the 202 kg N ha<sup>-1</sup> fertilizer plots than in the zero fertilizer plots (not significant). A Pearson correlation analysis of soil enzyme activity and mass remaining in litterbags (10 weeks after burial) indicated a weak, negative correlation (NAG: r<sup>2</sup>=-0.37, p = NS; TAP: r<sup>2</sup>=-0.25, p = NS).

There was no detectable effect of WCC use or N fertilizer rate on NAG and TAP activity in soils (Table 1.12). The overall experimental site contained 15-fold more NAG activity than TAP across all plots. Pearson correlation analysis found both NAG and TAP activity to be correlated to soil macroaggregate content (NAG: r<sup>2</sup>=0.49, p = NS; TAP r<sup>2</sup>=0.36, p = NS).

## 1.4 Discussion

### 1.4.1 *Weather data*

An important opportunity afforded by this long-term study is to explore how diverse weather patterns influence cover crop growth and consequences for soil quality parameters. This is relevant to realistic field crop rotation sequences, as integration of WCC is particularly challenging within the narrow planting window between harvest of the cash crop and the onset of winter. Cereal rye is uniquely suited to this window, as it can germinate at temperatures as low as 1.1 C (34 F) (Rector et al., 2002).

Average daily temperatures in November and GDD data over the 9 years of this study suggest that generally there was sufficient warmth for rye germination and establishment during the month of November. Most cereal rye cultivars need a minimum of 260 - 350 GDD to produce a satisfactory stand as a WCC. However, growing requirements can vary by species and cultivar (Nuttonson, 1958; Mirsky et al., 2009). Late fall and early spring months weather at this site accumulates enough GDD for satisfactory WCC growth, which may entice farmers to adopt WCC into a cropping rotation with corn.

### 1.4.2 *WCC Biomass*

Biomass accumulations of winter rye and weeds from WCC plots and weeds from fallow plots that were measured in 2007, 2010 and 2013 are reported for the 2006-2013 duration of this experiment (Figure 1.1). There was no difference in biomass accumulated in WCC and the fallow plots in 2013, providing an opportunity to primarily assess impact of previous years WCC management (Fig. 1.1). Weeds consisted mostly of annual bluegrass (*Poa annua* L.) and

Shepard's purse (*Capsella bursapastoris* L.). Overall, the amount of biomass accumulated in WCC and fallow plots by April of each year was modest, and ranged between 0.53 to 1.46 Mg ha<sup>-1</sup>. Similar results of cereal rye WCC accumulation have been observed by Strock et al. (2004) and Snapp et al. (2005) in Minnesota and Michigan field studies. The rye biomass observed in this study was similar to 0.51 to 2.9 Mg ha<sup>-1</sup> range observed in a 8-year field study with a N-fertilizer gradient, at a Washington State site (Kuo and Jellum, 2000). However, in a 3-yr field experiment was conducted on sandy loam soils in SW Michigan, Rasse et al. (2000) found that rye WCC accumulated between 1.1 to 6.2 Mg ha<sup>-1</sup> when WCC was planted before corn harvest. Late plating of WCC within a corn-corn-soybean rotation sequence provides a short window for growth in Northern U.S. and differences in seasonal weather are key reasons for low cereal rye biomass accumulation (Miguez and Bollero, 2005; Farsad et al., 2011; Acuña and Villamil, 2014).

More aboveground WCC biomass accumulated at higher N fertilizer rates (101 and 202 kg N ha<sup>-1</sup>) compared to zero fertilized plots (Figure 1.1). Ruffo et al. (2004) and Andraski and Bundy (2005) reported similar results of high WCC dry matter production after high N fertilizer rates applied to a preceding crop, which also resulted in higher residual N uptake. The initial 2 years of this study was reported on by McSwiney et al. (2010), in which they estimated 2.3 times more belowground biomass in WCC plots compared to fallow plots (0.46 vs. 0.20 Mg ha<sup>-1</sup>). Unfortunately, measuring belowground biomass was not feasible every year. However, it is still important to note that root biomass affect soil structure and water stable aggregates (Rasse et al., 2000a).

#### *1.4.3 Soil Nutrient Availability (2013 baseline measurements)*

Results from soil texture and chemical analysis are shown in table 1.5. Nine years of WCC treatment did not influence soil properties, but N application did influence soil pH, CEC, Bray-1 P,  $K^+$ ,  $Ca^{+2}$ , and  $Mg^{+2}$  content (Table 7). Soil pH, CEC, Bray P,  $K^+$ ,  $Ca^{+2}$ , and  $Mg^{+2}$  content were negatively influenced by N application rate, with increasing acidification and slightly lower cation levels with higher N doses. Nitrification of  $NH_4$  to  $NO_3$  after the use of ammonium-based fertilizers is a well-documented cause of soil acidification (Ruffo et al., 2004; Liebig et al., 2014). Lower concentrations of Bray-1 P,  $K^+$ ,  $Ca^{+2}$ , and  $Mg^{+2}$  may be attributed in part to nutrients removed from the system in the form of grain (Karlen et al., 2014). Loss of these secondary nutrients from soils could also explain the reduced CEC.

#### *1.4.4 Bulk density (BD) and total porosity (TP)*

The 9-year cumulative use of WCC and N fertilizer over the study period did not affect BD (Table 1.5). Total porosity was calculated based on BD, thus no treatment effects were observed on TP either. Results of BD data are similar to those reported by Crum and Collins (1995). However, our results show that BD decreased with depth, which may be explained in large part by progressively higher sand content at this site, whereas Crum and Collins (1995) report a trend of increased bulk density with depth. Williams and Wiel (2004) suggest that WCC use can help alleviate the effects of soil compaction, likely due to the extensive, fibrous root system of cereal rye, but we did not observe this. WCC use has also been shown to support gains in organic SOC, ultimately influencing BD (Kong et al., 2005; Blanco-Canqui et al., 2011; Steele et al., 2012).

However, our results and those of others (Colla et al., 2000; Williams and Weil, 2004; Chen and Weil, 2011) indicate that impacts of WCC on compaction are not always discernable.

Contradictions in the literature concerning WCC effects on soil compaction are likely due to interactions with complex management practices, including tillage, and the long-time periods before BD changes become detectable. The ground was wet during sample collection, which likely affected measurements. Higher BD at the surface (0 - 20 cm) compared to deeper is likely the results of the amount of wheel traffic throughout the season, and soil water content during sample collection could have also had an effect (Chen and Weil, 2011).

#### *1.4.5 Soil water retention + Water stable aggregates*

Nine years of WCC did not influence soil water retention properties (Table 10). An effect for N fertilizer application rate was observed at -5, -10, -33 and -100 kPa water potentials ( $p = 0.10$ , Table 1.7). Soil water retention tended to be higher in subplots with lower N fertilizer rates (Table 1.7). Blanco-Canqui et al. (2011) also reported no effects on soil water retention due to the cumulative impact to 15 years of cover crop use in no-till. However, Villamil and colleagues (2006) found increases in soil water retention under WCC and no-till in silt loam Illinois soils. Including cereal rye WCC annually in a corn-soy rotation showed a significant increase in the volume of water held between saturation (0 kPa) and field capacity (-31 kPa) (Villamil et al., 2006). Impact at this level of the soil-water retention curve indicate that effects are likely from changes in SOM (Rawls et al., 2003) and also indicates increases in plant available water (e.g. available water capacity) (USDA NRCS, 1998).

Water stable aggregates were assessed in four class sizes. Of these, the largest WSA size

class 2 -4 mm (e.g. macroaggregates) and smallest WSA class size, 0.106 – 0.25 cm (e.g. microaggregates) were further analyzed to measure C and N content within WSA to understand some of the more complex relationships between soil aggregation and associated C and N pools (Sainju et al., 2003).

Overall, there were no effects of WCC on the proportions of soils in each WSA class size. Our results confirm those by Schutter and Dick (2002) who also found no effects of WCC on aggregate size distribution in an Oregon field study. Nitrogen fertilizer rate influenced size class distribution of WSA, with slightly lower proportion of macroaggregates being observed for zero N versus 202 kg N ha<sup>-1</sup>, (Figure 1.6, Table 1.7). Cover crops and N fertilization rates may influence WSA by increasing the amount of organic matter additions to soil and subsequently increasing microbial biomass and activities, as well as enhancing the substrate supporting polymer production (Mendes et al., 1999; Sainju et al., 2003; Liu et al., 2005). Yet our study found no enhancement of WSA with 9 consecutive years of WCC, possibly due to the modest amounts of biomass produced in the small window available within a corn-corn-soybean rotation. A review by Bronick and Lal (2005) suggest that the effect of different cover crops and crop rotations reflect changes in aggregate dynamics, but these effects are short-lived under conventional tillage regimes. The tillage practices in this study may be diminishing the effects of improved WSA with WCC in rotation by increasing mineralization rates (Six et al., 2000; Grandy and Robertson, 2006). Additionally, the chemical killing of WCC may also be reducing C and N inputs, limiting SOC growth (Snapp and Borden, 2003). Contradictory results of effects of WCC on WSA from this study and another long-term study by Villamil et al. (2006) can be due to differences in amount of biomass produced and differences in tillage management in both



studies (Six et al., 1999; Six et al., 2000).

Result from ANOVA reveals several influences of WCC on N fertilizer rate and subsequent effects on C, N and C/N ratio on both macroaggregates and microaggregates. The macroaggregates from 202 kg ha<sup>-1</sup> N fertilizer plots contained a slightly lower percent C than zero fertilizer plots (0.95 vs. 1.01), fig. 1.6. whereas the microaggregates from 202 kg ha<sup>-1</sup> N fertilizer plots contained a high percent C compared to zero fertilizer plots, fig. 1.6. Kong et al. (2005) reports that finding increases in C inputs over 10 years of organic material reflected in decreases in microaggregate-associated C. Because biomass accumulation in WCC is influenced by the N fertilizer rate of the preceding crop, results from this study confirm Kong et al. (2005) and provides further evidence that increases in C inputs can have an inverse effect on microaggregate-associated C.

Changes in C and N content in macro- and microaggregates are reflected in the C/N ratio. Macroaggregates contained lower C/N ratios than microaggregates, fig. 1.6. Microaggregates in plots receiving the 202 kg ha<sup>-1</sup> N fertilizer rate had a higher C/N ratio than those in plots receiving zero N fertilizer, fig. 1.6.

#### *1.4.6 Biological soil quality indicators: Labile SOC pools, soil enzyme activity, litter bag decomposition*

Soil organic matter (SOM) is an established indicator of soil quality (Karlen et al., 1997). Labile SOM pools can be assessed with POM (Cambardella and Elliott, 1992) and POXC (Culman et al., 2012), both known to be sensitive to short-term management changes. Particulate organic matter (POM) is a SOM pool of partially decomposed litter and roots that are physically

protected in soil aggregates (Wander, 2004). Potassium permanganate can be used to fractionate SOC via oxidation reaction (Loginow et al., 1987).

This study evaluated POM in two size fractions: large (210 - 2000  $\mu\text{m}$ ) and small (53 - 210  $\mu\text{m}$ ), and also assessed the C, N and C/N ratio. The use of WCC for 9 years did not affect the amount of POM content in WCC vs. fallow plots for either size fraction. Results of long-term effects of WCC use on POM confirm those by Steele et al. (2012). In a long-term row-crop rotation study in Maryland, USA by Steele et al. (2012), WCC promoted the accumulation of soil labile C compared with winter fallow during the first sampling year, but not the second year. The cumulative effects of WCC over a 13-year period did not find detectable changes in labile SOC under WCC compared to fallow treatments (Steele et al., 2012).

An interesting finding was that in the large POM fraction, POM-N values were significantly higher in WCC plots, in the presence of N fertilizer ( $p=0.05$ ), fig. 1.7 and table 1.10. This is consistent with a modest but persistent effect of a WCC legacy, as the rye cover crop in 2013 had no more biomass than the weedy fallow, but the effect was still detectable. Because POM samples were collected before the incorporation of WCC for 2013, POM-C reflects potentially mineralization N that the soil is able to supply for the 2013 growing season (Nissen and Wander, 2003). Additionally, N enrichment in large POM fractions, realized through lower C/N ratios in large fraction POM pools was observed with higher N fertilizer rates (Figure 1.7, Table 1.10). Treatment plots receiving zero and 101 kg N ha<sup>-1</sup> had C/N ratios of 23 and 21, respectively, which is drastically different with the highest N fertilizer (202 kg N ha<sup>-1</sup>), which had an average C/N ratio of 4 in large fraction POM. Overall, soils contained 65% more large fraction POM than small fraction (Table 1.9). These findings indicate that a substantially higher

content of readily mineralizable SOM has accumulated in high N plots compared to other plots. Increases in SOM are directly related to increases in potentially mineralizable N in soil (Doran and Jones, 1996). Increases in SOM from increased C inputs with increased N fertilization is commonly reported (Gregorich et al. 1996; Mazzoncini et al., 2011).

In the small POM fraction, there were no clear trends in the effects of WCC and N fertilizer rate interaction on POM-C, POM-N, and ratio in small POM fraction (Figure 1.7, Table 1.10). Plots receiving zero and 101 kg N ha<sup>-1</sup> fertilizer had higher POM-N in WCC plots compared to fallow plots in the small POM fraction pool, fig. 1.7. Interestingly, plots receiving 202 kg N ha<sup>-1</sup> fertilizer had lower POM-N in WCC compared to the fallow plots. Overall, in the small POM fractions, N applications increased the C/N ratio (Figure 1.7, Table 1.10).

Permanganate oxidizable C (POXC) provided another means to assess labile C. POXC was measured during corn grain fill (August 2013) and after corn harvest (November 2013). At both sampling times, high N fertilizer resulted in the lowest POXC. These results conflict with the results of Mazzoncini et al., (2011) who found high N fertilizer rates increased SOC under conventional tillage. Culman et al., (2013) compares POXC under three management systems in an adjacent long-term field trial, the Living Field Laboratory: conventional, integrated (fertilizer) and compost, and reported that POXC values were as high as 500 µg C (kg soil)<sup>-1</sup> in rotations with compost (Culman et al., 2013). Our results are similar to the POXC values Culman et al. (2013) reported in soils from a 20-year trial under an integrated management treatment that historically followed low-input practices, including reduced applications of herbicide and N fertilizer, and chisel plow tillage. In this adjacent trial reported on by Culman and colleagues

(2013), the POXC values reported for a conventional corn production system were  $\sim 200 \mu\text{g C (kg soil)}^{-1}$ , considerably lower than values in our study (Table 1.11).

The only detectable effects of WCC on soil labile SOC pools were found in POM-C in the large fraction POM. Similar effects of WCC were not reflected in POXC. Differences in sensitivities of WCC on POM and POXC is likely due to difference in sampling times in 2013; POM was measured before WCC incorporation and POXC were measured later in the growing season. Our results agree with the increase sensitivity to N fertilizer management of POC than POXC measurements reported in previous literature (Plaza-Bonilla et al., 2014). Results from this study challenge those presented in an 11-year study by Chen et al. (2009) that reported POXC to be twice as sensitive to soil management than POC. However, overall increases in POXC in WCC under corn-corn-soy rotation compared to POXC values reported by Culman et al. (2013) in adjacent plots in continuous corn and corn-soy-wheat without WCC demonstrates that, in general, POXC is responding to effects of WCC in labile C pools.

Soil extracellular enzymes play a key role in the mineralization of SOM and subsequent release of plant-available nutrients (Sinsabaugh, 1994). Soil enzymes are strongly correlated to soil microbial activity, soil organic C and total N content (Dick et al., 1988). Overall, extracellular enzyme activity and litter decomposition rates were not affected by the long-term use of WCC plots (Figure 1.8, Table 1.12). Allison and Vitousek (2005) propose that increasing supply of complex substrates in the soil environment should stimulate production of extracellular enzymes by the soil microbial community to exploit these new resources. This study find that a history of cereal rye WCC and modest incorporations of residue for 9 years did not alter soil

biology activity potential in these plots relative to fallow treatments (Table 1.12). There were also no detected effects of N fertilizer rate on extracellular enzyme activity litter decomposition rates (Figure 1.8, Table 1.12). These results confirm those by Grandy et al. (2013) where N fertilizer rate did not influence decomposition rates in litterbags. Grandy et al. (2013) suggests that the little impact of N fertilization on decomposition dynamics may be from the overriding effects of factors other than N on microbial community structure and function in agricultural land.

Soil enzyme activities often correlate with mass loss rate in litter and particulate organic matter, and with changes in SOM content in response to experimental manipulations (Sinsabaugh, 2010). A correlation analysis of soil enzyme activity and mass remaining of litter bags 10 weeks after burial had some correlation in NAG ( $r^2=0.37$ ) and TAP ( $r^2=0.25$ ). Unfortunately, soil enzyme activity was not assessed each time litterbag was collected, limiting any evaluation of soil enzyme activity and its effect on litterbag decomposition rates for the entire season.

## 1.5 Conclusion & Future recommendations

There were several lines of evidence that WCC modestly increased the winter cover biomass accumulated, but had no detectable impact on soil quality. WCC did not enhance soil structure (bulk density, water stable aggregates), soil-water relationships (total porosity, soil water retention), labile SOM pools (POM, POXC), and soil biological activity (soil enzyme activity, litterbag decomposition). The only effect of WCC was a modest gain in large fraction POM-C and no other effects were detectable within a corn-corn-soy rotation in the upper

Midwest. Although cereal rye has been shown to produce as much as 8 Mg ha<sup>-1</sup> biomass aboveground, large amounts of biomass on farmer fields is not likely due to the narrow planting window in Michigan after field crop harvest. An annual incorporation of 5 Mg ha<sup>-1</sup> plant residue (or 2 Mg C ha<sup>-1</sup>) has been reported as required to avoid net soil C losses in corn production (Larson et al., 1972; Johnson et al., 2006). We found less than 2 Mg ha<sup>-1</sup>, and it is unlikely that much more WCC biomass can be produced within Michigan corn-soybean production.

Conventional tillage practices in this study likely further diminished the effects of WCC biomass, as the physical mixing of soil enhances breakdown of organic residue leading to high rates of N and C mineralization (Yang and Wander, 1999; Six et al., 2000; Grandy and Robertson, 2007; Mazzoncini et al., 2011). Previous long-term field research has focused on WCC within no-till systems (Villamil et al., 2006; Blanco-Canqui et al., 2011; Stelle et al., 2012). Further research could address the high variability of soil biophysical and chemical properties across both space and time, which enhances the difficulty in detecting soil quality response. Inclusion of a rotational crop with a summer harvest period, such as winter wheat, would provide a substantial expansion in the window available for cover crop establishment and growth. In northern climates, aerial seeding WCC into standing crops maybe another way to increase the time for germination and growth (Wilson et al., 2013). Other avenues for enhancing organic matter amendment could also be pursued, such as application of manure or conservation tillage (e.g. no-till, strip-till).

## APPENDIX

**Table 1.1** Cropping rotation summary at the study site from 2005 – 2013.

Year	Cropping History	
	Cover Crop	Cash Crop
2005	Winter wheat †	--
2006	Winter rye ‡	Corn §
2007	Winter rye	Corn
2008	Winter rye	Soy
2009	Winter rye	Corn
2010	Winter rye	Corn
2011	Winter rye	Soy
2012	Winter rye	Corn
2013	Winter rye	Corn

† Winter wheat was planted at a rate of 168 kg seed ha<sup>-1</sup>

‡ Winter rye was planted at a rate of 134 kg seed ha<sup>-1</sup>

§ Corn was planted at a rate of 81,543 seeds ha<sup>-1</sup>



**Table 1.2** General timeline of agronomic management operations during growing season from 2006 – 2013

Operation	Approximate timing
Cover planted	After corn harvest
Cover termination	Early May
P and K fertilizer application†‡	2 weeks after cover termination
Cover crop incorporation §	1 -2 d after K application
Corn planting #	Early May – early June
N fertilizer application	July (plant V6 stage)
Corn harvest	Mid October - November

† P fertilizer was applied only to subplots receiving 0 kg N ha<sup>-1</sup> fertilizer in 2012 and 2013

‡ K fertilizer at rate of 78.4 kg ha<sup>-1</sup> was applied to all subplots

§ Cover crop, P and K fertilizers were incorporated with chisel plow at depth of 20 cm

# Field was prepped for planting with soil finisher (cultivator plow) same day as planting

**Table 1.3** Soil quality indicators that were assessed in this study and the rationale for selecting indicator.

Soil quality indicator	Rationale for selection
Soil pH	Influences nutrient availability, microbial community, enzyme activity, CEC, plant growth
Bray-1 P	Essential plant nutrient; positive effect on yield
Available K <sup>+</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup>	Nutrient availability for plants; affects yields
Cation Exchange Capacity	Capacity of soil to hold nutrients; influenced by SOM
Bulk density	Plant root penetration and limited root mobility can affect yields, impacted by SOM
Total porosity	Plant root penetration and water movement can affect yields, impacted by SOM
Soil water retention	Ability of soil to hold water (plant-available water); affected by management and SOM; influences yields
Water-stable aggregates	Soil structure, erosion resistance, crop emergence and early indicator of soil management effect; influences yields; impacted by SOM
Particulate organic matter	Important labile-SOM pool; affect potentially mineralizable N and SOM
Permanganate oxidizable C	Reflects labile-SOC pool; affect potentially mineralizable N and SOM
Litter bag microbial decomposition	Biological activity indicator; estimate of biomass activity; influences SOM mineralization
Soil enzyme activity	Influenced by microbial activity that control decomposition of SOM

**Table 1.4** Monthly summary of daily average temperatures(C) from May – November for 2006, 2009, and 2012 and historical averages from 1981 – 2010 at Kellogg Biological Station in SW Michigan.

Year	Month	Daily average temp. °C
2006	May	14.1
	June	19.6
	July	23.0
	August	21.2
	September	15.2
	October	8.5
	November	5.2
2009	May	14.9
	June	19.3
	July	19.2
	August	19.8
	September	16.9
	October	8.6
	November	6.6
2012	May	17.2
	June	21.1
	July	25.3
	August	20.7
	September	16.5
	October	9.8
	November	3.2
1981 – 2010	May	22.4
	June	27.4
	July	29.4
	August	28.1
	September	24.0
	October	17.1
	November	5.0

**Table 1.5** Treatment means and ANOVA results effects of WCC, N fertilizer rate, interaction on % clay, % sand, soil pH, CEC, Bray-1 P, and cation saturation of available K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> measured April 2013 at the Kellogg Biological Station in SW Michigan after 9 years of treatments. Significant effects are denoted.

N Fertilizer Rate	Clay	Sand	pH	C.E.C.	P	Cation Saturation		
						K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>
kg ha <sup>-1</sup>	%	%		meq/100 g	Bray-1	ppm	ppm	ppm
0	16.9	37.3	6.3	6.3	20.6	60.6	138.8	756.3
34	16.8	39.9	6.5	6.3	19.6	56.8	129.4	768.8
67	16.1	41.6	6.3	6.0	16.4	55.5	130.0	718.8
101	16.9	37.6	6.2	5.9	17.1	54.6	125.6	700.0
134	16.7	39.1	6.1	6.0	16.9	50.0	130.0	712.5
168	16.9	40.3	5.8	5.8	18.3	45.0	116.9	637.5
202	15.8	42.6	5.9	5.5	18.4	47.0	116.3	643.8
Overall Average	16.58	39.75	6.16	5.97	18.18	52.8	126.7	643.8
Factor	Clay	Sand	pH	C.E.C.	P	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>
WCC	0.61	0.08	0.17	0	0.09	0.03	0.39	0.13
N Fertilizer Rate (N)	2.14*	4.82***	12.88***	2.39**	2.83**	4.52**	2.46**	3.58**
WCC × N	0.31	0.27	0.95	1.64	0.29	0.61	0.84	1.96

\* Significant at the  $\alpha = 0.10$

\*\* Significant at the  $\alpha = 0.05$

\*\*\* Significant at the  $\alpha = 0.01$

**Table 1.6** F-value test statistics of three-way ANOVA showing effects of WCC, N fertilizer rate, depth, and interactions on bulk density and porosity from soils collected after corn harvest in November 2013. Significant effects are denoted.

