NITROGEN FERTILIZATION EFFECTS ON LITTER DECOMPOSITION DYNAMICS AND SOIL CARBON IN AGRICULTURAL SYSTEMS

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ABSTRACT

NITROGEN FERTILIZATION EFFECTS ON LITTER DECOMPOSITION DYNAMICS AND SOIL CARBON IN AGRICULTURAL SYSTEMS

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I examined the effects of three N applications (0,134,291 Kg N /ha) on corn (*Zea* mays) and wheat (*Triticum* aestivum) litter decomposition rates, soil and litter enzymes, soil C pools, and litter chemistry at the W.K. Kellog Biological Station Long-Term Ecological Research (LTER) site. There were no changes in litter chemistry after decomposition under N fertilization and the litter decomposition rates did not respond to N fertilization but there was significantly (P = <0.001) greater mass loss in corn (73% mass loss) than wheat (63% mass loss). Laboratory incubations indicated that soil C-mineralization significantly declined (P=0.0001) under 291Kg N/ha in comparison to 0N. There were no significant differences in light fraction (LF) C and N between treatments receiving 0 and 291 Kg N/ha. The C concentration (mg/g of LF) of the LF organic matter increased by 8% in June and 1.6% in Aug in the high N treatment in comparison to 0N, and the N concentration (mg/g of LF) increased by 16 % in June and by 8% in Aug under 291 Kg N/ha treatment in comparison to 0N. The fertilizer N had no effect on total soil C or chemically labile organic matter while the activity of hydrolase and oxidase enzymes in soil and in both types of litter showed temporally variable response to N. The results of this study indicate that N fertilization does not increase litter decomposition rates under no-till management systems, had variable effects on soil enzymatic activity, and substantially reduced soil CO_2 flux in the lab.

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CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Increased N availability often increases plant biomass and litter inputs (Liu & Greaver, 2010). These above ground litter inputs represent the major contributions to the soil organic matter pool (Sullivan et al., 2007) but below ground soil C may also increase (Alvarez, 2005), decrease (Khan et al., 2007) or remain unchanged (Halvorson et al., 2002) in response to N addition. This variation depends on the changes in the chemical composition of the litter and /or the microbial activity in the soil. For example, Khan et al. (2007) reported that N fertilization reduced soil C despite increased residue incorporation under continuous corn and reported an even greater decline under corn-soybean and corn-oat rotation. Contrary to this finding, Gregorich et al. (1996) reported higher C storage with increased fertilizer use and residue inputs under continuous corn for 30 years. Similarly, a study conducted in temperate and boreal forests in the Northern hemisphere showed an increase in C uptake by these forests over a wide range of N deposition rates (Magnani et al., 2007). Due to the inconsistency of these results, and the importance of soil organic matter dynamics in local, regional and global processes, there is an urgent need to better understand the mechanisms associated with the sequestration of soil organic carbon (SOC).

Agricultural soils in the US alone have lost nearly 30 to 50 Mg C/ha of SOC due to cultivation (Lal, 2002). There is a growing concern about how high N availability in the soil profile will affect C storage. Soil organic carbon (SOC) is the basis for most key soil functions, plays a key role in the global C cycle, and is the foundation upon which sustainable agricultural

systems have historically been built. SOC influences soil quality by improving water holding capacity of the soil and studies suggest that for every 1g increase in the SOM there is a 1 to 10g increase in soil moisture (Emerson, 1995). SOC increases nutrient availability to plants by increasing the cation exchange capacity (Johnston, 1986), and enhances soil structure and aggregation (Feller & Beare, 1997). For all the aforementioned benefits of SOC, soils with high SOC have the potential to give higher crop yields (Ganzhara, 1998; Zhukov et al., 1993). Further, C sequestration has been suggested by many researchers as a means to restore degraded soils and increase their productivity (Lal, 2006). Thus not only is SOC important for improving soil quality and productivity but its storage in soil can help offset atmospheric increases in CO_2 (Pretty & Ball, 2001).

SOC sequestration depends on the additions and losses of carbon in soils (Christopher & Lal, 2007). The balance between additions in the form of net primary production (NPP) of crops or other organic inputs such as manure and the losses by decomposition determines the quantity of SOC storage (Russell et al., 2009).

$$\Delta SOC = \text{additions} - \text{losses}$$

However, these gains and losses in SOC are influenced by many factors including moisture, temperature, soil type and land management factors such as tillage and fertilization (Russell et al., 2009).

Nutrient inputs in the form of fertilizer can have a profound impact on soil carbon through:

I. increasing net primary production

II. influencing the decomposition rate of SOC (increase or decrease) and

III. changing the chemistry of plant tissues, which can influence their decomposition rate.

Historically, it was widely thought that SOC would increase – at least in agricultural systems – with N enrichment because of the increased productivity and residue inputs. However, highly variable results across a number of studies suggests that there is an uncertainty regarding the response of soil carbon to N enrichment due to N deposition from fossil fuel combustion and the intensive use of N fertilizer in the agricultural systems. If N enrichment influences the rate of decomposition by changing microbial activity or the microbial community composition and the chemistry of the litter, soils could either lose or gain soil C and its associated functions