	Bulk Density	Porosity
<b>Factor</b>	<b>F-value</b>	
WCC	0.78	0.74
N Fertilizer Rate (N)	0.7	0.70
WCC $\times$ N	0.87	0.87
Depth (D)	24.22***	24.21***
WCC $\times$ D	1.52	1.53
N $\times$ D	0.35	0.34
WCC $\times$ N $\times$ D	1.08	1.08

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

**Table 1.7** Treatment means± standard errors of treatment means and ANOVA results effects of WCC, N fertilizer rate, interaction on volumetric water content ( $\text{cm}^3 \text{ cm}^{-3}$ ) at 0, -5, -10, -33 and -100 kPa under cereal rye cover crop (WCC) and fallow at 0, 34, 67, 101, 134, 168, and 202  $\text{kg ha}^{-1}$  N fertilizer rates, measured November 2013.

N Fertilizer Rate	Water Potential (kPa)				
$\text{kg ha}^{-1}$ N	0	-5	-10	-33	-100
0	0.39	0.34	0.33	0.31	0.30
34	0.38	0.32	0.30	0.28	0.27
67	0.38	0.32	0.30	0.29	0.28
101	0.39	0.33	0.32	0.30	0.29
134	0.38	0.33	0.32	0.30	0.29
164	0.37	0.34	0.33	0.31	0.30
202	0.38	0.32	0.31	0.29	0.28
<b>Factor</b>	0	-5	-10	-33	-100
WCC	NS	1.01	0.08	0.05	0.06
N Fertilizer Rate (N)	NS	2.35**	2.00*	2.11*	2.02*
WCC × N	NS	0.13	0.32	0.31	0.25

\* Significant at the  $\alpha = 0.10$  level (two-tailed)

\*\* Significant at the  $\alpha = 0.05$  level (two-tailed)

**Table 1.8** F-value test statistics of three-way ANOVA showing effects of WCC, N fertilizer rate, size class, and interactions on water stable aggregate proportions, C %, N % and C/N ratio; significant effects are denoted.

Factor	Water Stable Aggregates			
	Proportion †	C % ‡	N % ‡	C/N Ratio ‡
	F Value			
WCC	1.19	0.17	1.19	0.37
N Fertilizer Rate (N)	0.03	1.31	0.03	1.26
WCC × N	1.82	0.84	1.82	0.01
Size Class (SC)	29.4 ***	0.04	29.4 ***	14.49 ***
WCC × SC	0.3	0.75	0.3	0.3
N × SC	3.49 *	8.09 ***	3.49 *	2.67
WCC × N × SC	1.21	0	1.21	0.61

† Includes four size classes: 2 – 4 mm, 1 – 2 mm, 0.250 – 1 mm and 0.106 – 0.250 mm

‡ Includes two size classes: 2 – 4 mm and 0.106 – 0.250 mm

\*Significant at the  $\alpha = 0.10$  level (two-tailed)

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

**Table 1.9** Averages of soil surface (0 – 20 cm) small POM (210 – 53  $\mu\text{m}$ ) and large POM (2000 – 210  $\mu\text{m}$ ) content  $\pm$  standard errors under cereal rye winter cover crop (WCC) and no cover crop (fallow) in 0, 101 and 202 kg N ha<sup>-1</sup> fertilizer rates in April 2013.

Size Fraction	N Fertilizer Rate	Particulate Organic Matter	
		WCC	Fallow
	kg N ha <sup>-1</sup>	g POM kg <sup>-1</sup> soil	
Small (210 – 53 $\mu\text{m}$ )	0	89.62 $\pm$ 1.32	95.44 $\pm$ 0.75
	101	103.23 $\pm$ 1.43	100.78 $\pm$ 2.27
	202	102.20 $\pm$ 2.00	104.65 $\pm$ 1.01
Large (2000 – 210 $\mu\text{m}$ )	0	269.54 $\pm$ 3.93	287.4 $\pm$ 13.48
	101	283.95 $\pm$ 3.15	274.59 $\pm$ 4.27
	202	319.22 $\pm$ 3.66	311.17 $\pm$ 4.95



**Table 1.10** Results of two-way ANOVA F values of treatment effects on small and large fraction POM content, C content in POM (POM-C), N content in POM (POM-N) and C/N ratio; significant effects are denoted.

Factor	Small fraction POM			
	POM	POM-C	POM-N	C/N Ratio
	F Value			
WCC	0.24	0.10	0.30	0.10
N Fertilizer Rate (N)	3.21*	0.86	3.65**	0.86
WCC $\times$ N	0.48	5.22***	8.53**	5.22***
Factor	Large fraction POM			
	POM	POM-C	POM-N	C/N Ratio
	F Value			
	POM	POM-C	POM-N	C/N Ratio
WCC	0.02	10.73**	3.15	1.21
N Fertilizer Rate (N)	2.13*	37.68***	115.72***	139.18***
WCC $\times$ N	0.39	1.73	0.65	1.06

\* Significant at the  $\alpha = 0.10$

\*\* Significant at the  $\alpha = 0.05$

\*\*\* Significant at the  $\alpha = 0.01$

**Table 1.11** Treatment means $\pm$  standard errors of treatment means and ANOVA results effects of WCC, N fertilizer rate, interaction on permanganate oxidizable C (POX-C) under cereal rye cover crop (WCC) and fallow at 0, 34, 67, 101, 134, 168, and 202 kg ha<sup>-1</sup> N fertilizer rates, measured in August and November 2013.

Date	N Fertilizer Rate kg N ha <sup>-1</sup>	POX-C		Factor	F Value
		WCC $\mu\text{g C kg}^{-1}\text{soil}$	Fallow $\mu\text{g C kg}^{-1}\text{soil}$		
August†	0	280.02 $\pm$ 12.37	259.06 $\pm$ 14.35	WCC	0.17
	101	331.24 $\pm$ 34.9	300.68 $\pm$ 27.41	N Fertilizer Rate (N)	7.52*
	202	244.79 $\pm$ 11.69	267.66 $\pm$ 17.06	WCC $\times$ N	1.55
November‡	0	383.82 $\pm$ 26.43	381.3 $\pm$ 18.39	WCC	0.37
	101	364.53 $\pm$ 16.36	385.49 $\pm$ 18.45	N Fertilizer Rate (N)	5.78*
	202	329.73 $\pm$ 6.28	342.44 $\pm$ 7.02	WCC $\times$ N	0.33

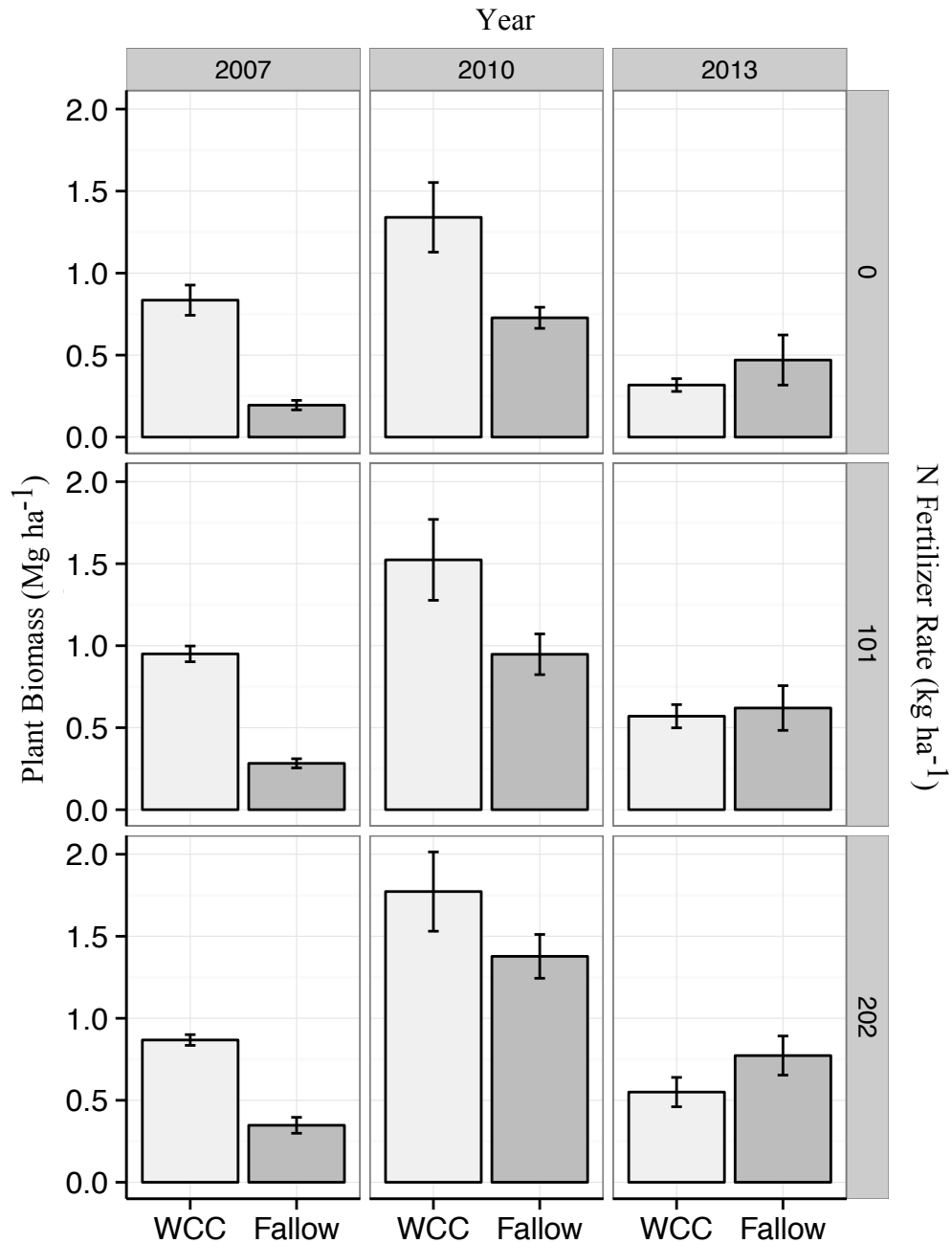
† Corn at grain fill stage in 2013

‡ After corn harvest in 2013

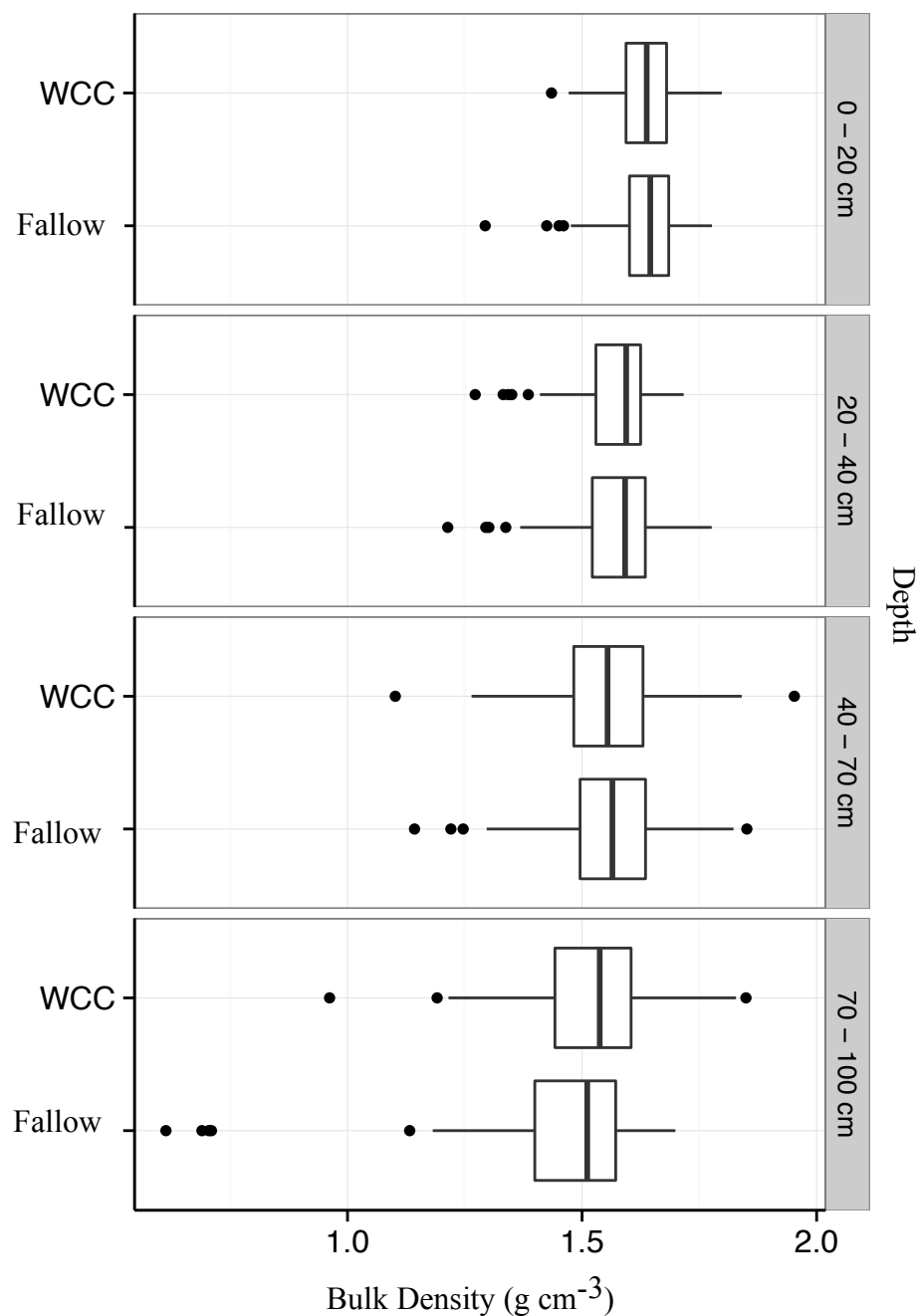
\*Significant at the  $\alpha = 0.10$  level (two-tailed)

**Table 1.12** Treatment means $\pm$  standard errors of treatment means and ANOVA results effects of WCC, N fertilizer rate, interaction on Extracellular enzyme activity of N-acetyl- $\beta$ -glucosaminidase (NAG) and tyrosine amino acid peptidase (TAP) under cereal rye cover crop (WCC) and fallow at 0 and 202 kg ha<sup>-1</sup> N fertilizer rates, measured in August 2013.

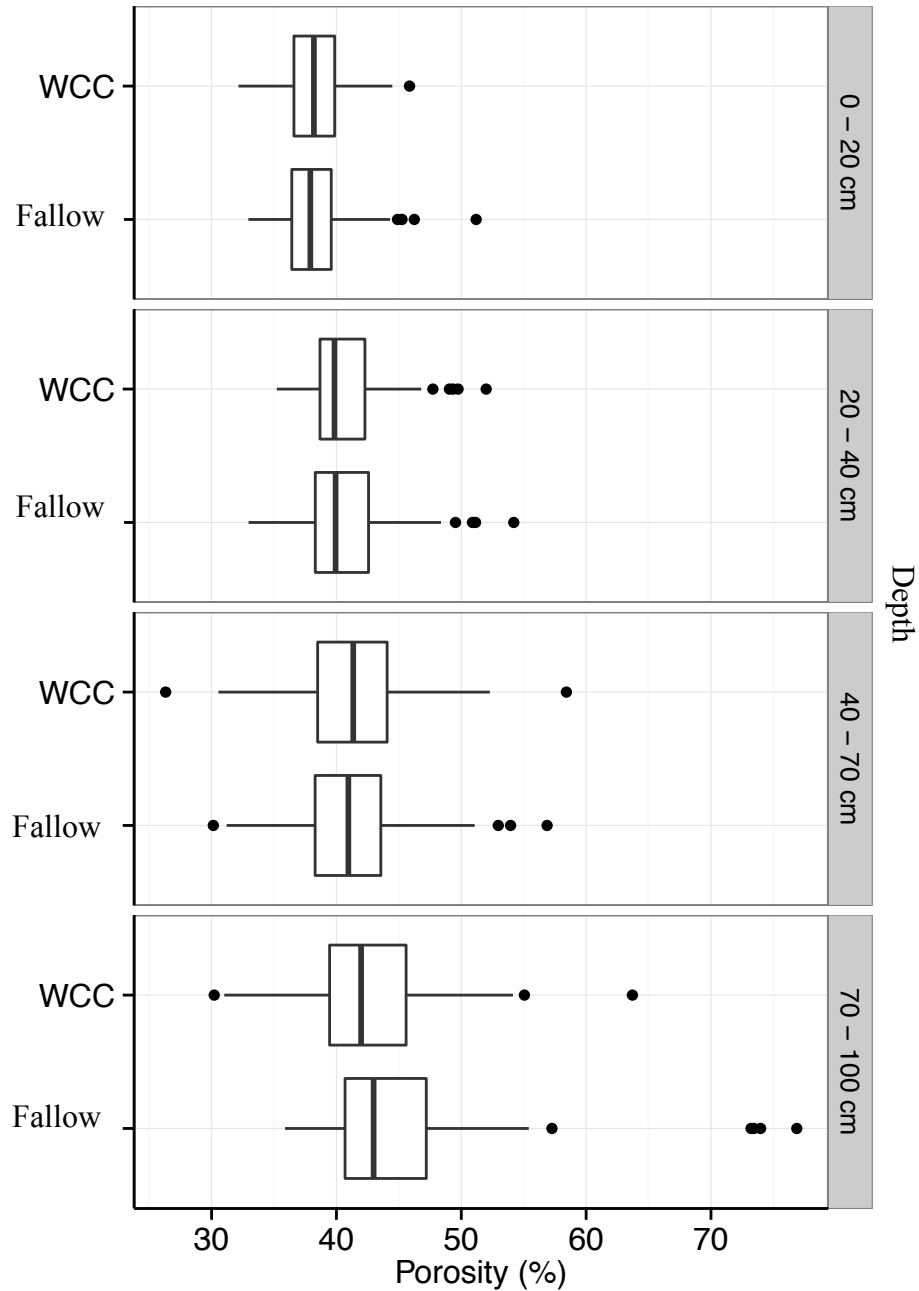
Soil Enzyme	N Fertilizer Rate	Enzyme Activity			
		WCC	Fallow	Factor	F Value
	kg ha <sup>-1</sup>	nmol h <sup>-1</sup> g <sup>-1</sup> soil			
NAP	0	38.8 $\pm$ 7.6	37.1 $\pm$ 2.9	WCC	0.06
	101	45.5 $\pm$ 3.1	45.1 $\pm$ 4.0	N Fertilizer Rate (N)	1.57
	202	42.6 $\pm$ 2.0	42.1 $\pm$ 3.0	WCC $\times$ N	0.01
TAP	0	2.5 $\pm$ 0.8	2.7 $\pm$ 0.2	WCC	0.56
	101	3.1 $\pm$ 0.4	3.3 $\pm$ 0.5	N Fertilizer Rate (N)	0.97
	202	2.2 $\pm$ 0.4	2.8 $\pm$ 0.6	WCC $\times$ N	0.07



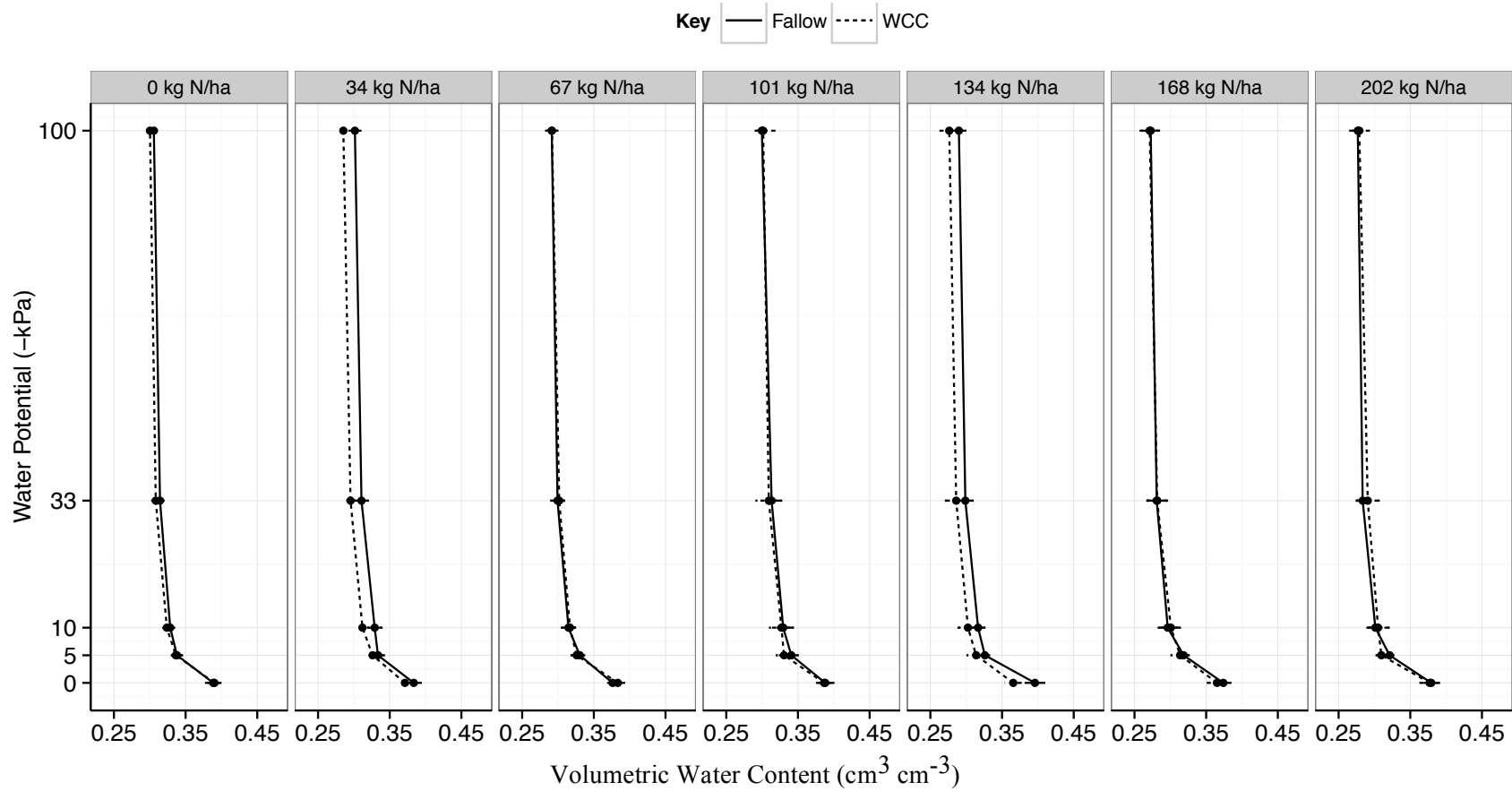
**Figure 1.1** Average plant biomass (Mg ha<sup>-1</sup>) under cereal rye cover crop (WCC) and fallow at 0, 101, and 202 kg ha<sup>-1</sup>N fertilizer rates, measured April of 2007, 2010 and 2013 at the Kellogg Biological Station in southwest MI. WCC plots consisted of cereal rye and weeds, fallow plots of weeds. Error bars represent ± standard errors of treatment means.



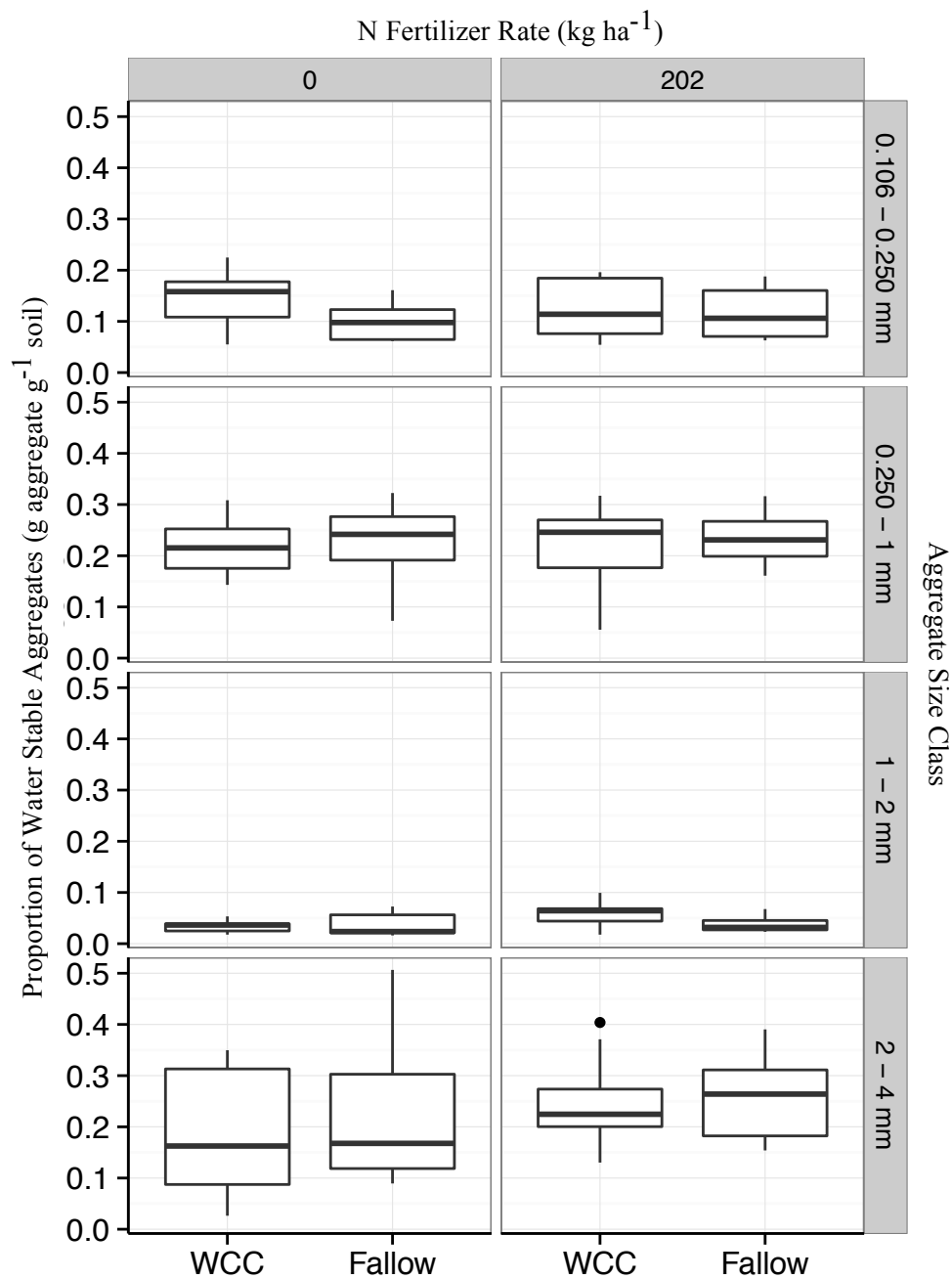
**Figure 1.2** Boxplots of soil bulk density (g cm<sup>-3</sup>) under cereal rye cover crop (WCC) and fallow combined across 7 N fertilizer rate treatments. Bulk density was measured by 0-20, 20-40, 40-70, and 70-100 cm depths in November 2013 after 9 consecutive years of WCC use at the Kellogg Biological Station in southwest MI.



**Figure 1.3** Boxplots of total porosity (%) under cereal rye cover crop (WCC) and fallow combined across 7 N fertilizer rate treatments. Porosity was measured by 0-20, 20-40, 40-70, and 70-100 cm depths in November 2013 after 9 consecutive years of WCC use at the Kellogg Biological Station in southwest MI. Porosity was calculated as a function of bulk density where particle density was assumed to equal  $2.65 \text{ g cm}^{-3}$ .

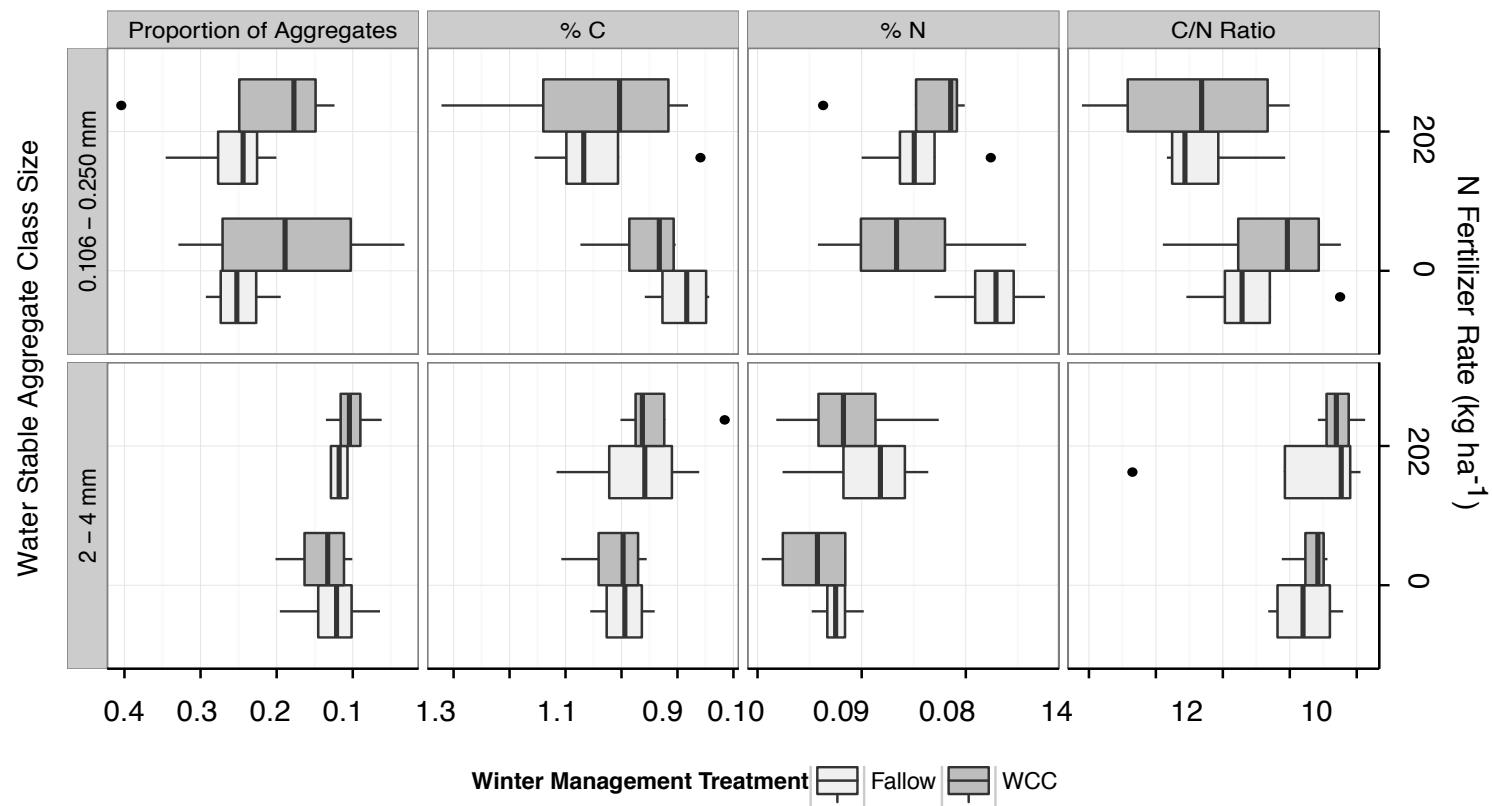


**Figure 1.4** Soil water retention curves represented by volumetric water content ( $\text{cm}^3 \text{cm}^{-3}$ ) between 0 to -100 kPa under cereal rye cover crop (WCC) and fallow at 0, 34, 67, 101, 134, 168, and 202  $\text{kg ha}^{-1}$  N fertilizer rates, measured November 2013 at the Kellogg Biological Station in southwest MI after 9 yrs of treatments at the Kellogg Biological Station in southwest MI. Error bars represent  $\pm$  standard errors of treatment means

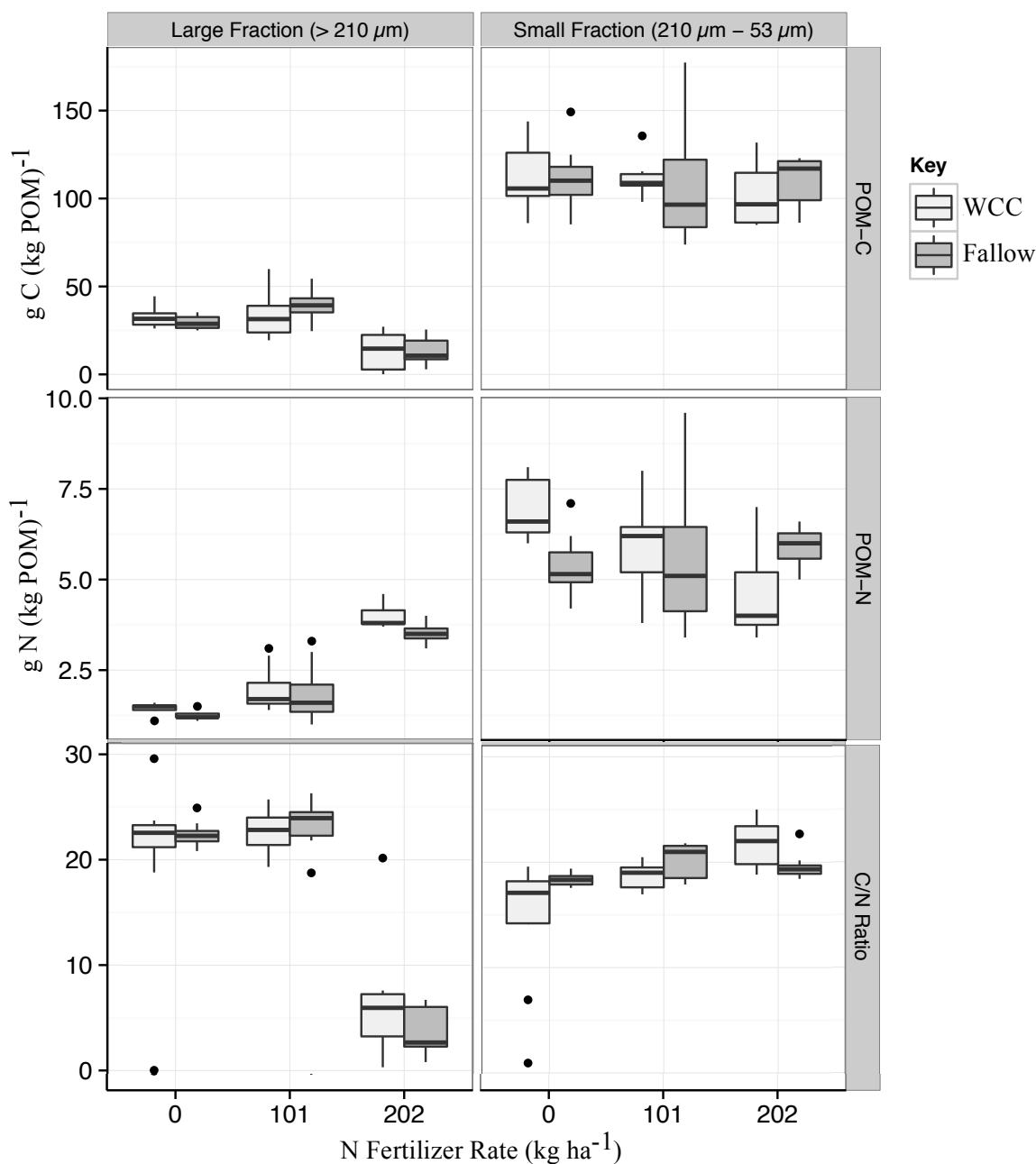


**Figure 1.5** Boxplots of proportion of wet-sieved soil in four water stable aggregate size classes: 2 – 4 mm, 1 – 2 mm, 0.250 – 1 mm and 0.106 – 0.250 mm under cereal rye cover crop (WCC) and fallow in 0 and 202 kg ha<sup>-1</sup> N fertilizer rates. Proportion of aggregates represent the 0 – 10 cm plow layer from soil collected on 29 October 2013 from in-row corn at the Kellogg Biological Station in southwest MI.

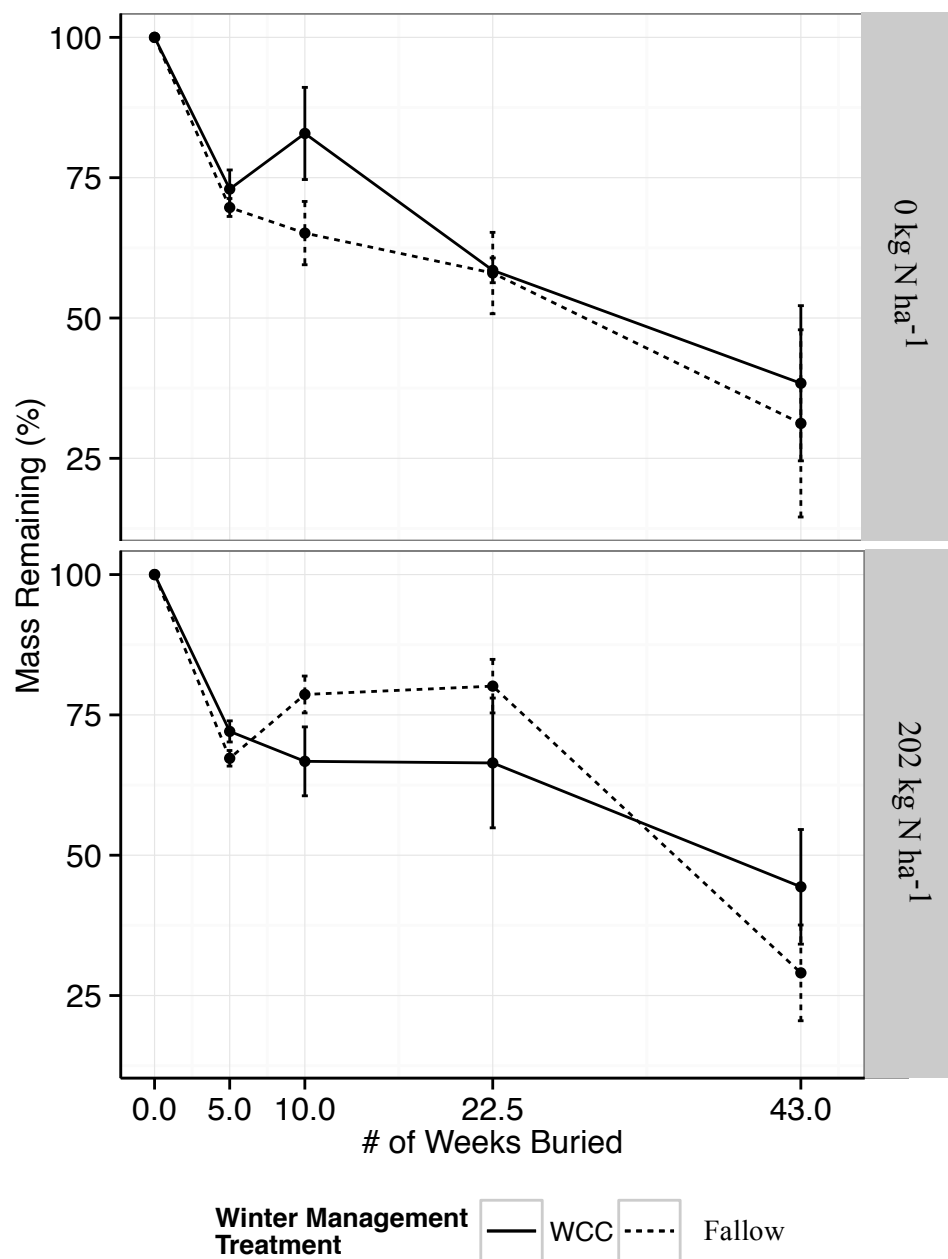




**Figure 1.6** Boxplots of proportion of aggregates, C concentration (%), N concentration (N) and C/N ratio in 2 – 4 mm and 0.106 – 0.250 mm water stable aggregate size classes under cereal rye cover crop (WCC) and fallow at 0 and 202 kg ha<sup>-1</sup> N fertilizer rates. All values represent the 0 – 10 cm plow layer from soil collected on 29 October 2013 from in-row corn at the Kellogg Biological Station in southwest MI.



**Figure 1.7** Boxplots of C content (POM-C), N content (POM-N), and C/N ratio of small fraction (56 – 210 μm) and large fraction (2 mm – 210 μm) in particulate organic matter (g POM kg<sup>-1</sup> soil) under winter rye cover crop (WCC) and no cover crop (fallow) in 0, 101 and 202 kg ha<sup>-1</sup> N fertilizer rates in April 2013.



**Figure 1.8** Percent mass remaining of winter rye leaf litter at 5, 10, 22.5 and 43 weeks after initial burial under cereal rye cover crop (WCC) and fallow at 0 and 202 kg ha<sup>-1</sup>N fertilizer rates. Error bars represent  $\pm$  standard errors of treatment means. Mass remaining (%) was calculated on an air-dried basis as ((initial mass – final mass)/initial mass) X 100.

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## CHAPTER 2: LONG-TERM IMPACT OF CEREAL RYE WINTER COVER CROP ON SOIL N AVAILABILITY AND YIELDS ACROSS A NITROGEN FERTILIZER GRADIENT IN RAINFED MICHIGAN CORN

### ABSTRACT

In the U.S. Midwest, the inclusion of a cereal rye as a winter cover crop (WCC) has been recognized as a potential nutrient management tool to mitigate environmental consequences of intensive crop production and high synthetic N fertilizer uses. The objectives of this 9-yr field study from 2006-2013 in SW Michigan were two-fold: first, measure N supply and soil N pools after cereal rye WCC incorporation and its impacts on corn N availability across an N fertilizer gradient (0, 34, 67, 101, 134, 168, or 202 kg ha<sup>-1</sup>) during the 2013 growing season, and second, assess yield response to above under diverse weather environments. This experiment was a RCBD with a main factor of winter cover and sub-factor N rate, conducted from 2006-2013 at Michigan State University's W.K. Kellogg Biological Station located in SW Michigan.

Between 2006 and 2013, the annual aboveground cereal rye WCC accumulation ranged between 0.53 to 1.46 Mg ha<sup>-1</sup>. Nitrogen retained in WCC biomass was influenced by the N fertilizer rate and residual soil N from preceding crop. Practical limitations to WCC biomass growth are likely from late planting after long-season row crops and year-to-year weather variability in this region. Our results find evidence of synchrony of N mineralization to crop N demand after cereal rye WCC compared to fallow plots during the 2013 corn growing season, indicating historical effects of WCC use on subsequent crop growth. The increase in soil NO<sub>3</sub><sup>-</sup>-N is supported by evidence from higher net mineralization rates and increases in soil NO<sub>3</sub><sup>-</sup>-N

adsorption in ion exchange resin strips. Benefits of increased soil  $\text{NO}_3^-$  were found after N fertilization of crops 5 weeks after planting (corn at V6) in WCC compared to fallow treatment plots. However, the synchrony of N mineralization with crop N uptake did not result in any yield benefits in 2013. In addition, we found no effects of WCC on biomass accumulation and N concentration in grain at harvest. Overall, after the 2013 harvest, there was generally more residual N under WCC plots that received N fertilizer compared to fallow plots.

Over the 9-yr period of this study, WCC posed no negative impacts on corn yields compared to fallow treatment plots. Nitrogen response to corn grain yields from 2006 to 2013 under WCC vs. fallow treatments were further analyzed by fitting quadratic response with plateau regression models to determine EONR and yield at EONR. Corn yields at the EONR under WCC were not different to fallow treatments, resulting in no yield benefit following WCC. These findings suggest that farmers do not experience yield losses under cereal rye WCC.

## 2.1 Introduction

Growing problems with N contamination of environmental resources in the U.S. Midwest are most notably due to decreased diversity in crop rotation, increased use of synthetic N fertilizers, increased tillage, and artificially drained areas (Tilman, 1999; Dinnes et al., 2002). Environmental consequences of these destructive land use practices have been identified as the cause of increased nitrate ( $\text{NO}_3^-$ ) contamination of surface water and increased soil erosion, leading to loss of soil sediments and valuable nutrients (Burkart and James, 1999; Rabalais et al., 2001). Increased awareness of the impact of N loading from agricultural fields on the environment has prompted research on farm management practices that are both environmentally sustainable and economically viable. In the U.S. Midwest, the inclusion of a cereal rye as a winter cover crop (WCC) has been recognized as a potential nutrient management tool to mitigate environmental consequences of intensive crop production (Ruffo and Bollero, 2003). Cereal rye is known for its winter-hardiness and its exceptional ability to scavenge residual N (Wagger, 1989; Shipley et al., 1992; Ruffo et al., 1994).

Growing cover crops between cash crops have been shown to reduce the potential for  $\text{NO}_3^-$  leaching from farm fields by immobilizing excess N fertilizer, thus retaining it in the system (Tonitto et al., 2006; McSwiney et al., 2010). Ruffo et al. (2004) and Wagger et al. (2008) demonstrated that cereal rye responded to high N availability and was able to take up large amounts of soil mineral N. During wet fall and early spring months, winter rye immobilizes the N in the system by taking up residual N after corn. Subsequently, when biomass is incorporated into the soil in the spring, microbial decomposition convert dead organic material into soluble forms of nutrients for plant uptake. This soluble N is potentially available for the subsequent

cash crop.

Decomposition includes two important processes: mineralization and immobilization, which govern the release of nutrients from organic residue additions. The fate of N released from decomposition is generally determined by the residue quality, or N content in the biomass, reflected by the C/N ratio (Ranells and Waggoner, 1996; Trinsoutrot et al., 2000; Kuo and Jellum 2002; USDA NRCS, 2011). Residues with higher N content have a lower C/N ratio ( $< 24:1$ ), which leads to mineralization and subsequent release of nutrients for plant uptake. Residues with lower N contents have a higher C/N ratio ( $> 24:1$ ), and are characterized with immobilization, where mineral N is temporarily moved into the microbial pool and unavailable for plant uptake. Soil organic matter (SOM) includes the plant and animal residues and all of its various decay products. Though the C/N ratio is one of the best predictors of potential mineralization or immobilization of nutrients after organic residue incorporation (Vigil and Kissel, 1991), other environmental factors (moisture, temperature) and management practices (planting date, kill date, incorporation method) also influence rate of decomposition (Kuo and Sainju, 1997; Lundquist et al., 1999; Schroder et al., 2000; Crandall et al., 2005; Steenwerth and Belina, 2008).

The effect of environmental factors on mineralization cannot be controlled in rainfed systems, therefore estimates of soil N supplying capacity are only a potential source of N for crops (Mikha et al., 2006). Potentially mineralizable N is known to be correlated with the SOM concentration (Kuo et al., 1996). Often times, general estimates of N mineralization could be made based on SOM content for a given climatic region (Schepers and Mosier, 1991). Soil N mineralization has been shown to provide 20 to 80% of the N required by plants (Broadbent, 1984). WCC can be an economically advantageous nutrient management tool because WCC

residue additions can increase labile SOM pools (Cassman et al., 2002). However, the dynamic nature and transient pools of soil N make it an ongoing challenge to characterize the patterns of N release from WCC to increase synchrony between soil N mineralization and crop N demand. Challenges in determining N mineralization potential of soils are further compounded with changes in climate that have increased erratic weather events such as much heavier rainfall but fewer rainfall events. While there is no way to control seasonal variability in climate, farmers can improve N synchrony with WCC with careful management decisions in regards to timing and method of incorporation to increase synchrony of WCC with crop N demand (Baggs et al., 2000).

Ultimately, WCCs can be a beneficial nutrient management strategy. The incorporation of organic residues from WCCs increases labile SOM pools and thus increases soil N mineralization potential. Because mineralized N can provide a substantial amount of N to crops, WCC may provide economic benefits to yields by supplying extra N, or by reducing N rate applications as unused residual N is recycled with WCC incorporation. The effectiveness of a WCC to provide benefits to subsequent crop is through its ability to synchronize N mineralization with crop N demand.

A range of effects of cereal rye WCC on subsequent cash crop yields have been observed in numerous studies. Some studies have reported no subsequent yield benefit with WCC use (Wagger, 1989; Kuo and Jellum, 2002); others concluded there was no yield penalty with WCC use (Ball-Coelho and Roy, 1997; Ritter et al., 1998; Rasse et al., 2000; Strock et al., 2004); and some studies found WCC to slightly increase corn yields compared to fallow plots (Kuo et al., 1996). Most of these studies evaluated the effect of WCC over short duration, generally less

than 5 years. In addition, the lack of consensus on the effect of WCC on corn yields may be due to the year-to-year variability on climatic conditions (Andresen et al., 2001; Cassman et al., 2002; Kravchenko et al., 2005, Smith et al., 2007). Seasonality effects are high in rainfed systems and a long-term field trial may provide further insights into the relationship between climate, corn yield, and the effect of WCC rotations on N availability. A longitudinal analysis of the effect of WCCs is necessary to further understand the effect of climate on potential N availability and N synchrony to fully realize potential benefits in using WCC as a nutrient management tool.

The objectives of this 9-yr field study from 2006-2013 in SW Michigan were two-fold: first, to measure N supply and soil N pools after cereal rye WCC incorporation and its impacts on corn N availability across an N fertilizer gradient during the 2013 growing season, and second, assess the impact of long-term cereal rye WCC on corn yield performance across an N fertilizer gradient in a rainfed agroecosystem under a corn-corn-soy rotation. We hypothesized that (1) management practices and soil moisture conditions determine the impact of WCC on soil N availability and yield performance and (2) that 9-yrs of WCC improves synchrony of N availability in soil in relationship to corn N demand, thus providing yield benefits with adequate rainfall during the growing season.



## 2.2 Materials & Methods

### 2.2.1 *Site description and weather data collection*

This experiment was conducted at Michigan State University's W.K. Kellogg Biological Station (KBS) located in SW Michigan (42°24' N, 85°24' W, elevation 288 m), in the eastern portion of the U.S. Corn Belt. The soil at this site is a well-drained sandy loam dominated by Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) soil series with A, E and B soil horizons (Crum & Collins, 1995). Mean annual temperature is 10.1 C with an annual precipitation of 1,005 mm, about half of which falls as snow, as described at KBS Long Term Ecological Research Station which can be found online at <http://lter.kbs.msu.edu/datasets>.

### 2.2.2 *Experimental design*

In fall of 2005, this study was established as a split-split plot randomized complete block design that included 14 treatments and 4 replications. The main plot consisted of two winter management systems: winter cover crop (WCC) or no cover crop (fallow) with subplots receiving a nitrogen fertilizer gradient. That is, main plots were split into seven subplots, which were randomly assigned N fertilizer rates of 0, 34, 67, 101, 134, 168, or 202 kg N ha<sup>-1</sup>. The size of each subplot was 9 × 9 m, for a total of 56 subplots. Each subplot contained six rows. The subplot treatments remained the same for the 9-year duration of the experiment under a corn-corn-soybean rotation. Cropping rotation history at this site is described in Table 2.1. Data were only collected in years that corn was grown in the rotation.

### 2.2.3 Agronomy

From 2006 to 2013, all experimental plots received the same agronomic management (Table 2.2). After crop harvest in the fall, cereal rye was drilled at 134 kg seed ha<sup>-1</sup> on plots receiving WCC treatment, which date generally occurred in November. Winter cereal rye (*Secale cereale* L.) was planted as cover crop for all except the first year of the study when winter wheat (*Triticum aestivum* L.) was planted. Site was managed as described previously in McSwiney et al. (2010). The management was as follows: in early May, the winter cover crop was sprayed with glyphosate (0.46 AI ha<sup>-1</sup>) to chemically kill cover crop and weeds in all plots. Two weeks later, potash (78.3 kg K ha<sup>-1</sup>) was applied to the entire field. Dead biomass residue and potash were incorporated with a chisel plow at an approximate 20 cm depth. Corn (*Zea mays* L.) planting date varied depending on weather, but planting dates were between early May to early June. The soil bed was prepared with a soil finisher (cultivator) and corn was planted without starter N fertilizer at 81,543 seeds ha<sup>-1</sup> (33,000 plants ac<sup>-1</sup>). In all years, a mixture of S-metolachlor, mesotrione, and atrazine were applied and incorporated the same day or day after planting for early-season and residual weed fallow. Corn plant populations were thinned to 69,160 plants ha<sup>-1</sup> (28,000 plants ac<sup>-1</sup>) at around the V6 stage of development. Nitrogen fertilizer treatments were applied after plant thinning as anhydrous ammonia (NH<sub>3</sub>), ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) or urea ((NH<sub>2</sub>)<sub>2</sub>CO). Shortly after N fertilizer was applied, glyphosate was again applied to control weed growth.

#### 2.2.4 *Cover crop biomass assessment*

Biomass samples of cereal rye and weeds in WCC plots and weeds in fallow were collected before glyphosate was applied in the spring. It is important that weeds in fallow plots were measured since they too can uptake residual N from previous crop and contribute to subsequent crop (Ritter et al., 1998). Biomass samples were collected in 2007, 2010, and 2013. In all years data was collected, aboveground biomass was harvested from two  $0.5 \times 0.5$  m quadrats per subplot. Samples were oven-dried at 60 °C to constant mass, and weighed. The samples were ground to pass a 1-mm screen in a Christy-Turner 8-inch (20.3 cm) Lab Mill (Ipswich, Suffolk, UK) and then analyzed for total C and N using a Costech Elemental Combustion Analyzer (ECS4010, Valencia, California, USA). Cover crop biomass data are reported as dry biomass weight ( $\text{Mg ha}^{-1}$ ). Nitrogen uptake by WCC is reported as  $\text{kg ha}^{-1}$ .

#### 2.2.5 *Corn plant biomass assessment*

Destructive samples of total aboveground biomass were collected from randomly selected of whole plants from rows two and five. Samples were collected three times during the growing season: pre N application, post N application, and at physiological maturity. In 2013, before N fertilizer application, four whole-plant samples were collected randomly and composited from WCC and fallow plots that received 0, 101, and 202  $\text{kg N ha}^{-1}$  fertilizer rates (total 24). In 2013, four whole plants for pre N application were collected 9 July, post N application on 15 August, and physiologically mature plants were harvested on 8 November.

Whole plants collected at physiological maturity were divided into three fractions: vegetative tissue (leaf and stem), reproductive organs (husk, cob, shank and tassel), and grain. The silage portion was weighed fresh, shredded, and subsampled for further analyses. Ears from

whole plants were hand-shelled to measure weight of grain only; removed cobs were added to the reproductive support fraction. All collected whole-plant biomass and biomass fractions were dried to constant mass at 60 °C, and analyze for dry matter and N content. All corn plant samples collected in this study were ground to pass a 1-mm screen in a Christy-Turner 8-inch (20.3 cm) Lab Mill and then analyzed for total C and N using a Costech Elemental Combustion Analyzer.

#### *2.2.6 Yield assessment*

Corn grain yields were estimated by hand-harvesting 5.3 m of two center rows in all corn growing years of the study (2006, 2007, 2009, 2010, 2012 and 2013). Rows three and four were designated yield rows and were only used to collect samples at physiological maturity for grain yield measurements. In 2013, corn ears were shelled with grain shell equipment (Almaco Manufacturers, Nevada, IA). Total grain mass was weighed and subsample was removed to measure grain moisture, with a grain moisture tester (DICKEY-john Corp., Auburn, IL). Another subsample was oven-dried at 60 C to constant mass, weighed. Yields were adjusted to 15.5% moisture content and were quantified as kg ha<sup>-1</sup> grain.

#### *2.2.7 Corn N availability*

##### *2.2.7.a SPAD chlorophyll meter measurements*

Chlorophyll readings of 30 corn plants were taken on an unblemished spot of the leaf above the ear leaf with a Konica Minolta SPAD 502 meter (Ramsey, NJ). Only plants in WCC and fallow plots that received 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer rate were measured (total 24). Chlorophyll meter readings took place on 31 July and 16 August in 2006, 26 June, 1 August and

3 September in 2007, and 21 August and 4 September in 2013. These readings represented plant N status during 10 to 14 d and 24 to 28 d after pollination.

#### *2.2.7.b Available inorganic N and potentially mineralizable N*

Soil mineral N was measured throughout the growing season in WCC and fallow subplots receiving 0, 101, and 202 kg N ha<sup>-1</sup> N fertilizer rates (total 24). Eight soil cores were collected from the 0 to 20 cm depth and composited. Sampling times were selected to correspond to known changes in N availability throughout growing season. Soils were collected -5, 5, 8, 13.5, 18.5 and 26 weeks after planting. Sampling 5 wks before planting was used as a baseline soil sampling for the 2013 growing year. Plants ere approximately at V6 stage 5 weeks after planting, before N fertilizer was applied. Soils were sampled again after N fertilizer application, 8 weeks after planting. Plants were at grain fill 13.5 weeks after planting and had reached physiological maturity at 18.5 weeks after planting. Soils were sampling again after harvest, 26 weeks after initial planting. All fresh soils were sieved through a 6-mm sieve and homogenized. Soils were stored in 4 C until analysis, but were generally analyzed within 2 d of sample collection.

Two 10-g subsamples were used to determine potentially mineralizable N and another subsample was taken for gravimetric soil moisture analysis. The first 10-g soil sample was shaken with 100 mL of 1 M potassium chloride (KCl) for 1 min; solids were allowed to settle overnight, shaken again, allowed to settle for 1 h, and filtered through Whatman No. 1 filter paper (GE Healthcare Bio-Sciences, Pittsburg, Pennsylvania) into 20-mL scintillation vials. The second 10-g subsample was combined with 25 mL of deionized water and placed in a 28 C incubator for 7 d to assess anaerobic N mineralization potential. Incubated samples were

extracted with 75 mL of 1.33 M KCl for a final concentration of 1 M KCl. All samples were frozen at 4 C until analyses to determine nitrate and ammonium.

#### *2.2.7.c Cation and anion ion strip exchangeable N*

In 2013, soil N availability was also monitored using ion-exchange resin strips in WCC and fallow subplots receiving 0, 101 and 202 kg N ha<sup>-1</sup> fertilizer rates (total 24). Cation and anion-exchange membranes (Ionics, Waterville, MA, USA) were cut into 2.5 x 10 cm strips, and then charged for 1 h in 0.5 M HCl while shaken at 40 rpm. Strips were then soaked in 0.5 M NaHCO<sub>3</sub> for 5 h while shaken at 40 rpm. After charged strips had been washed with deionized water, they were inserted into vertical slots in the soil, and which firmly closed to ensure that the ion-exchange resin strip was in full contact with the soil.

Three sets of cation and anion strip pairs were placed randomly in each subplot. Ion strip pairs were replaced every 3 wks in 2013 from late June to early October. Five total readings were taken throughout the season from 7 June to 28 June, 28 June to 19 July, 23 July to 14 August, 14 August to 4 September, and 10 September to 4 October. Immediately after collecting strips, adhering soils was washed off with deionized water. Ion resin strips from subplots were composited and 70 mL of 2.0 M KCl was added per each strip. Ion strips with KCl solution were shaken at 40 rpm for 1 h and transferred to 20-mL scintillation vials. Samples were stored at 4 C until nitrate and ammonium analysis.

#### *2.2.7.d Nitrate and ammonium colorimetric assay*

Nitrate ( $\text{NO}_3^-$ -N) was determined as described by Doane and Horwath (2003), while extracted ammonium ( $\text{NH}_4^+$ -N) was determined as described by Sinsabaugh et al. (2000). Samples were analyzed for soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N concentration on a Multi-Skan Ascent 96-well plate reader (MTX Lab Systems Inc., Vienna, VA) at 540 nm and at 630 nm, respectively. Concentrations were converted from ppm and are reported as  $\text{kg N ha}^{-1}$  soil.

#### *2.2.8 Statistical analysis*

Experimental design was a split-split plot randomized complete block design with a main treatment of winter cover crop and split-treatment of N fertilizer rate. All statistical analysis was conducted in SAS 9.3 (SAS Institute, 2002). For all analysis used in GLIMMIX, block and all interactions with main effects were considered in the model as a random effect. Cover crop biomass was analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects and year as a repeated measures effect. Percent C, percent N, and C/N ratio in cover crop biomass for 2013 was analyzed using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. Model parameters for corn plant biomass (pre N, post N and physiologically mature plant partitions) were estimated using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects. Surface soil gravimetric moisture, soil  $\text{NO}_3^-$ -N, soil  $\text{NH}_4^+$ -N, PMN and ion strip adsorption rates were analyzed as a repeated measures analysis using the GLIMMIX procedure with WCC and N fertilizer rate as fixed effects and sampling time as the repeated measures variable. Deep soil  $\text{NO}_3^-$ -N after corn harvest was analyzed using the

GLIMMIX procedure with cover crop use and N fertilizer rate as fixed effects with depth as an additional fixed effect. Soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N data were not normally distributed and were transformed using logarithm base 10. Normality was attained through these transformations, and were confirmed with the Shapiro-Wilk test at the  $\alpha = 0.05$  level (Razali & Wah, 2011). Data presented in tables are back-transformed. Model parameters for corn grain yield from 2006 – 2013 were obtained using the repeated measures analysis in GLIMMIX procedure with cover crop use and N fertilizer rate as fixed effects and year as the repeated term. Quadratic models with plateau to represent corn yields were estimated with the NLIN procedure to obtain critical rate of N fertilizer rate (ENOR) at the yield at ENOR (P). Yield plateaus were further analyzed using the GLIMMIX procedure with cover crop use and year as fixed effects. Residuals of all variables were assessed for normality using the UNIVARIATE procedure in SAS 9.3 (SAS Institute, 2002). F-values for all effects and included with indication of effects that were significant. Significant effects are discussed at the  $\alpha = 0.01$ ,  $\alpha = 0.05$  and  $\alpha = 0.10$  probability levels. The covariance structure type selected was compound symmetry and based on BIC criteria. Least square means for fixed effects were separated using appropriate standard errors with output from the PDIF option of the LSMEANS statement in the GLIMMIX procedure in SAS 9.3. Graphs were created using R version 3.1.1 (R Core Team, 2014).



## 2.3 Results

### 2.3.1 *Weather data*

Climatic conditions for the experimental years 2006 to 2013 are presented in Table 2.3. The field experiment involved a rotation sequence with 2 yrs of corn followed by soybean, where the focus of this study is on the corn phases (2006, 2007, 2009, 2010, 2012, and 2013). Growing conditions were mesic during 2006 and 2010, which had above-average rainfall (Figure 2.1). In contrast, below-average rainfall occurred in 2007, 2009 and 2012, with 2012 being a drought year throughout the Midwest with 36% below-average rainfall at our site, combined with abnormally high temperatures in March and cooler temperatures in September which caused erratic yields in this year. In 2007, a month and half long dry spell occurred in the middle of the growing season, and supplementary irrigation was used in the fields to ensure plant growth. Rainfall was inconsistent during the 2009-growing season, with very low rainfall in July. Rainfall conditions were very close to the long-term average for 2013, the year of detailed soil and plant monitoring reported in this study.

### 2.3.2 *Cover crop biomass growth and weather data*

Biomass accumulation of cereal rye and weeds in WCC plots and weeds in fallow plots were measured for three N rate treatments (0, 101 and 202 kg N ha<sup>-1</sup>) in April of 2007, 2010 and 2013, just before it was killed with glyphosate (Figure 2.2). A three-way ANOVA revealed significant effects of WCC ( $F=54.46$ ,  $p = 0.01$ ), N fertilizer rate ( $F=4.85$ ,  $p=0.01$ ), year ( $F=157.32$ ,  $p=0.01$ ), and WCC  $\times$  year ( $F=29.95$ ,  $p=0.01$ ) on the amount of WCC biomass accumulated when collected in April of each year. An average of 0.53 to 1.46 Mg ha<sup>-1</sup> of cereal

rye grew over all years of the study, whereas an average of 0.28 to 1.04 Mg ha<sup>-1</sup> of weed biomass grew in the fallow over all years of the study. Weeds in all plots primarily consisted of annual bluegrass (*Poa annua* L.) and Shepard's purse (*Capsella bursapastoris* L.) (data not shown).

In 2007, the overall average biomass of cereal rye and weeds was 0.88 (0.03) Mg ha<sup>-1</sup> in WCC plots while fallow plots only accumulated 0.28 (0.02) Mg ha<sup>-1</sup> weedy biomass across all N rates that were measured (Figure 2.2). Biomass accumulation was high in 2010, about two-fold higher compared to other years for both WCC and fallow plots with an average of 1.46 (0.08) Mg ha<sup>-1</sup> biomass accumulated in WCC, and only 1.05 (0.06) Mg ha<sup>-1</sup> in fallow plots (Figure 2.2). In 2013, the average biomass in WCC and fallow plots was 0.53 (0.03) and 0.59 (0.04) Mg ha<sup>-1</sup>, respectively (Figure 2.2). There was more biomass accumulation in WCC plots than fallow plots in 2007 and 2010, but not in 2013. There was a treatment effect of WCC (vs. weedy fallow) on the cumulative organic biomass incorporated in 2007, 2010 and 2013 ( $F=54.46$ ,  $p = 0.01$ ). The amount of biomass grown was positively impacted by the N fertilizer treatment ( $F=4.85$ ,  $p=0.01$ ). More biomass accumulated in plots with increasing N fertilizer rate treatments, compared to zero fertilized plots in 2010 and 2013.

In 2013, there was no effect of WCC on the overall N uptake and C/N ratio of the cereal rye WCC and weeds biomass. The C/N ratio of biomass across all plots was 26 (data not shown). There was, however, an effect of N fertilizer rate on plant N uptake ( $F=4.85$ ,  $p=0.01$ ) and C/N ratio ( $F=3.23$ ,  $p=0.01$ ). The interaction of WCC  $\times$  N fertilizer rate also had an effect on the C/N ratio ( $F=1.86$ ,  $p=0.10$ ). In general, as N fertilizer rate increased, so did plant N uptake, which narrowed the C/N ratio. The overall N uptake by WCC and weeds in the spring of 2013 ranged

from 5.64 kg N ha<sup>-1</sup> in the zero fertilizer plots to as much as 11.35 kg N ha<sup>-1</sup> in the high N (202 kg N ha<sup>-1</sup>) fertilizer plots (data not shown).

### *2.3.3 2013 Corn biomass analysis*

Treatment means corn plant biomass, tissue N concentration (%) and C/N ratios from whole plant corn samples collected before N fertilizer application (5 weeks after planting) and after N fertilizer application (10 weeks after planting) during the 2013 growing-season are reported in Table 2.4. Whole corn plants at physiological maturity were partitioned into its vegetative components, reproductive organs (husk, silk, cob), and grain. Treatment means of biomass, tissue N concentration (%) and C/N ratios partitioned plants at physiological maturity (18.5 weeks after planting) are reported in Table 2.5. The use of cereal rye WCC for 9 yrs had no effect on corn plant growth when measured 5, 10 and 18.5 weeks after planting (Table 2.4, 2.5).

Effects of N fertilizer rate ( $p=0.05$ ) were detected on plant N uptake and C/N ratio, in addition to an interaction of WCC  $\times$  N fertilizer rate ( $p=0.01$ ) on plant N uptake in corn plants before N fertilizer application (Table 2.4). Historic use of N fertilizer had a positive effect on corn biomass N content when measured five weeks after planting, when corn was at V6 stage, but before fertilizer application (Table 2.4). There was an advantage of higher N concentration in corn plants under WCC compared to fallow plots that historically received zero fertilizer (Table 2.4). There were no advantages of WCC compared to fallow plots in plots that have historically received N fertilizer (Table 2.4).

Only treatment effects of N fertilizer rate ( $p=0.01$ ) were detected on plant N uptake after N fertilizer application (Table 2.4). In zero fertilized plots, there were no differences in amount of

corn plant biomass when measured 10 weeks after planting in WCC and fallow plots. Interestingly, there was an apparent 24% increase in corn plant N concentrated when growing under cereal rye WCC treatment plots compared to fallow plots under zero N fertilizer application (Table 2.4). Corn plants collected 10 weeks after planting that received 101 and 202 kg ha<sup>-1</sup> N fertilizer also had higher N concentration under WCC plots compared to fallow plots (Table 2.4).

Whole corn plants at physiological maturity (harvest) were partitioned into its vegetative components, reproductive organs (husk, silk, cob), and grain. Treatment means of biomass, tissue N concentration (%) and C/N ratios partitioned plants at physiological maturity (18.5 weeks after planting) are reported in Table 2.5. There was no effect of cereal rye WCC on biomass, N concentration, and C/N ratio in all of the mature corn plant partitions measured (Table 2.5). There was a positive effect of N fertilizer rate ( $p=0.01$ ) and interaction of WCC  $\times$  N fertilizer rate ( $p=0.05$ ) on vegetative biomass (g plant<sup>-1</sup>) of corn plants at harvest (Table 2.5). In addition, N fertilizer rate had a positive effect on N concentration (%) of vegetative biomass of corn plants at harvest, which resulted in a narrower C/N ratio with increasing N fertilizer rates when compared to zero fertilizer treatments (Table 2.5).

There were treatment effects of N fertilizer rate ( $p=0.01$ ) on N concentration of biomass and the C/N ratio of the of mature corn plants at harvest. N fertilizer rate had a positive effect on N concentration (%) of reproductive organ components of corn plants at harvest, which resulted in a narrower C/N ratio with increasing N fertilizer rates when compared to zero fertilizer treatments (Table 2.5). Corn grain biomass and grain N concentration were both enhanced by increasing N fertilizer rate treatments ( $p=0.05$ ) (Table 2.5). Higher N concentration in grain

resulted in a narrower C/N ratio with increasing N fertilizer rates when compared to zero fertilizer treatments (Table 2.5).

#### *2.3.4 SPAD Chlorophyll*

SPAD chlorophyll contents were collected in 2013 were combined with data collected in 2006 and 2007 in the same study that are reported in McSwiney et al. (2010). SPAD chlorophyll meter did not detect any differences in plant N uptake with WCC across all 3 yrs of data (Table 2.6). N fertilizer rate, year, sampling time, N fertilizer rate  $\times$  sampling time, year  $\times$  sampling time  $\times$  N fertilizer rate, and year  $\times$  sampling were found to have an effect on SPAD chlorophyll content (Table 2.6). In 2013, there is higher SPAD chlorophyll content in WCC plots at both samplings indicating higher tissue N concentration in WCC compared to fallow plots (Figure 2.3). There was generally higher SPAD chlorophyll content when plants were measured 10 - 14 days after pollination (R2 or blister stage) than 24 - 28 days after pollination (R4 or dough stage) all years SPAD was measured (Figure 2.3).

#### *2.3.5 2013 season-long moisture and inorganic N & post harvest deep soil N*

Surface soil (0 – 20 cm) were collected 5 weeks before planting and 5, 8, 13.5, 18.5 and 26 weeks after planting were measured for soil gravimetric moisture content, inorganic soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N WCC did not affect soil gravimetric moisture content at any point in the growing season (Table 2.7). There were effects of N fertilizer rate ( $p=0.01$ ), sampling time ( $p=0.01$ ), and interaction of N fertilizer rate  $\times$  sampling time ( $p=0.10$ ) on soil gravimetric moisture (Table 2.7). Although soil gravimetric moisture varied between 9.8 – 18% throughout the season, zero

fertilizer plots were less variable and tended to maintain higher moisture compared to fertilized plots (Figure 2.4).

Analysis detected treatment effects of N fertilizer rate, sampling time, WCC  $\times$  sampling time, N fertilizer rate  $\times$  sampling time, and WCC  $\times$  N fertilizer rate  $\times$  sampling time (all  $p=0.01$ ) on surface soil  $\text{NO}_3^-$ -N (Table 2.7). Sampling time was the only treatment effect detected on surface soil  $\text{NH}_4^+$ -N (Table 2.7). In 2013, there was more inorganic N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) available with WCC and N fertilizer application during the growing season compared to fallow plots (Figure 2.5, 2.6). When no fertilizer was applied, there was more  $\text{NH}_4^+$ -N in soil from 8 to 18.5 weeks after planting but less  $\text{NO}_3^-$ -N than fallow plots (Figure 2.5, 2.6).

There were no differences detected in soil  $\text{NO}_3^-$ -N both times sampled before fertilizer application (Figure 2.5, 2.6). When soils were sampled 5 weeks before planting, average soil inorganic N ranged from 0.16 – 0.17 kg  $\text{NO}_3^-$ -N ha<sup>-1</sup> and 4.2 – 6.3 kg  $\text{NH}_4^+$ -N ha<sup>-1</sup> across all treatment plots (Figure 2.5, 2.6). When soils were sampled again 5 weeks after plating, but before N fertilizer application, average soil surface (0 – 20 cm) inorganic N ranged from 32.7 to 45.2 kg  $\text{NO}_3^-$ -N ha<sup>-1</sup> and 4.2 to 5.8 kg  $\text{NH}_4^+$ -N ha<sup>-1</sup> across all treatment plots (Figure 2.5, 2.6).

WCC plots receiving 101 and 202 kg N ha had higher  $\text{NO}_3^-$ -N available for plant uptake compared to fallow plots after fertilizer application (Figure 2.5). The 101 kg N ha<sup>-1</sup> treatment plots with WCC had 77.3 kg  $\text{NO}_3^-$ -N ha<sup>-1</sup>, which was higher than fallow plots that only had 42.8 kg  $\text{NO}_3^-$ -N ha<sup>-1</sup> in the soil surface fertilizer application (Figure 2.5). The 202 kg N ha<sup>-1</sup>

treatment plots with WCC also had higher  $\text{NO}_3^-$ -N compared to fallow plots (117.9 vs. 67.7 kg  $\text{NO}_3^-$ -N  $\text{ha}^{-1}$ ) (Figure 2.5). There were no detected effects of WCC or N fertilizer rate on soil surface (0 – 20 cm)  $\text{NH}_4^+$ -N after N fertilizer application; average surface soil  $\text{NH}_4$ -N ranged from 9.2 – 29.4 kg  $\text{NH}_4^+$ -N  $\text{ha}^{-1}$  across all treatment plots (Figure 2.6).

At corn grain fill (13.5 weeks after planting), surface soil (0-20 cm)  $\text{NO}_3^-$ -N was four-fold in WCC plots compared to fallow plots (120.5 vs. 32.8 kg  $\text{NO}_3^-$ -N  $\text{ha}^{-1}$ ) in 202 kg  $\text{ha}^{-1}$  N fertilizer application plots (Figure 2.5). By corn plant maturity (18.5 weeks after planting), there was almost two-fold higher soil  $\text{NO}_3^-$ -N in fallow plots compared to WCC plots (129.3 vs. 79.0 kg  $\text{NO}_3^-$ -N  $\text{ha}^{-1}$ ) (Figure 2.5). There were no detectable differences in surface soil  $\text{NO}_3^-$ -N under cereal rye WCC and fallow plots in 0 and 101 kg  $\text{ha}^{-1}$  N fertilizer application plots after corn grain fill (13.5 weeks after planting) (Figure 2.5).

No effects of cereal rye WCC use on soil surface (0 - 20 cm)  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N after 2013 corn harvest (26 weeks after planting) were detected (Table 2.8). There was also no effect of WCC use on  $\text{NO}_3^-$ -N in deep soil profile (0 -100 cm) after 2013 corn harvest (26 weeks after planting) (Table 2.8). Both soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were affected by N fertilizer rate ( $p=0.01$ ) (Table 2.8). Soil  $\text{NO}_3^-$ -N was also influenced by depth ( $p=0.01$ ), and the interaction of N fertilizer rate  $\times$  depth ( $p=0.01$ ) (Table 2.8). Averages of total inorganic N ( $\text{NO}_3^-$ -N +  $\text{NH}_4^+$ -N) in soil surface was highest in 101 and 134 kg N  $\text{ha}^{-1}$  fertilizer plots (Table 2.9). There was generally more residual N in WCC plots that received N fertilizer, but this was not the case for

zero fertilizer plots with WCC which averaged a lower residual soil N in the surface than fallow plots (7.0 vs. 15.1 kg N ha<sup>-1</sup>), table 2.9.

A majority of NO<sub>3</sub><sup>-</sup>-N was found on the soil surface (0 - 20 cm) compared to the combined 20 - 100 cm intervals (17.3 vs 2.2 kg N ha<sup>-1</sup> across all plots), Fig. 2.7. The average NO<sub>3</sub><sup>-</sup>-N in the 70 - 100 cm depth interval was only 0.30 kg N ha<sup>-1</sup> (Figure 2.7). No cover crops were planted fall of 2013. Plots with 101 and 134 kg N ha<sup>-1</sup> fertilizer rates the highest combined residual NO<sub>3</sub><sup>-</sup> from to the 1 m depth, averaging 6.6 and 6.0 kg N ha<sup>-1</sup>, respectively. Similar to surface soil inorganic N, it was surprising to find that zero (2.2 kg N ha<sup>-1</sup>) and 202 kg N ha<sup>-1</sup> (4.8 kg N ha<sup>-1</sup>) fertilizer plots had lower residual N than fertilizer rates compared to 101 kg N ha<sup>-1</sup> (6.5 kg N ha<sup>-1</sup>).

### *2.3.6 Potentially Mineralizable N*

PMN was measured 5 weeks before planting, and 8, 13.5 and 18.5 weeks after planting. There was no effect of WCC on PMN throughout the growing season (Figure 2.8). There was an effect of N fertilizer rate (p=0.01), weeks after planting (p=0.01), and the interaction of N fertilizer rate × weeks after planting (p=0.01) (Table 2.7). PMN in all plots was lowest 5 weeks before planting and highest at week 8 (Figure 2.8). Although not significant, there was increased PMN in plots with WCC than fallow at all levels of N gradient 8 weeks after planting (Figure 2.8). After N fertilizer application (8 weeks after planting) on average, 1.20 (0.46), 18.70 (8.01) and 31.38 (5.81) kg N ha<sup>-1</sup> week on plots with zero, 101 and 202 kg N ha<sup>-1</sup> fertilizer treatment plots, respectively (Figure 2.8).



The average PMN was reduced 13.5 weeks after planting in all N gradients. The zero fertilizer plots at 13.5 weeks after planting had similar rates to 5 weeks before planting at 0.43 (0.13) kg N ha<sup>-1</sup> week (Figure 2.8). Plots with 101 kg N ha<sup>-1</sup> fertilizer rate reduced the most from 8 to 13.5 weeks after planting and were measured to be mineralizing 0.99(0.22) kg N ha<sup>-1</sup> week<sup>-1</sup>. The average PMN in plots receiving 202 kg N ha<sup>-1</sup> were mineralizing at 22.98 (9.10) and 1.11(0.36) kg N ha<sup>-1</sup> week and in WCC and fallow plots, respectively (Figure 2.8). WCC plots at 18.5 weeks after planting had similar or less PMN than fallow plots. Zero fertilizer plots had the highest rate of PMN when corn was at physiological maturity, 18.5 weeks after planting with an average of 2.62 (0.14) kg N ha<sup>-1</sup> week<sup>-1</sup> (Figure 2.8). This was similar to PMN observed in plots with 101 kg N ha<sup>-1</sup> which were averaging 2.54 (0.07) kg N ha<sup>-1</sup> week<sup>-1</sup> (Figure 2.8). High N fertilizer rate plots averaged 19.21 (6.57) kg N ha<sup>-1</sup> week<sup>-1</sup>. The higher rates of PMN at 18.5 weeks after planting (corn maturity) compared to PMN at grain fill (13.5 weeks after planting) (Figure 2.8).

### *2.3.7 Ion resin strip inorganic N adsorption*

There was no effect of WCC on soil inorganic N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) adsorption. However, analysis revealed that N fertilizer rate (p=0.05), sampling time (p=0.01), and the interaction of N fertilizer rate × sampling time (p=0.01) treatment effects on the adsorption rates of soil NO<sub>3</sub><sup>-</sup> (Table 2.7). Soil NH<sub>4</sub><sup>+</sup> ion resin strip adsorption rates were affected by N fertilizer rate (p=0.01), the interaction of WCC × N fertilizer rate (p=0.01), sampling time (p=0.01), and the interaction of N fertilizer rate × sampling time (p=0.01) (Table 2.7).

The total season averages of  $\text{NO}_3^-$  adsorption rates ranged from 0.30 to 0.58  $\mu\text{g NO}_3^-/\text{cm}^2$  soil/day (Figure 2.8). The highest adsorption rates occurred between 23 July and 14 August (0.58  $\mu\text{g NO}_3^-/\text{cm}^2$  soil/day), followed by 28 June and 19 July (0.51  $\mu\text{g NO}_3^-/\text{cm}^2$  soil/day) and 14 August and 4 September (0.48  $\mu\text{g NO}_3^-/\text{cm}^2$  soil/day) (Figure 2.8). Because ion-exchange resin strips simulate plant root adsorption, high  $\text{NO}_3^-$  adsorption at these time periods (28 June and 4 September) indicate that high rates of  $\text{NO}_3^-$  were being taken up by the plant. Though the lower  $\text{NO}_3^-$  adsorption rates (0.39  $\mu\text{g NO}_3^-/\text{cm}^2$  soil/day) at the earlier in the growing season (7 June to 28 June) is likely due to the fact that this was before N fertilizer was applied to the soils (Figure 2.8). The lowest adsorption of  $\text{NO}_3^-$  occurred between 10 September and 4 October, indicating that lower N was in the soil due to high N uptake from plants.

The total season  $\text{NH}_4^+$  adsorption rates were much more variable than  $\text{NO}_3^-$  adsorption rates and ranged from as low as 0.09 to as high as 0.65  $\mu\text{g NH}_4^+/\text{cm}^2$  soil/day (Figure 2.8). The average highest  $\text{NH}_4^+$  adsorption rate were between 28 June and 19 July (0.65  $\mu\text{g NH}_4^+/\text{cm}^2$  soil/day), followed by the sampling period between 23 July and 14 August (0.39  $\mu\text{g NH}_4^+/\text{cm}^2$  soil/day) (Figure 2.8). The lowest  $\text{NH}_4^+$  adsorption rates were between 10 September and 4 October, with only 0.09  $\mu\text{g NH}_4^+/\text{cm}^2$  soil/day (Figure 2.8). There were no observable differences in WCC and fallow plots of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  adsorption rates in the zero and 101 kg N ha<sup>-1</sup> fertilizer plots. Using WCC seemed to give an advantage with higher rates of  $\text{NO}_3^-$  -N

adsorption from 28 June to 4 October in 202 kg N ha<sup>-1</sup> fertilizer plots, but no trends in adsorption rates in the sample plots (Figure 2.8).

### 2.3.8 *Corn grain yields*

Importantly, there was no effect of WCC on corn yields between 2006 and 2013 (Figure 2.9). However, there were effects of N fertilizer rate ( $F=82.35$ ,  $p=0.01$ ), year ( $F=208.27$ ,  $p=0.01$ ) and the interaction between N fertilizer rate  $\times$  year ( $F=12.43$ ,  $p=0.01$ ) on corn grain yields. On average, across all N fertilizer rates, the drought year 2012 had the lowest yields (3.54 Mg ha) (Figure 2.9). Other years (2006, 2007, 2009, and 2010) averaged yields between 5.65 - 6.92 Mg ha (Figure 2.9). 2013 had the highest yield, with an overall average of 7.58 Mg ha (Figure 2.9). There were no years over the 9-yr period of this study where WCC posed negative impacts on corn yields compared to fallow treatment plots.

Grain yields from 2006-2013 and N fertilizer rates were fit to quadratic response with plateau regression models to find the N fertilize rate in which corn yields plateau. The N fertilizer rate at where the model yields plateau is the economic optimum N rate (ENOR) (Warncke et al., 2004). Table 2.10 shows the ENOR and yield at ENOR that were determined from regression model. Although there were not clear trends associated with WCC on ENOR, but there were some years where WCC achieved the same ENOR yield at a lower N fertilizer rate. In 2013, yields at ENOR were both 9.4 Mg ha; however the average ENOR with WCC was 23 kg N ha<sup>-1</sup> less than fallow plots (166 vs. 143 kg N ha<sup>-1</sup>) (Table 2.10). In 2010, 2012 and 2013, same yields were observed at lower ENOR in WCC plots compared to fallow plots; this was not true for 2006, 2007 and 2009 (Table 2.10).

## 2.4 Discussion

### 2.4.1 Cover crop accumulation

Biomass accumulation of cereal rye (+ weeds) and weedy fallows varied each year and this was likely due to weather conditions and planting dates. Weather conditions and planting dates can affect the overall cover crop growing period, often resulting in a reduction in biomass accumulation (Kuo and Jellum, 2000). WCC biomass in this study varied between years (0.53 to 1.46 Mg ha<sup>-1</sup>) (fig. 2.2), and similar results were observed in a field crop study at a sandy site in Wisconsin, where WCC yields ranged between 0.6 to 1.4 Mg ha<sup>-1</sup> (Andrask et al., 2005). Other studies have reported even more variable results, as in Snapp et al. (2005) in which rye produced between 0.8 and 2.9 Mg ha<sup>-1</sup> biomass when fall-sown in Michigan. Year to year variation in biomass accumulation of WCCs appears to be high, even in similar regions and environments.

Biomass accumulation was higher in years subsequent to soybean production compared to years after corn. Spring biomass accumulation was high in 2010, about two-fold higher compared to the other years for both WCC and fallow plots (fig. 2.2). This may be in part due to plant growth response to soil N fertility from the preceding crop (Sawyer et al. 2012). In addition, soybean is harvested earlier in the season than corn in Michigan (USDA NASS, 1997). As a result, in soybean years the fall cover crop is planted earlier than in corn years and the cover crop growing period is longer.

There was more spring biomass accumulation in WCC plots than fallow plots in 2007 and 2010, but this was not in 2013. The low amount of biomass accumulated in WCC plots in 2013 was expected due to poor conditions for rye establishment in the fall of 2012 including a late planting date (12 November, 2012), low temperatures and minimal rainfall. There was very low

residual N uptake by the cereal rye WCC from fall of 2012 to spring 2013. Additionally, weedy fallow plots were also able to accumulate as much biomass as cereal rye plots (0.59 and 0.53 Mg ha<sup>-1</sup>) and had similar N uptake (8.7 vs. 8.1 kg N ha<sup>-1</sup> biomass) in samples collected April 2013 (Fig. 2.2).

Years of higher biomass production indicates that more N was immobilized in biomass from pervious year. In 2007, 0.78 Mg ha<sup>-1</sup> of residue biomass contributed 14.4 kg N ha<sup>-1</sup> in WCC plots, and 0.88 Mg ha<sup>-1</sup> weed biomass in fallow plots contributed 13.9 kg N ha<sup>-1</sup> (McSwiney et al, 2010). Results from this study Our results were slightly higher than those reported by Baggs et al. (2000) where N uptake by WCC varied between 1 to 5 kg N ha<sup>-1</sup> between December and March in Scotland. The higher N uptake in biomass in this study may be due to the extended growth time since biomass in plots were collected in April of each year. Ritter et al. (1998) also found evidence suggesting that weedy fallows can be as effective at taking up residual soil N as a WCC in a sandy loam.

Winter cover crops can also contribute N benefits through N accumulations in root biomass (Isse et al., 1999). In the same study, McSwiney et al (2010) reports a 0.46 Mg ha<sup>-1</sup> accumulation of belowground biomass in WCC plots and only 0.20 Mg ha<sup>-1</sup> in fallow plots accumulated in 2007. Cereal rye is known to have more biomass than other potential WCCs (Kuo et al., 1997). Although belowground biomass accumulations were not measured each year, it is important to note that these contributions also affect N contributions after incorporation.

The overall C/N ratio of biomass of both winter rye and weeds across all plots ranged

between 24 and 28 (data not shown). This C/N ratio is similar to that obtained by (Kuo and Jellum, 2000). There were effects of N fertilizer rate on plant N uptake ( $F=4.85$ ,  $p=0.01$ ) and C/N ratio ( $F=3.23$ ,  $p=0.01$ ). The interaction of WCC  $\times$  N fertilizer rate also had an effect on the C/N ratio ( $F=1.86$ ,  $p=0.10$ ). There was a positive effect of N fertilizer rate on WCC biomass accumulation compared to zero fertilizer treatments (Fig. 2.2), which is consistent with other field studies (Shipley et al., 1992; Ditsch et al., 2008). In general, as N fertilizer rate increased, so did plant N uptake, which narrowed the C/N ratio (data not shown). This was clearly demonstrated in 2013 where detailed measurements of soil and plant N dynamics were undertaken. The consequences of N enrichment of WCC tissues include altered residue quality, notably the C/N ratio, and subsequent impacts on timing of N release through microbial mineralization. Because the C/N ratio of both the WCC and weeds were under 30, residues were expected to be associated with temporary immobilization, and release in time sufficient to meet crop demand the subsequent year, a practice that may be useful to farm management to reduce N losses (Waggoner, 1989; Vigil and Kissel, 1991; McSwiney et al., 2010).

#### *2.4.2 Corn Growth and N status in 2013*

The use of WCC for 9 years had no effect on corn plant growth when measured 5 (before fertilizer application), 10 (after fertilizer application) and 18.5 (corn at physiological maturity) weeks after planting (Table 2.4, 2.5). Historic use of N fertilizer positively influenced corn biomass and N concentration when measured prior to fertilization, 5 weeks after planting, at V6 growth stage. This may be due to residual N in soil from previous year's N application. Although minimal amounts of inorganic N from previous years are generally available during the growing

season, Ditch et al. (2008) suggests a caveat providing evidence of increased residual nitrogen from in the rooting zone after drought-like years.

In this study, the effects of N fertilizer application in early season corn growth may be from 2012 fertilizer application that accumulated in rooting zone from the abnormally low rainfall into spring of 2013.

Overall, corn tissue N concentration results show that there may be some benefit to early corn growth with WCC use when inorganic fertilizer is not used (Table 2.4). Enhanced corn tissue N concentration under WCC compared to fallow treatment also appears 10 weeks after planting plots under zero N fertilizer application (Table 2.4). Interestingly, there was an apparent 24% increase in corn plant N concentration when growing under cereal rye WCC treatment plots compared to fallow plots under zero N fertilizer application (Table 2.4). Although there were no benefits to corn biomass and N uptake under WCC in historically fertilized plots (before 2013 fertilizer application), there were benefits of increased soil surface inorganic N concentration under WCC plots compared to fallow plots after fertilizer application in 2013 (Table 2.4).

The benefits of WCC noted by increased N concentration in corn tissue in historically non-fertilized plots is suggestive that WCC uptake of residual N and release the next growing season is a process underway at all N fertilizer rates, including zero; however, benefits increase when fertilizer is applied (Fig. 2.7). In sum, this process may be important for preventing environmentally harmful losses of N, as supported by an earlier report from this study (McSwiney et al., 2010), but it did not appear to involve sufficient amounts of N to influence corn growth or justify reduction in N fertilizer application. These results conflict with those found by Tollenaar et al (1993), which report that corn growth and development was reduced when corn was preceded by a winter rye cover crop.

Additional influences of WCC and N fertilizer rate were measured with SPAD chlorophyll content. There was generally higher SPAD chlorophyll content when plants were measured 10 - 14 days after pollination (R2 or blister stage) than 24 - 28 days after pollination (R4 or dough stage) for all years SPAD was measured (Figure 2.3). When corn plants reach R3 (milk stage), the plant has reached maximum N uptake, making it one of the most popular pre-harvest yield prediction methods (Nielsen et al., 2004; Abendroth, 2011). Because chlorophyll content measurements have been shown to correlate with tissue N concentrations in the plant tissue, it has the ability to predict grain yields (Wood et al. 1992). After R3, the plant leaves may decrease in N content because the plant has completed N uptake from roots and is mobilizing excess N from leaves into the grain. This explains why the second SPAD chlorophyll meter readings are generally lower than the first reading. This trend was most noticeable in zero fertilizer plots and years in which inadequate rainfall stressed the plants. This is a critical time for plants to not experience water or N stress, if corn grain development is to be optimum (R1-R6).

#### *2.4.3 Soil moisture and inorganic N status during 2013 growing season*

Soil N status was assessed by monitoring soil surface (0 – 20 cm) inorganic N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N), PMN, and ion resin strip absorption. Soil samples for gravimetric moisture and soil extracted  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N, were collected 5 weeks before planting and 5, 8, 13.5, 18.5 and 26 weeks after planting. The soil environment, namely soil temperature and moisture, control the release of N from organic sources. Because the soil environment cannot be controlled in rainfed systems, it is challenging to predict patterns and amounts of N that could be released throughout the growing season. Since there were no treatment differences in soil moisture throughout the



2013 field season, it is likely that soil moisture did not mediate the differences in N mineralization observed (Fig. 2.4). Additionally, the favorable rainfall conditions during the 2013 growing season did not limit the N mineralization potential.

Because 2012 was a drought year and yields were low across all N rates, there was likely a  $\text{NO}_3^-$ -N accumulation in the rooting zone from the previous year (Ditsch et al., 2008). When soils were sampled 5 weeks before planting, average soil inorganic N ranged from 0.16 to 0.17 kg  $\text{NO}_3^-$ -N ha<sup>-1</sup> and 4.2 to 6.3 kg  $\text{NH}_4^+$ -N ha<sup>-1</sup> across all treatment plots (Figure 2.5, 2.6). These results are comparable to results found by Sainju and Singh (2001) who reported  $\text{NO}_3^-$  concentration ranged from 4.5 to 6.0 mg kg<sup>-1</sup> and  $\text{NH}_4^+$  ranged from 1.3 to 2.5 mg kg<sup>-1</sup> when measured 4 to 7 d before cover crop kill on a sandy loam in central Georgia. Higher  $\text{NO}_3^-$  may be from increased net mineralization from warmer spring temperatures in GA compared to MI. Residual N from preceding drought year may have been the main contribution to the increases in  $\text{NO}_3^-$  witnessed in treatment plots from 5 weeks before planting to 5 weeks after planting (Fig. 2.5). Because soil  $\text{NH}_4^+$  does not change from the first two samplings, this may be reflecting the clay “fixed”  $\text{NH}_4^+$  pool, fig. 2.6 (Stevenson, 1994).

It rained hours after urea fertilizer was applied to the field at peak corn N uptake (V6 stage) in 2013 and likely made a majority of the urea fertilizer applied readily available and actively taken up by the growing corn plants. The higher  $\text{NH}_4^+$  after N fertilizer application compared to the first two samplings, before application are expected results (Fig. 2.6). The lack of an effect of N fertilizer rate on differences in  $\text{NH}_4^+$  in plots receiving N fertilizer treatments indicate that

soil conditions were ideal for crop N uptake and applied fertilizer was readily hydrolyzed to  $\text{NH}_4^+$  and then further oxidized for  $\text{NO}_3^-$ . Under adequate soil moisture and temperature, 75% of the urea applied is often converted within 7 d after application (Honeycutt et al., 1991; Agehara and Warncke, 2005).

Results showed trends of additional soil  $\text{NO}_3^-$ -N WCC compared to fallow treatment plots when fertilizer was applied (Fig. 2.5). However,  $\text{NO}_3^-$ -N was lower in WCC than fallow plots when fertilizer was not applied. This suggests that N fertilization is stimulating N mineralization, also known as the “priming effect” (Jenkinson et al., 1985). Lundquist et al. (1999) reported that microbial biomass C increased substantially and rapidly following cover crop incorporation in all. WCC residues were incorporated at least two weeks before corn planting and this likely enhanced N mineralization synchrony, reflected by the increase of soil  $\text{NO}_3^-$ -N from the first two soil samplings, fig. 2.5 (Kuo and Jellum, 2000). However, because there were no difference in residue biomass additions between WCC and fallow treatments in 2013, it is unlikely that this was the direct impact of the priming effect. Differences in soil  $\text{NO}_3^-$  in WCC vs. fallow in 2013 may have been from previous year’s residue additions and long-term cover crop use impacts on labile and recalcitrant soil N pools (Sainju et al., 2003).

Overall, soil inorganic N results were consistent with synchronization of WCC N release to peak crop N uptake and shown when measured at V6 stage (5 weeks after planting) and V8-12 (8 weeks after planting) compared to fallow plots. (Figure 2.4). Results suggest that the effect of WCC comes from its influence on soil N availability since relatively little benefits were found in corn biomass and N concentrations. These results confirm those found by Vyn et al. (2000) and

Kuo and Jellum (2002) showed that the benefits of WCC is from its influence on soil N availability but has little impact on subsequent row crop growth.

A barrier to farmers adopting WCC is determining the rate of N fertilizer to apply in the absence of an ability to predict if WCC will have positive or negative effects on N mineralization. This was a top concern of Michigan farmers diversifying potato rotations with cover crops (Snapp et al., 2005). Grass cover crops in particular pose a risk of immobilizing applied soil N fertilizer (Ranells and Waggoner, 1996). Results from this study show N management with WCC with synchrony of N release to crop N demand is possible. Conflicting results reported in scientific literature is likely due to the year-to-year variations in weather that reflect soil moisture and temperature, thus affect N mineralization rates. Brennan et al. (2013) suggests that even after 8 years of CC use, year-to-year weather variability influence decomposition rates and soil mineral N dynamics more than biomass addition amount. In addition, Vigil and Kissel (1991) report that regardless of the amount of biomass incorporated, the C/N ratio of crop residue was the best predictor of the amount of N mineralization in residue-amended soils. In Michigan, the effects of immobilization may not be as large of a concern when N fertilizer is applied since biomass accumulations remain relatively low from short bare periods for WCC due to long duration row-crops in rotation. However, negative effects of immobilization from WCC should be further investigated in drought years.

The large amount of residual soil  $\text{NO}_3^-$ -N at corn physiological maturity is consistent with asynchrony of N mineralization in fallow plots, and potential for loss later in the year. When soils were sampled again after corn harvest, 26 weeks after planting for the season, soil  $\text{NO}_3^-$  was measured to the 1 m depth. Nitrate does not bind to cation exchange sites in the soil,

allowing it to readily leach out of the soil surface. This is especially true on this study site which became increasingly sandy at > 30 cm depth. Ammonium was only measured in the soil surface (0 - 20 cm). Overall, after the 2013 harvest, there was generally more residual N under WCC plots that received N fertilizer compared to fallow plots. However, the zero fertilizer plots under WCC averaged a lower residual soil N in the surface than fallow plots.

The N mineralization potential of soil is used to reflect the amount of N available for corn uptake within a growing season (Kuo et al., 1996). Plant available N from mineralization has been shown to provide 20 to 80% of the N required by plants (Broadbent, 1984). Results prior to N fertilizer application confirm the net mineralization rates that varied between 0.25 and 1.50 kg N ha<sup>-1</sup> day<sup>-1</sup> as reported by (Schroder et al., 2000) (Fig. 2.7). Soil net mineralization potential for the same study collected in end of May of 2007 averaged 14 (1.9) kg N ha<sup>-1</sup> 28 d<sup>-1</sup> in WCC plots while only 8 (1.9) was observed in fallow plots (McSwiney et al. 2010). Although season N mineralization rates were similar in both 2007 and 2013, there were smaller differences in net mineralization between WCC vs. fallow plots in 2013 (Fig. 2.7). The lack of differences from historic N fertilizer treatment (before fertilizer was applied) in net mineralization rates (may be from the relatively cooler spring temperatures that may be limiting mineralization when soils were tested in April 2013).

Increases in PMN are consistent with an active organic matter pool that is a readily mineralizable source of N (Kuo and Jellum, 1996; Wander, 2004; Steenwerth and Belina, 2008). The greater net N mineralization rates in the WCC treatments demonstrated that these soils had greater potential N availability compared to fallow plots and provides evidence for the differences in soil NO<sub>3</sub><sup>-</sup>-N witnessed between WCC and fallow plots under both 101 and 202 kg

N ha<sup>-1</sup> fertilizer rates (Fig. 2.5, 2.7). Throughout the growing season, there was generally more potentially mineralizable N in plots with WCC than fallow at all levels of N gradient that was tested (0, 101 and 202 kg N ha<sup>-1</sup>) 8 weeks after planting, suggesting that N mineralization is in synchrony with plant uptake (Fig. 2.7). However, in zero fertilizer plots, season highest net mineralization took place 18.5 weeks after planting, providing evidence for asynchrony in mineralization of N to crop N uptake when is applied; this also explains the higher residual N observed under zero fertilizer plots after corn harvest in November 2013 (Fig. 2.7, Table 2.8).

The high rates of net mineralization even at 18.5 weeks after planting (corn maturity) compared to net mineralization at grain fill (13.5 weeks after planting) observed in plots reviewing 202 kg ha<sup>-1</sup> N fertilizer consequently increased residual N that remained and was potentially lost after corn harvest (Fig. 2.7, Table 2.8). Higher to mineralization observed at 8, 13.5 and 18.5 weeks after planting may be effects of increased mineralization after spring tillage practices and residue incorporation (Rice and Havlin, 1994; Grandy and Robertson, 2007). General variations in net mineralization throughout growing season may also be from weather, soil conditions, crop rotation, and management of crop residues and cover crops (Schroder et al., 2000).

Ion exchange resins are used to measure the amounts of plant-available nutrient ions in soils and the rates at which they are released (Qian and Schoenau, 2001). Ion exchange resins were deployed in three-week periods from early June to early October in 2013. Trends in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> adsorption were inversed in fertilized vs. non-fertilized plots (Fig. 2.8). Ammonium adsorption rates were more variable than nitrate adsorption in fertilized plots, whereas nitrate absorption rates were more variable than ammonium adsorption in zero fertilized plots (Fig. 2.8).

In zero fertilized plots, there was generally more  $\text{NO}_3^-$  adsorption than  $\text{NH}_4^+$  adsorption throughout the entire growing season (Fig. 2.8). However, when fertilizers applied, there is slightly more  $\text{NH}_4^+$  adsorption than  $\text{NO}_3^-$  adsorption. Overall, there were no detectable differences in N adsorption from WCC and N fertilizer rate treatments in 2013 (Fig. 2.8, Table 2.4). In 2007, as a part of the same study, McSwiney et al. (2010) observed no differences in cumulative N availability in ion exchange resin strips under WCC vs. fallow plots.

Because ion-exchange resin strips simulate plant root adsorption, high  $\text{NO}_3^-$  adsorption at these time periods (28 June and 4 September) indicate that high rates of  $\text{NO}_3^-$  were being taken up by the plant. The lowest adsorption of  $\text{NO}_3^-$  occurred between 10 September and 4 October, indicating that lower N was in the soil due to high N uptake from plants. Although measurements can be converted to an area basis, it is important to note that these values represent only general fluxes of nutrient ions towards to the resin. Overall, results from ion exchange strip adsorption seem support the differences in soil N availability when compared to soil extracted inorganic N and net N mineralization from 2013 growing season (Fig. 2.4, 2.7, 2.8).

#### *2.4.4 Post-corn harvest residual N status in 2013*

There was generally more residual N under WCC plots that received N fertilizer compared to fallow plots after corn harvest in 2013 (Table 2.9). However, the zero fertilizer plots under WCC averaged a lower residual soil N in the surface than fallow plots. Almost 85-90% of residual soil  $\text{NO}_3^-$ -N was found on the soil surface (0 - 20 cm) compared to the combined 20 - 100 cm intervals (17.3 vs 2.2 kg N ha<sup>-1</sup> across all plots) (Table 2.9). Because  $\text{NO}_3^-$  is the main interest,

the focus on residual N will be mostly in terms of  $\text{NO}_3^-$ . There was less almost 68% less  $\text{NO}_3^-$ -N in soil surface (0 – 20 cm) in WCC plots than fallow plots in zero fertilizer (Table 2.9). However, there were no differences in soil  $\text{NO}_3^-$ -N under WCC and fallow plots in plots with N fertilizer use (Table 2.9). There was actually slightly more  $\text{NO}_3^-$ -N in soil surface (0 – 20 cm) under all WCC plots compared to fallow plots, except for those that received  $134 \text{ kg N ha}^{-1}$  (Table 2.9).

The difference in soil  $\text{NO}_3^-$  from corn plant maturity and post harvest soil sampling is alarming, and a large amount of N is unaccounted for, especially in higher N fertilized plots (Fig. 2.5). The percent of inorganic  $\text{NO}_3^-$ -N in soil surface (0-20 cm) remaining after corn harvest in 2013 ranged from 7% to 40% (Table 2.9). Gardner and Drinkwater (2009) report that on average, 38% of applied fertilizer is unaccounted for in crops and soil at the end of one growing season. In both WCC and fallow plots, the percent of soil  $\text{NO}_3^-$ -N in soil surface (0-20 cm) was inversely proportional to N fertilizer rate. When  $34 \text{ kg N ha}^{-1}$  fertilizer was applied, about 40% of the N applied was found at the end of the growing season under both WCC and fallow plots (Table 2.9). Of the  $202 \text{ kg N ha}^{-1}$  that was applied, the same plots averaged only 7% of that applied N rate in the soil surface was in the soil surface (Table 2.9). However, the residual N found in soil profile after harvest cannot be directly related to N from fertilizer application since methods used cannot separate fertilizer-derived  $\text{NO}_3^-$ -N from mineralized N sources. The inverse relationship of reduced residual N with increasing N fertilizer application contradicts results reported by LeClerc (1987) and Pearson et al. (2003); they both found fall soil  $\text{NO}_3^-$ -N

contents increased with increasing N rates on sandy loam soils.

Across all treatment plots, the average  $\text{NO}_3^-$ -N in the 70 - 100 cm depth interval was very low ( $0.30 \text{ kg N ha}^{-1}$ ). Low  $\text{NO}_3^-$ -N in 70 – 100 cm below soil may be because not very much  $\text{NO}_3^-$ -N was leaching out of the system since much of it was in the soil surface (0-20 cm), or much of the residual N had already leached out of the system in the wet, cool fall in 2013. Because this was the final year of the study, WCC was not planted in fall of 2013, and cannot determine the effectiveness of WCC use on reducing N leaching. A field experiment by Rasse et al. (2000) on sandy loam soils in southwest Michigan reported that 80% of total annual leaching had already occurred by August. Ritter and others (1998) suggest that the cover crop should probably be planted by October 1 to maximize nitrogen uptake rates in the fall. The same study also concluded that cereal rye as a WCC cannot be counted on as a best management practice for reducing nitrate leaching in the Mid-Atlantic states on sandy loams (Ritter et al., 1998).

#### *2.4.5 Corn grain yields*

There were no years where WCC posed negative impacts on corn yields during this 9-year study, as compared to fallow plots (Table 2.9). These results confirm other published research. Ball-Coelho and Roy (1997), Ritter et al. (1998) and Strock et al. (2004) also report no corn yield penalty with cereal rye WCC compared to fallow/bare fields. Corn biomass accumulation samplings, plant tissue N concentration, and SPAD chlorophyll content results support these findings in 2013, as there were no observed reductions in early or mid-season growth under WCC compared to fallow plots (Table 2.4, 2.5; Fig. 2.3). Results from this study confirm those



observed by Andraski and Bundy (2005) in a 3-year study on coarse-textured soils in Wisconsin; this study concluded that whole-plant corn dry matter yields and N uptake were not significantly affected by the presence of a cereal rye WCC.

Compared to seasonal weather variation, corn yield was less responsive to N rate applied, but had little detectable response to WCC, except in select N fertilizer rates in 2006, 2007 and 2009 (Fig. 2.1, 2.9). There were some exceptions in some years where WCC caused disadvantages in subsequent corn yields. In the first year of the study, 2006, low fertilizer plots (0 and 34 kg N ha<sup>-1</sup>) had lower yields under WCC than fallow plots (Fig. 2.9). In 2007, plots receiving 34 and 67 kg N ha<sup>-1</sup> had lower yields under WCC than fallow plots (Fig. 2.9). In 2009, N application rates of 0, 67, 101, 168 and 202 kg N ha<sup>-1</sup> all experienced a yield disadvantage with WCC (Fig. 2.9). Higher biomass accumulations of winter wheat WCC in 2005-2006 and cereal rye WCC in winter and early spring of 2008-2009 likely also widened the residue C/N ratio (Wagger, 1989), causing temporarily immobilization in soil N McSwiney et al. (2010), causing asynchrony in soil N availability with plant uptake, fig 2.1 (Ranells and Wagger, 1996; Vyn et al., 2000). In Iowa corn, Carlson et al. (2010) found no affect on grain yields across 8 site-years, except for those years when WCC not properly managed prior to corn planting. Results from this study highlight the importance of proper management practices to ensure optimal use from WCC inclusion in corn rotations to influence soil N status to provide advantages to N supply during corn growth. It is important to note that there are some studies that have reported to finding reduced yields in subsequent crop after WCC when not managed properly (Sainju and Singh, 2001; Tonitto et al., 2005).

Nitrogen response to corn grain yields from 2006 to 2013 under WCC vs. fallow

treatments were further analyzed by fitting quadratic response with plateau regression models (Table 2.10). The N fertilizer rate where the model yields plateau is the economic optimum N rate (ENOR), a tool often promoted to optimize cost of N return in yield (Warncke et al., 2004). Table 2.10 shows the EONR and yield at EONR determined from the regression model. Researchers have determined that a quadratic-with-plateau is the most suitable response function for modeling corn yield response to N (Cerrato and Blackmer, 1990; Boyer et al., 1990; Bulluck III et al., 2002). Our findings are consistent with this model, as corn yields did not linearly increase with N fertilizer rate application, but rather plateaus at a specific N rate each year (Fig. 2.9). Corn yields at the EONR under WCC were not different to fallow treatments, resulting in no yield benefit following WCC (Table 2.10, Fig. 2.9). However, there were interesting findings in numerically lower EONR in later years of WCC use (Table 2.10). Substantial benefits at a lower EONR with unaffected yields at ENOR were observed in 2013. Model showed that yield at EONR was 9.4 Mg ha<sup>-1</sup> while the average ENOR with WCC was 23 kg N ha<sup>-1</sup> less than fallow plots (166 vs. 143 kg N ha<sup>-1</sup>) (Table 2.10). In a 3-yr experiment on Central Sands region of Wisconsin where Andraski and Bundy (2005) reported that ENOR was an average of 32 kg N ha<sup>-1</sup> lower for corn following WCC compared with corn following winter fallow treatment. Our findings and the findings of Andraski and Bundy (2005) suggest that winter rye can provide yield benefits to the subsequent corn crop at slightly lower N fertilizer rates, and these effects may become stronger and more reliable over time. In a 4-year study by Sawyer et al. (2012) estimated an average 10 lb N/acre higher economic optimum N rate and a 5 percent lower corn yield with the rye cover crop. Another important finding from this study is the lack of effect of WCC on yields at ENOR even during a drought year as experienced in 2012.

Overall, our results suggest that year-to-year weather variation has a marked influence on corn grain yield, fig. 2.1, 2.9 (Andresen et al. 2001; Cassman et al., 2002; Kravchenko et al., 2005; Smith et al., 2007). The impacts of year-to-year variability in climate, primarily with rainfall amount and pattern affect soil water and its availability is of greatest relative importance on crop performance (Andresen et al. 2001). Soil water also has a substantial influence on soil microbial activity, decomposition processes and mobility of plant nutrients. As previously stated, the primary advantages of WCC come from the increased ability to ecologically manage N and maintain the N in the rooting zone, thus reducing N losses via leaching and influencing soil N availability to subsequent crops (Sainju et al. 1998; Baggs et al. 2000; Vyn et al. 2000; Kuo and Jellum, 2002). Management practices such as chemically killing WCC in timely manner and breakdown of residue with tillage incorporation can accelerate the release of N from residue amendments, providing some control over N mineralization to synchronize with crop N demand (Wagger, 2002). However, ultimately, the microbial activity and subsequent release of plant nutrients is determined by soil moisture and temperature. In rainfed systems, a challenge that is needed for maximize benefits of WCC rely in the ability to predict seasonal weather more accurately to determine and control mineralization of N release to synchronize with crop uptake.

Advantages to grain yields with lowered N rates as shown by lower EONR without affecting yields under WCC offers the greatest practical benefit to growers, especially in coarse-textured soils with high leaching potentials (Andraski and Bundy, 2005). Providing farmers with a “benefit to cost ratio” of a more judiciously selected N fertilizer rate with WCC on rotation can influences farmer adoption rate of practice (Cassman et al., 2002). Overall, EONR analysis and increased  $\text{NO}_3^-$ -N synchrony with corn N demand from 2013 results in this study encourage the

need of combining the growing technology of increased accuracy in predicting short-term (seasonal) weather forecasts and in considering N application rates when using WCC. Combining management practices (kill date, incorporation date, planting date) with more accurate short-term (seasonal) weather can increase the ability to synchronize N mineralization in soil with crop N demand. By better determining potentially mineralizable N, farmers can improve decisions regarding N fertilizer application rates. Understanding N mineralization can help increase reliability of WCC as a management practice to influence soil N availability during growing season. Ultimately, improving the decisions related to the selection to maximize profitable and lower rate of fertilization on a field scale has economic benefits to the farmer as well as environmental benefits on a regional and national scale (Cerrato and Blackmer, 1990).

## 2.5 Conclusions & Future Directions

Between 2006 and 2013, the annual aboveground cereal rye WCC accumulation ranged between 0.53 to 1.46 Mg ha<sup>-1</sup> at this site in SW Michigan. Nitrogen retained in WCC biomass was influenced by the N fertilizer rate and residual soil N from preceding crop. Practical limitations to WCC biomass growth are likely from late planting after long-season row crops and year-to-year weather variability in this region. Because cereal rye growth remained in its vegetative stage when killed and incorporated, the C/N ratio of residue was under 30 and likely experienced net mineralization in fertilized plots (Sainju and Singh, 2001). Our results find evidence of synchrony of N mineralization to crop N demand after cereal rye WCC compared to fallow plots during the 2013 corn growing season. The increase in soil NO<sub>3</sub><sup>-</sup>-N is supported by evidence from higher net mineralization rates and increases in soil NO<sub>3</sub><sup>-</sup>-N adsorption in ion

exchange resin strips. Benefits of increased soil  $\text{NO}_3^-$  were found after N fertilization of crops 5 weeks after planting (corn at V6). However, the synchrony of N mineralization with crop N uptake did not result in any yield benefits in 2013. In addition, we found no effects of WCC on biomass accumulation and N concentration in grain at harvest.

The percent of inorganic  $\text{NO}_3^-$ -N in soil surface (0-20 cm) remaining after corn harvest in 2013 ranged from 7% to 40% and there was generally more residual N under WCC plots that received N fertilizer compared to fallow plots. Because this was the final year of the study, WCC was not planted in fall of 2013, and cannot determine the effectiveness of WCC use on reducing N leaching. Rasse et al. (2000) reported that 80% of total annual leaching had already occurred by August on sandy loam soils in southwest Michigan. Because corn N uptake ceases at R3, it may be beneficial to areal seed cereal rye into standing corn to increase effectiveness of residual N uptake. There is some evidence of success of increase N uptake of WCC when cereal rye seeds are overseeded into corn systems (Ball-Coelha and Roy, 1997; Rasse et al., 2000; Wilson et al., 2013). Further research is still needed to investigate how to effectively establish rye stands in corn without reducing yields.

Over the 9-yr period of this study, WCC posed no negative impacts on corn yields compared to fallow treatment plots. Nitrogen response to corn grain yields from 2006 to 2013 under WCC vs. fallow treatments were further analyzed by fitting quadratic response with plateau regression models to determine EONR and yield at EONR. Corn yields at the EONR under WCC were not different to fallow treatments, resulting in no yield benefit following WCC. However, there were interesting findings in numerically lower EONR in 2010, 2012 and 2013 under WCC compared to fallow treatment plots. This was not true for 2006, 2007 and 2009, suggesting that the use of

WCCs do provide to be advantageous for yields, but may take 4+ yrs of WCC use in a corn-corn-soy rotation. These findings suggest that farmers may find economic benefits with consistent yields at lower N fertilizer rates under WCC.

However, there still remain challenges in utilizing WCC as a N management tool during row crop production. It can be difficult to predict and control potential N from mineralization in rainfed systems since it is controlled by environmental factors. Additionally, is need for further research with more consistent results of yield benefits with lower N rates with WCC use under conventional tillage management. Showing yield increases with lower N fertilizer rates would encourage farmers to adopt WCCs since “benefit to cost ratio” of new management adoption has large influence on farmer adoption rates (Cassman et al., 2002).

## APPENDIX

**Table 2.1** Cropping rotation summary at the study site from 2005 – 2013.

Year	Cropping History	
	Cover Crop	Cash Crop
2005	Winter wheat †	--
2006	Winter rye ‡	Corn §
2007	Winter rye	Corn
2008	Winter rye	Soy
2009	Winter rye	Corn
2010	Winter rye	Corn
2011	Winter rye	Soy
2012	Winter rye	Corn
2013	Winter rye	Corn

† Winter wheat was planted at a rate of 168 kg seed ha<sup>-1</sup>

‡ Winter rye was planted at a rate of 134 kg seed ha<sup>-1</sup>

§ Corn was planted at a rate of 81,543 seeds ha<sup>-1</sup>



**Table 2.2** General timeline of agronomic management operations during growing season from 2006 – 2013, and management dates for 2013 season.

Operation	Approximate timing	2013
Cover planted	After corn harvest	10 Nov 2012
Cover termination	Early May	8 May
P and K fertilizer application†‡	2 wks after cover termination	14 May
Cover crop incorporation §	1 -2 d after K application	15 May
Corn planting #	Early May – early June	4 Jun
N fertilizer application	July (plant V6 stage)	9 -10 Jul
Corn harvest	Mid October - November	12 Nov

† P fertilizer was applied only to subplots receiving 0 kg N ha<sup>-1</sup> fertilizer in 2012 and 2013

‡ K fertilizer at rate of 78.4 kg ha<sup>-1</sup> was applied to all subplots

§ Cover crop, P and K fertilizers were incorporated with chisel plow at depth of 20 cm

# Field was prepped for planting with soil finisher (cultivator plow) same day as planting

**Table 2.3** Monthly summary of growing season (May – October) weather data for corn growing years in rotation and historical averages from 1981 – 2010 at Kellogg Biological Station in SW Michigan.

Year	Month	Total precipitation mm	Daily average temp. °C	Average daily max. temp °C	Average daily min. temp °C
2006	May	140.72	14.11	20.64	8.26
	June	51.05	19.63	26.53	13.19
	July	79.25	23.02	29.29	16.92
	August	148.97	21.16	27.53	14.96
	September	100.20	15.20	21.00	9.90
	October	127.64	8.53	14.51	3.11
2007	May	65.02	16.82	23.79	9.78
	June	46.10	21.10	27.56	13.95
	July	19.30	21.61	28.22	14.61
	August	171.70	21.92	27.81	16.31
	September	47.37	18.11	25.04	11.24
	October	147.45	13.97	19.48	8.68
2009	May	49.54	14.85	21.69	7.84
	June	95.50	19.34	25.87	13.03
	July	7.36	19.16	25.17	12.72
	August	173.99	19.77	25.75	14.55
	September	32.00	16.88	23.92	10.73
	October	122.17	8.51	13.35	4.36
2010	May	134.88	16.06	22.32	10.20
	June	184.13	20.25	26.04	14.88
	July	148.84	23.55	29.90	17.45
	August	34.04	22.53	28.55	17.19
	September	66.55	16.52	22.21	11.36
	October	48.01	11.59	17.95	5.61
2012	May	30.23	17.19	23.89	9.82
	June	22.86	21.05	27.86	13.39
	July	45.46	25.28	32.09	18.10
	August	70.11	20.66	27.55	13.98
	September	58.31	16.45	23.58	10.12
	October	143.00	9.82	15.79	5.77
2013	May	118.61	16.66	22.71	10.04
	June	107.94	19.41	25.28	13.88
	July	82.56	21.66	27.42	15.81
	August	117.09	20.07	26.49	14.19
	September	19.32	16.70	23.25	10.35
	October	106.16	11.00	16.86	5.84

**Table 2.3 (cont.)**

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1981 – 2010	May	99.06	22.44	8.72	15.61
	June	100.84	27.44	14.39	20.67
	July	97.79	29.44	16.22	22.83
	August	106.68	28.11	15.61	21.83
	September	119.13	24.00	11.22	17.61
	October	87.88	17.11	5.33	11.22

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**Table 2.4** Averages and standard errors (in parenthesis) of corn plant biomass ( $\text{Mg ha}^{-1}$ ), N concentration (%), and C/N ratio under cereal rye WCC and fallow at 0, 101, and 202  $\text{kg ha}^{-1}$  N fertilizer rates. Two-way ANOVA F values are also presented; statistically significant differences are denoted. Corn plants were sampled before and after N fertilizer rate application in 2013.

N fertilizer rate $\text{kg N ha}^{-1}$	Winter Management	Pre N Application Corn Plant (5 weeks after planting)			Post N Application Corn Plant (10 weeks after planting)		
		Biomass $\text{Mg ha}^{-1}$	N Uptake $\text{kg N ha}^{-1}$	C/N Ratio	Biomass $\text{Mg ha}^{-1}$	N Uptake $\text{kg N ha}^{-1}$	C/N Ratio
0	WCC	2.97 (0.31)	1.26 (0.07)	33.79 (1.62)	40.32 (3.21)	0.38 (0.04)	120.23 (12.03)
	Fallow	3.06 (0.24)	1.03 (0.05)	40.66 (1.65)	47.28 (7.22)	0.50 (0.04)	87.67 (6.83)
101	WCC	2.86 (0.38)	1.14 (0.05)	37.05 (1.54)	49.68 (3.31)	0.93 (0.02)	46.60 (0.98)
	Fallow	2.74 (0.58)	1.19 (0.04)	35.10 (1.04)	47.36 (3.59)	0.87 (0.04)	50.63 (2.49)
202	WCC	3.21 (0.30)	1.29 (0.05)	32.77 (1.15)	41.02 (2.35)	1.03 (0.07)	43.55 (2.68)
	Fallow	3.52 (0.45)	1.25 (0.04)	33.66 (1.03)	57.09 (2.65)	0.92 (0.03)	47.32 (1.43)
Factor		F Value					
WCC		0.09	0.64	3.2	3.41	2.31	0.09
N Fertilizer Rate (N)		1.06	3.03 *	5.29 **	0.63	24.23***	1.06
WCC $\times$ N		0.15	0.83	6.26 ***	2.51	4.66	0.15

\*Significant at the  $\alpha = 0.10$  level (two-tailed)

\*\*Significant at the  $\alpha = 0.05$  level (two-tailed)

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

**Table 2.5** Averages and standard errors (in parenthesis) of corn plant biomass ( $\text{Mg ha}^{-1}$ ), N concentration (%), and C/N ratio at harvest (physiological maturity) under cereal rye WCC and fallow at 0, 101, and 202  $\text{kg ha}^{-1}$  N fertilizer rates. Two-way ANOVA F values are also presented; statistically significant differences are denoted.

N fertilizer rate kg N/ ha	Winter Treatment	Physiologically Mature Plants								
		Vegetative Tissue			Reproductive Organs			Physiologically Mature Plant Grain		
		Biomass	N Conc.	C/N Ratio	Biomass	N Conc.	C/N Ratio	Biomass	N Conc.	C/N Ratio
		g / plant	%		g / plant	%		g / plant	%	
0	WCC	6.99 (0.63)	0.21 (0.03)	377 (14)	6.58 (0.21)	0.11 (0.00)	217 (23)	50.21 (6.13)	0.42 (0.01)	100 (2)
	Fallow	6.87 (0.73)	0.22 (0.03)	356 (31)	6.50 (0.25)	0.12 (0.01)	220 (22)	47.40 (3.53)	0.47 (0.04)	92 (8)
101	WCC	9.94 (0.18)	0.27 (0.02)	260 (17)	7.04 (0.35)	0.17 (0.01)	173(15)	105.38 (4.64)	0.78 (0.09)	58 (8)
	Fallow	10.16 (0.44)	0.28 (0.02)	211 (9)	6.85 (0.38)	0.21 (0.01)	164 (17)	107.26 (5.66)	0.65 (0.09)	70 (8)
202	WCC	12.23 (0.96)	0.29 (0.01)	155 (9)	6.89 (0.12)	0.28 (0.02)	153 (8)	120.56 (5.14)	0.75 (0.08)	58 (6)
	Fallow	13.19 (0.55)	0.28 (0.03)	148 (11)	6.94 (0.14)	0.30 (0.02)	165 (16)	124.67 (5.24)	0.77 (0.10)	59 (6)
<b>Factor</b>		<b>F Value</b>								
WCC		0.05	3.84	1.99	0.89	0.1	0.56	0.39	0.05	0
N Fertilizer Rate (N)		9.04 ***	56.35 ***	47.02 ***	35.22***	2.77	13.41***	117.81 ***	4.36 **	12.77 ***
WCC $\times$ N		4.47 **	2.5	0.45	1.49	0.15	0.31	1.82	0.35	0.46

\*\*Significant at the  $\alpha = 0.05$  level (two-tailed)

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

**Table 2.6** Results of multiple-effects ANOVA F values of treatment effects on SPAD chlorophyll; statistically significant differences are denoted.

Factor	F Value
WCC	0.02
N Fertilizer Rate (N)	417.28***
WCC $\times$ N	0.41
Year (Y)	66.94***
WCC $\times$ Y	1.49
N $\times$ Y	23.5***
WCC $\times$ N $\times$ Y	2.2
Sample (S)	239.28***
WCC $\times$ S	1.86
N $\times$ S	25.58***
WCC $\times$ N $\times$ S	0.64
Y $\times$ S	70.93***
WCC $\times$ Y $\times$ S	1.56
N $\times$ Y $\times$ S	7.86***
WCC $\times$ N $\times$ Y $\times$ S	0.53

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

**Table 2.7** Results of multiple-effects ANOVA F values of treatment effects on soil surface (0 – 20 cm) gravimetric moisture, inorganic N as NO<sub>3</sub>-N and NH<sub>4</sub>-N measured from soil extract and ion resin strips, and potentially mineralizable inorganic N (NO<sub>3</sub>-N+NH<sub>4</sub>-N) during 2013 growing season. Statistically significant differences are denoted.

Factor	Soil Moisture	Soil Extract N		Ion Resin Strip N		PMN
		NO <sub>3</sub> -N	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NH <sub>4</sub> -N	Total N
	F Value					
WCC	0.31	1.73	1.71	2.79	0.08	3.51
N Fertilizer Rate (N)	19.87***	35.5***	0.63	3.04**	33.91 ***	15.94***
WCC × N	0.04	1.89	0.56	0.09	6.61***	1.69
Sampling (S)	113.77***	18.16***	15.04***	3.47***	31.34 ***	10.44***
WCC × S	0.47	3.78***	1.21	0.24	0.59	1.16
N × S	1.74*	10.95***	1.16	3.21 ***	8.44***	3.22***
WCC × N × S	0.84	2.66***	0.73	0.71	0.57	0.72

\*Significant at the  $\alpha = 0.10$  level (two-tailed)

\*\*Significant at the  $\alpha = 0.05$  level (two-tailed)

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

**Table 2.8** Results of multiple-effects ANOVA F values of treatment effects on post harvest (November 2013) residual inorganic nitrate (NO<sub>3</sub>-N) and ammonium (NH<sub>4</sub>-N); statistically significant differences are denoted.

Factor	Post Harvest Inorganic N	
	NO3	NH4
	F Value	
WCC	0.1	0.08
N Fertilizer Rate (N)	40.02***	2.83 **
CC × N	0.86	0.56
Depth (D)	28.68***	--
WCC × D	0.38	--
N × D	9.07***	--
WCC × N × D	1.16	--

\*\*Significant at the  $\alpha = 0.05$  level (two-tailed)

\*\*\*Significant at the  $\alpha = 0.01$  level (two-tailed)

-- Indicates factor not tested

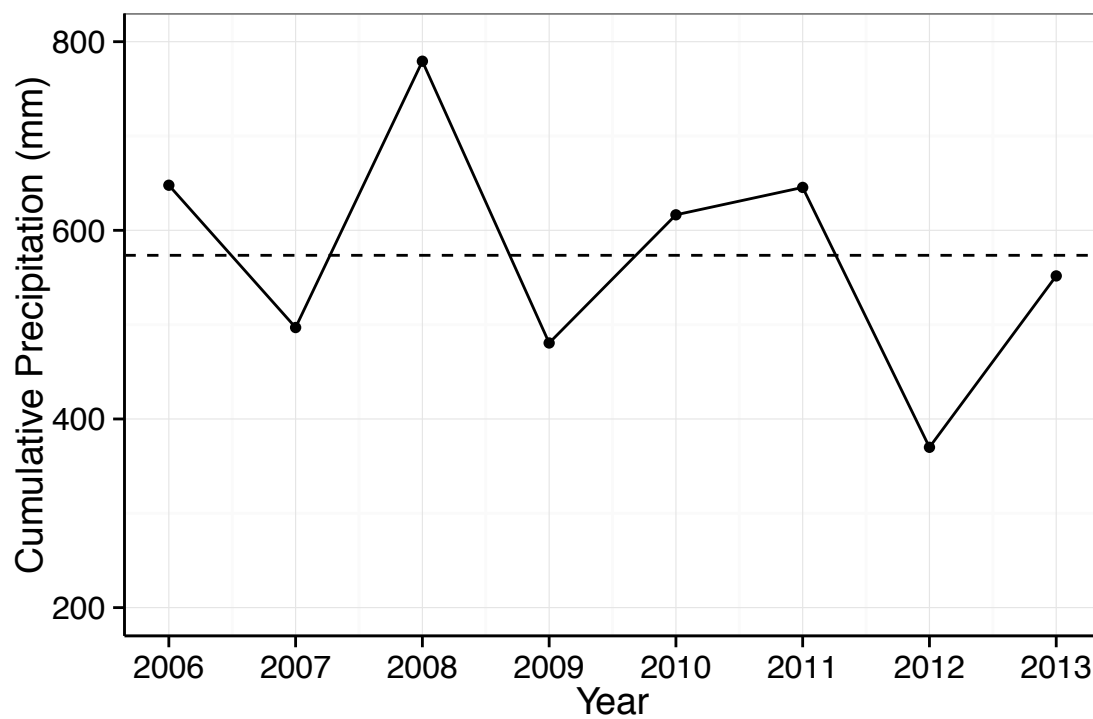


**Table 2.9** Averages and standard errors (in parenthesis) of inorganic soil N (NO<sub>3</sub>-N and NH<sub>4</sub>-N) measured after corn harvest in November 2013.

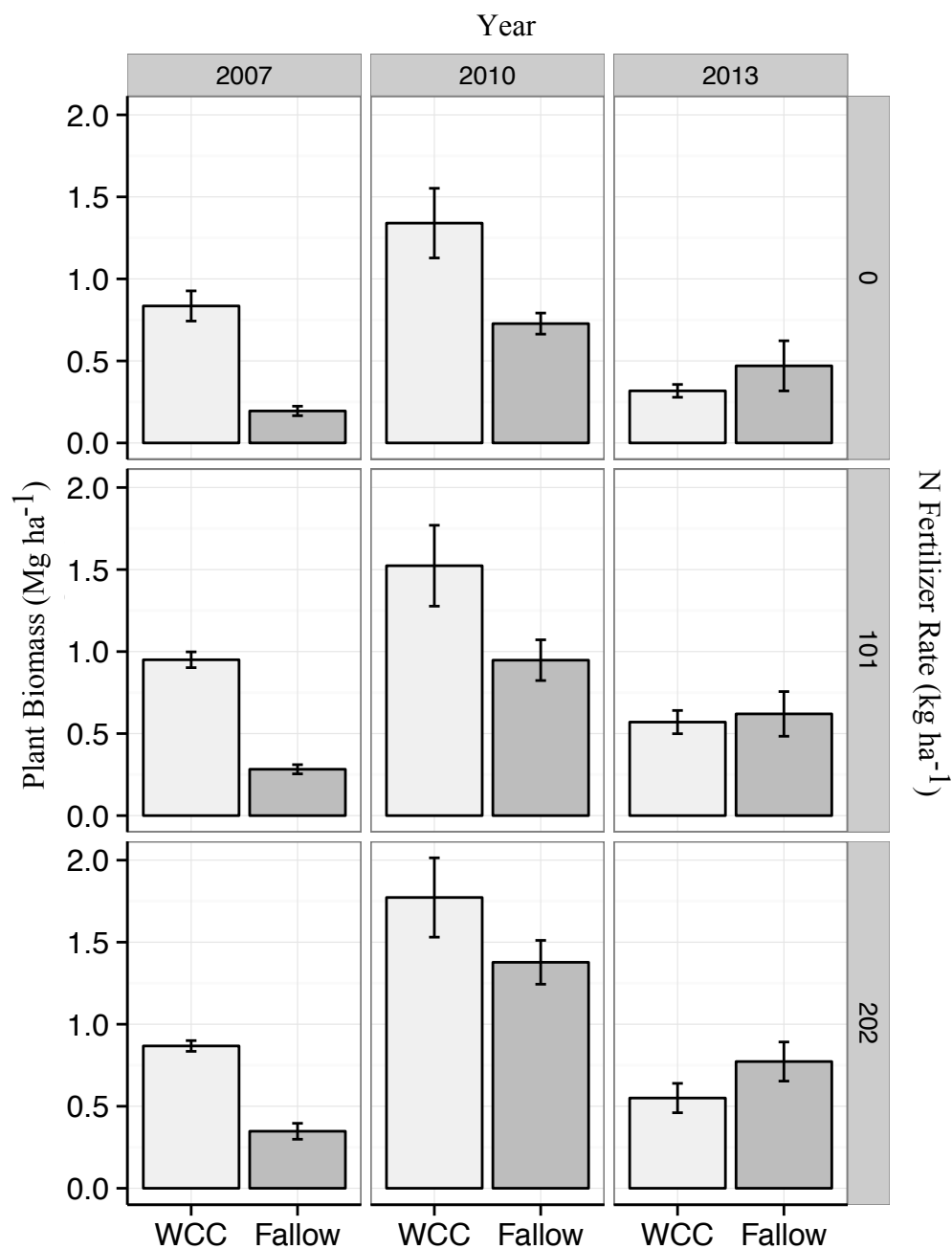
Depth	N Fertilizer Rate	Residual Soil Inorganic N			
		Fallow	WCC	Fallow	WCC
	kg ha <sup>-1</sup>	kg NO <sub>3</sub> -N ha <sup>-1</sup>		kg NH <sub>4</sub> -N ha <sup>-1</sup>	
0 - 20 cm	0	11.7(1.1)	3.8(1.0)	3.4(0.5)	3.2(0.1)
	34	12.6(1.3)	14.0(2.3)	3.1(0.2)	3.3(0.4)
	67	18.3(4.0)	19.7(1.2)	2.6(0.2)	2.9(0.2)
	101	21.9(1.9)	26.2(2.3)	3.4(0.2)	3.0(0.3)
	134	23.6(1.5)	19.5(1.8)	7.2(3.6)	4.6(2.1)
	168	19.9(5.8)	20.9(1.1)	1.6(0.3)	1.9(0.6)
	202	14.0(2.5)	15.8(4.6)	2.6(0.5)	3.5(0.4)
20 - 40 cm	0	0.5(0.1)	0.5(0.3)		
	34	0.3(0.0)	0.3(0.0)		
	67	0.7(0.3)	0.7(0.1)		
	101	1.0(0.2)	1.0(0.1)		
	134	2.0(0.7)	1.1(0.3)		
	168	1.8(1.1)	1.6(0.6)		
	202	2.5(1.0)	2.1(0.5)		
40 - 70 cm	0	0.2(0.0)	0.4(0.2)		
	34	0.3(0.1)	0.3(0.0)		
	67	0.6(0.2)	0.7(0.1)		
	101	1.1(0.3)	1.1(0.2)		
	134	0.7(0.2)	0.8(0.1)		
	168	0.6(0.1)	0.7(0.1)		
	202	0.8(0.3)	1.1(0.4)		
70 - 100 cm	0	0.1(0.0)	0.1(0.0)		
	34	0.1(0.0)	0.1(0.0)		
	67	0.1(0.1)	0.1(0.0)		
	101	0.1(0.0)	0.1(0.0)		
	134	0.4(0.2)	0.2(0.1)		
	168	0.7(0.3)	0.6(0.2)		
	202	1.2(0.3)	0.4(0.2)		

**Table 2.10** Equations represent the quadratic with plateau regression models of corn grain yield response to N fertilizer 2006 to 2013 at the Kellogg Biological Station in southwest MI. Model includes  $y$  as the yield of corn grain ( $\text{Mg ha}^{-1}$ ),  $x$  is the rate of N fertilizer application, (ENOR) is the economic optimum N rate (critical rate of fertilizer rate which occurs at the intersection of the quadratic response) and P is the plateau yield of corn grain at ENOR.

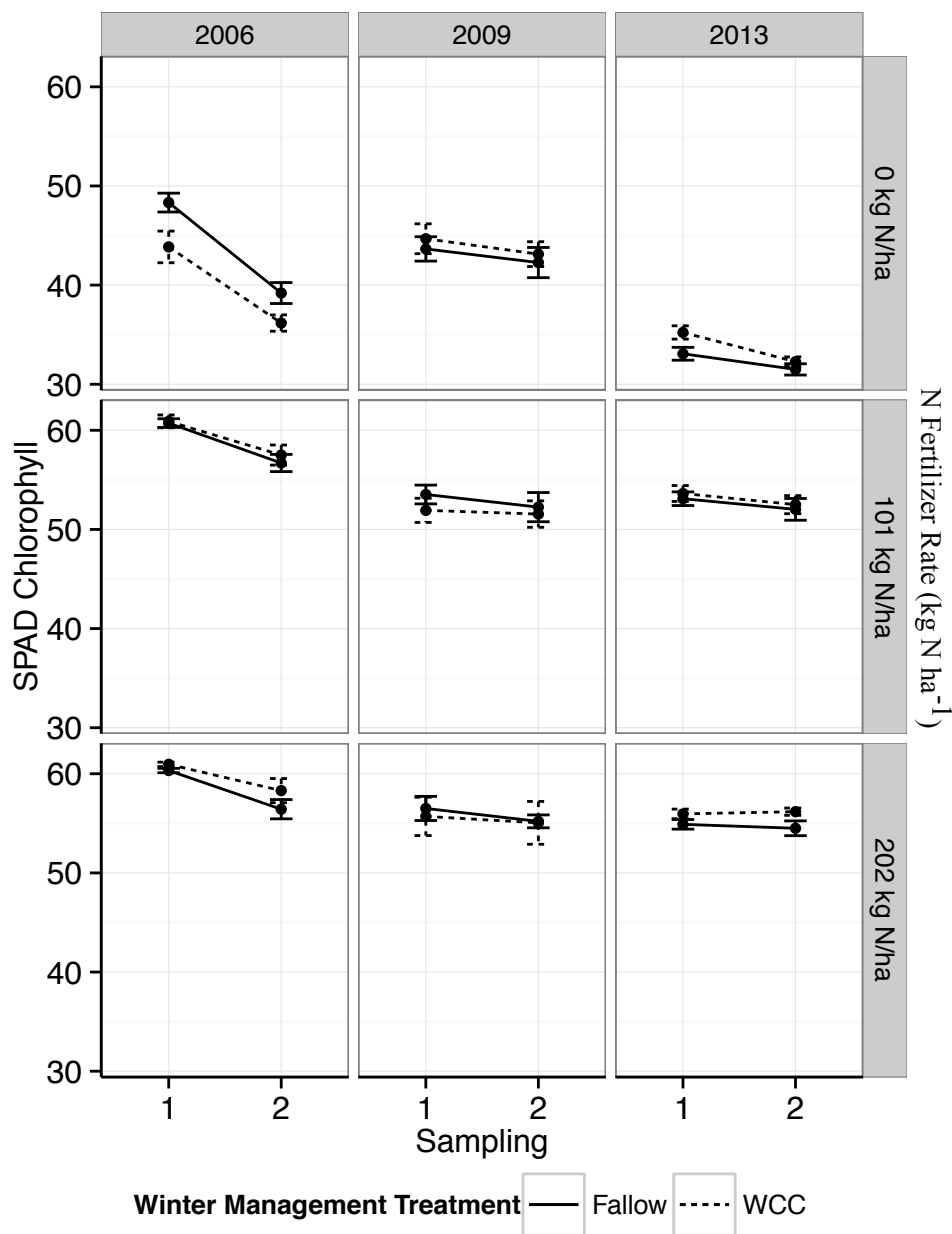
Year	Winter Treatment	$y = \alpha + \beta x + \gamma x^2$	P	ENOR
			$\text{Mg ha}^{-1}$	$\text{kg N ha}^{-1}$
2013	WCC	$y = 3.500 + 0.083 x - 0.00029 x^2$	9.4	142.97
2013	Fallow	$y = 3.218 + 0.075 x - 0.00023 x^2$	9.4	166.46
2012	WCC	$y = 3.223 + 0.063 x - 0.0025 x^2$	3.6	12.62
2012	Fallow	$y = 2.703 + 0.097 x - 0.0025 x^2$	3.6	19.45
2010	WCC	$y = 2.754 + 0.070 x - 0.00019 x^2$	9.1	181.48
2010	Fallow	$y = 2.645 + 0.068 x - 0.00018 x^2$	9.1	188.54
2009	WCC	$y = 3.488 + 0.036 x - 0.00012 x^2$	6.4	146.2
2009	Fallow	$y = 2.999 + 0.057 x - 0.00021 x^2$	6.4	136.33
2007	WCC	$y = 4.681 + 0.027 x - 0.00006 x^2$	7.3	212.46
2007	Fallow	$y = 4.255 + 0.089 x - 0.00071 x^2$	7.3	62.22
2006	WCC	$y = 2.480 + 0.088 x - 0.00033 x^2$	8.3	134.25
2006	Fallow	$y = 3.946 + 0.062 x - 0.00024 x^2$	8.0	129.17



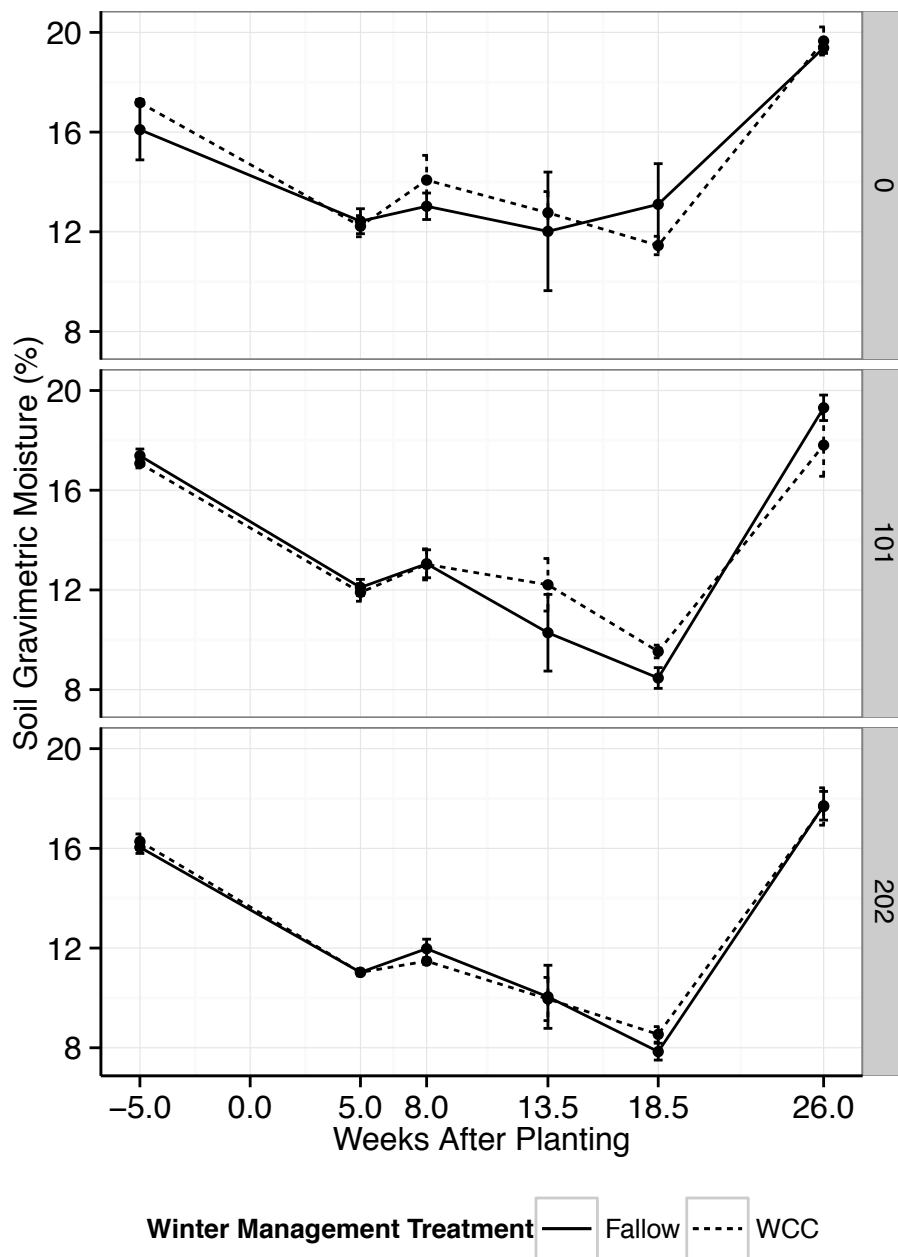
**Figure 2.1** Cumulative precipitations during growing season (May – October) from 2006 – 2013, Dashed line represents the 1981-2010 historical average growing season rainfall at Kellogg Biological Station in SW Michigan.



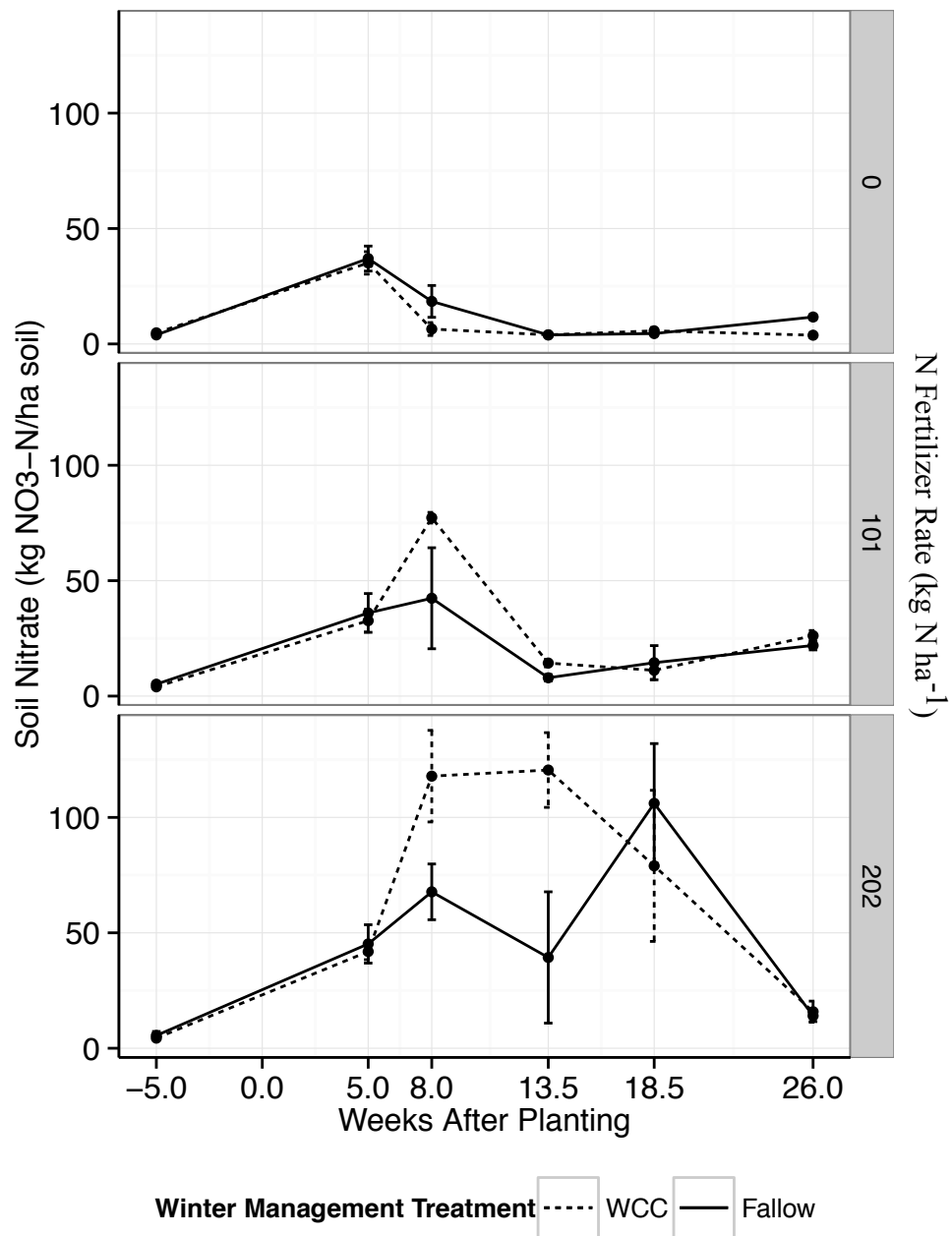
**Figure 2.2** Average plant biomass (Mg ha<sup>-1</sup>) under cereal rye cover crop (WCC) and fallow at 0, 101, and 202 kg ha<sup>-1</sup> N fertilizer rates, measured April of 2007, 2010 and 2013 at the Kellogg Biological Station in SW Michigan. WCC plots consisted of cereal rye and weeds, fallow plots of weeds. Error bars represent standard errors.



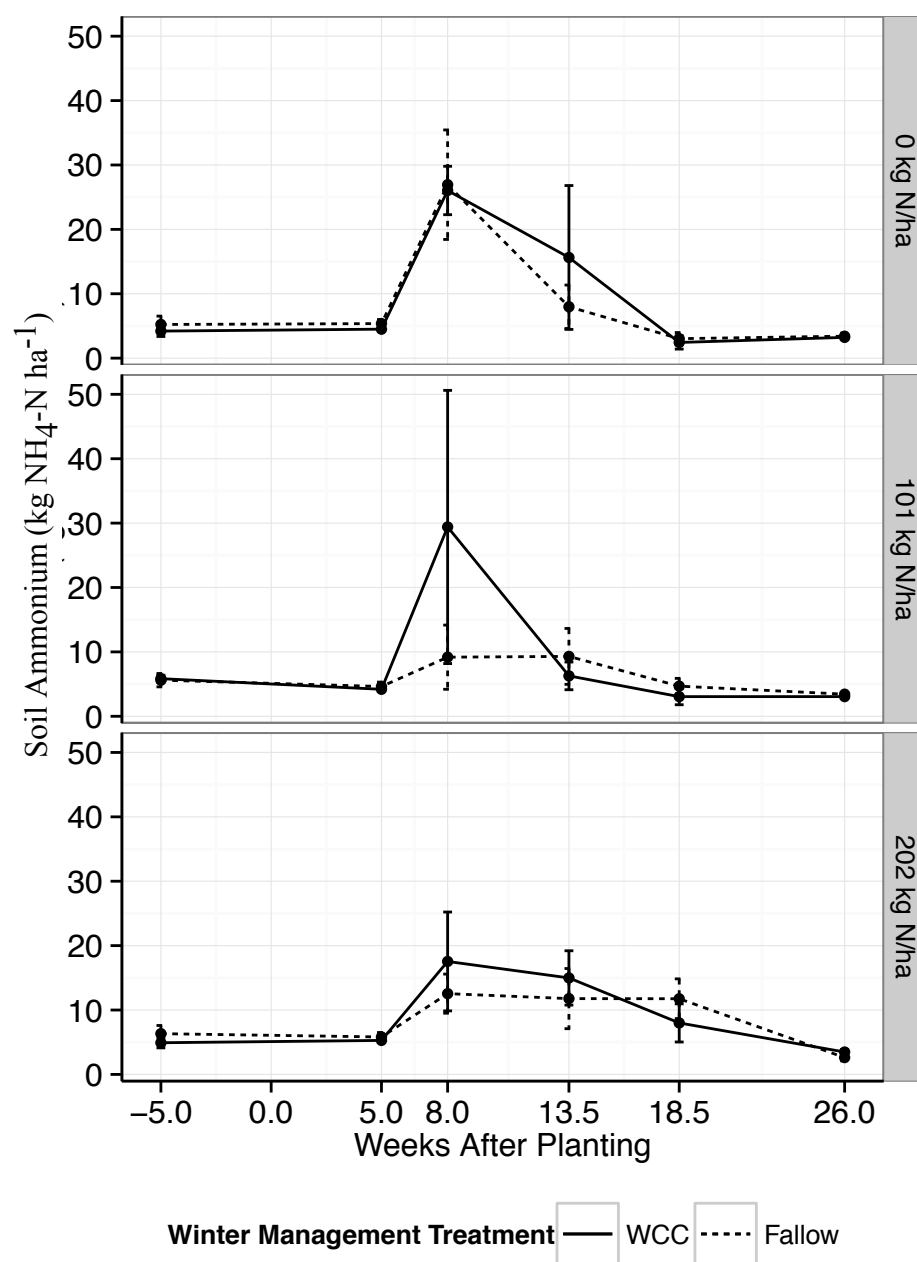
**Figure 2.3.** SPAD chlorophyll content under cereal rye cover crop (WCC) and fallow at 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer rates at two sampling times: 1=10-14 days after pollination and 2=24-28 days after pollination. Chlorophyll meter readings for stage 1 and 2 took place on 31 July and 16 August in 2006, 26 June, 1 August and 3 September in 2007, and 21 August and 4 September in 2013. Error bars represent standard error.



**Figure 2.4** Soil surface (0 – 20 cm) gravimetric moisture under cereal rye cover crop (WCC) and fallow at 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer rates from late April to mid-November in 2013. Error bars represent standard error.

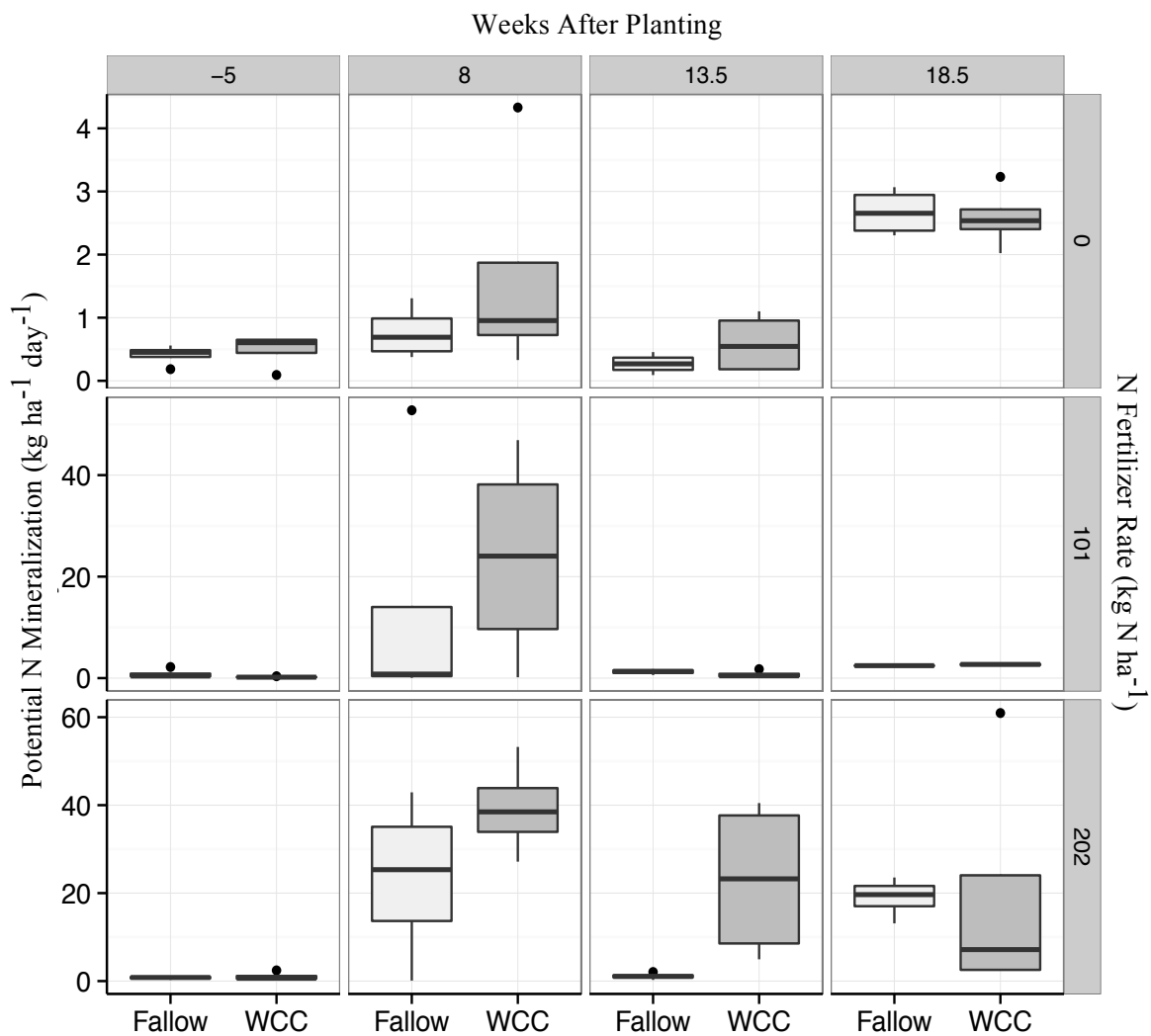


**Figure 2.5** Inorganic nitrate (NO<sub>3</sub>-N) in soil surface (0 – 20 cm) under cereal rye cover crop (WCC) and fallow at 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer rates from late April to mid-November in 2013. Error bars represent standard error.

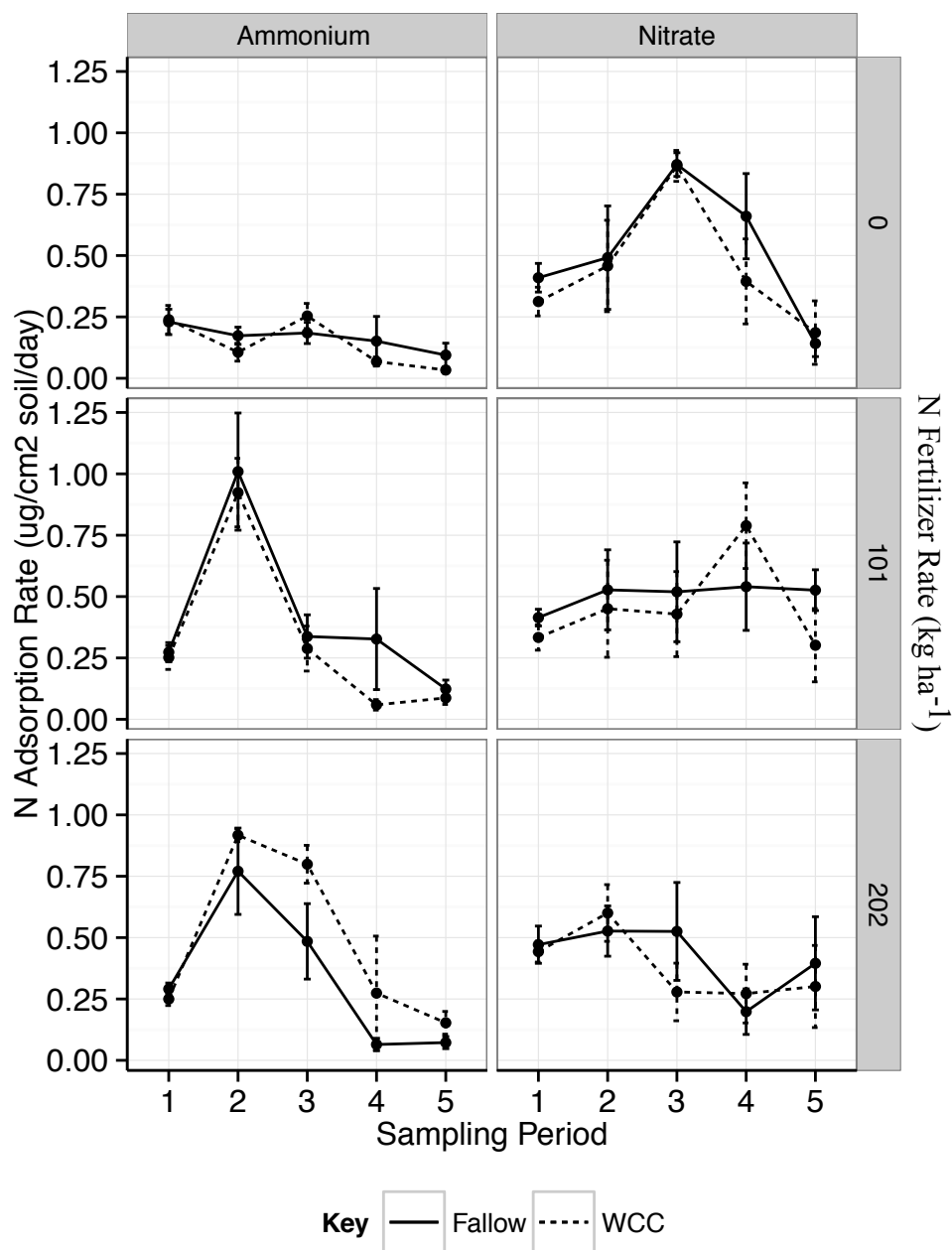


**Figure 2.6** Inorganic ammonium (NH<sub>4</sub>-N) in soil surface (0 – 20 cm) under cereal rye winter cover crop (WCC) and fallow at 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer rates from late April to mid-November in 2013. Error bars represent standard error.

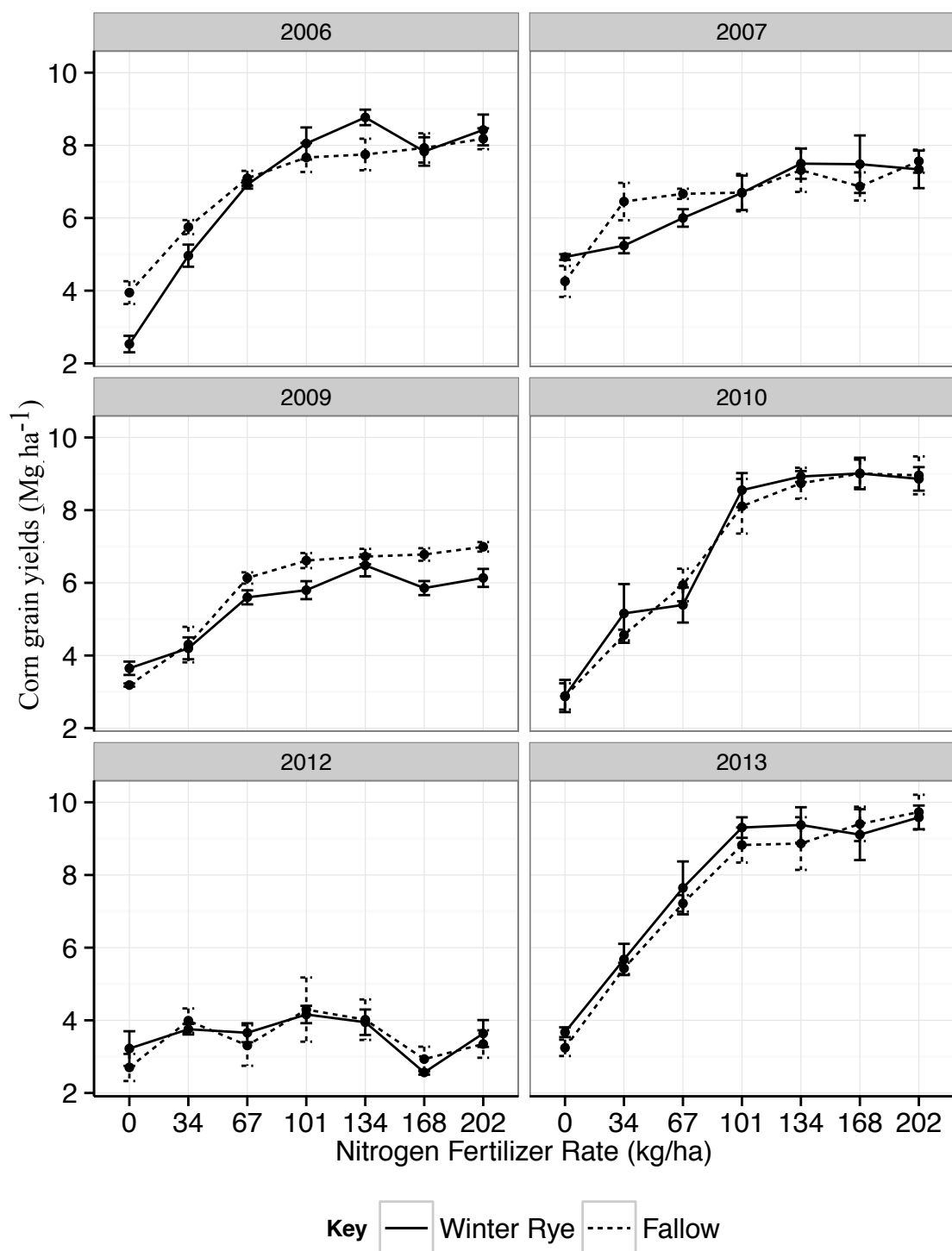




**Figure 2.7** Boxplots of potentially mineralizable N (kg N ha<sup>-1</sup> week<sup>-1</sup>) under cereal rye cover crop (WCC) and fallow at 0, 101, and 202 kg N ha<sup>-1</sup> fertilizer rates from soils in 2013.



**Figure 2.8** Ion exchange resin strips adsorption rates of nitrate (NO<sub>3</sub>-N) and ammonium (NH<sub>4</sub>-N) throughout the growing season from under cereal rye cover crop (WCC) and fallow at 0 and 202 kg ha<sup>-1</sup> N fertilizer rates. Sampling periods represent the following 2013: 1=7 June–28 June; 2=28 June–19 July; 3=23 July–14 August; 4= 14 August–4 September; and 5=10 September–4 October. Error bars represent ± standard errors of treatment means. Error bars represent standard error.



**Figure 2.9** Corn grain yields (Mg ha<sup>-1</sup>) from 2006 to 2013 under cereal rye cover crop (WCC) and fallow at 0, 34, 67, 101, 134, 168, and 202 kg ha<sup>-1</sup> N fertilizer rates at the Kellogg Biological Station in southwest MI. Error bars represent  $\pm$  standard errors of treatment means

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