Soil Organic Matter: Soil organic matter (SOM) formation is a complex process that includes the processing of leaf litter, plant residues, soil organisms and manure. The 'living matter' of the organic matter includes the plants, animals and microbes and it becomes a part of the 'non-Living matter' when it goes through the decay process after death. Cellulose and hemicellulose are the most abundant polysaccharides in plant tissues. Although these compounds are not as recalcitrant as lignin, they are often protected from microbial decomposition by aromatic compounds, (Carreiro et al., 2000). The different plant constituents are degraded by different

classes of enzymes produced by microbes in the following order, starting from least to most recalcitrant compounds:

Amino acids > Hemicellulose > Cellulose > Lignin

Reflecting the variation in plant tissue and microbial residue chemistry, organic matter is extremely heterogeneous and consists of an incredible diversity of compounds that range in their chemical structure, turnover time (Walcott, Bruce, & Sims, 2009), origin, and behavior in soil (Grandy et al., 2009). Soil scientists are always looking for ways to better understand SOM, its composition and behavior. Thus, a number of fractionation procedures have been developed that separate SOM into different fractions with discrete behavior. In a recent paper, Walcott et al. (2009) partitioned SOM into the following categories:

Dissolved organic matter: an energy source for microbial metabolism. It is a small fraction of the total soil carbon and its composition ranges from simple amino acids to complex high molecular weight molecules and comprises a small proportion of SOC at any one time (Neff & Asner, 2001).

Particulate Organic matter: is the partially decayed plant and animal tissue that is in a recognizable form it includes the organic matter at soil surface, the organic matter > 0.053mm in diameter and the light fraction that floats when the soil is wetted.

Humus: comprises about 50-60% of the total soil organic matter, is produced by the decomposition of plant and animal matter and is not in a recognizable shape and is dark colored.

Inert organic matter: also called the recalcitrant material that comes from burning and includes charcoal or charred material.

Furthermore SOM can be divided into light fraction (LF) and heavy fraction (HF) pools based on their chemical composition and their association with the different soil classes. The *'light fraction'* SOM, which is associated with coarse soil fraction is generally identifiable, unprotected young plant material with high C concentration, most of which is lost during initial degradation process (Ellert & Gregorich, 1996; Gleixner, Poirier, Bol, & Balesdent, 2002). The '*heavy fraction'* is stable and has lower C concentrations (Golchin et al.,1995; Golchin et al., 1995). It contains mostly the microbially-derived N compounds and stabilized aromatic compounds (Guggenberger et al., 1994) that are '*physically protected'* against microbial decomposition by association with clay surfaces and through encapsulation inside an aggregate. Therefore, well aggregated fine textured soils can typically accumulate more C than sandy soils. Hence based on the above discussion, the decomposability of LF and HF of SOM decreases in the soil classes in the following order:

sand > silt > clay

Increased soil N availability might influence the decomposition of SOM pools in different ways:

I. higher microbial activity in LF organic matter could lead to C losses, especially in coarse soils that provide little physical protection (Grandy & Neff, 2008);

II. accumulation of HF due to selective suppression of the microbial enzymes required to decompose complex molecules (e.g. lignin).

III. changes in the chemistry of the litter or the crop residue due to differential decomposition of polysaccharides and polyphenols in the LF and the HF pools; and

IV. N is known to bind with carbohydrates in soils and make '*melanoidins'* (Fog, 1988; Soderstrom, Baath, & Lundgren, 1983) which can increase the polymerization of polyphenols into *'brown'* compounds (Haider & Martin, 1967), both of which are resistant to microbial degradation, thus providing '*biochemical protection'* to SOC (Kogel-Knabner, 2002).

It is important to understand how the availability of inorganic N can change the dynamics of the decomposers and their activity in different SOM pools as the amount of C accumulated in one pool can be offset by the accelerated microbial degradation in another pool. Following is a discussion about how inorganic N might influence microbial activity and other associated mechanisms regarding SOC sequestration.

Mechanisms of Response:

Enzymes: Because of the complexity of SOC dynamics, in particular the interactions between soil communities, their enzyme systems, and different C compounds, the effect of N addition on decomposition remains uncertain. Microbial enzyme responses to N addition have been studied by many researchers to understand whether enzymes may help explain the variation in soil C responses to N. The long term addition of N can have neutral, positive or negative effects on decomposition rates, and the variability in enzyme responses to N may be one explanation for this variability as not all types of enzymes respond in similar way to the availability of N (Fog, 1988).

Enzymes are N-rich compounds and their production is regulated by the availability of inorganic N. Thus the variation in their response to N addition can be considered a good predictor of N effect. There are two classes of microbial enzymes, hydrolytic and oxidative enzymes, which degrade cellulosic and lignified compounds respectively. The hydrolytic enzymes include endoglucanases, exoglucanases and β-glucosidases (Table:1). These are produced by a large group of bacteria and certain fungal species and are associated with the decomposition of cellulose, chitin, and storage carbohydrates, whereas phenol oxidase and peroxidase are the oxidative enzymes responsible for the degradation of aromatic secondary phenolic compounds including lignin (Gallo et al., 2004). Oxidases are produced by fungi (Table:1).

Phenol oxidase uses oxygen to degrade phenolics whereas peroxidase uses H_2O_2 as an electron acceptor. Phenol oxidase is the only lignolytic enzyme produced by the white rot fungi in the basdiomycota and xylariaccous Ascomycota (Dix & Webster, 1995) (Table:1). Microbial lignin degradation results in the production of polyphenols. These are central reactive molecules in the formation of organic matter and can also be formed through microbial synthesis from a non-lignin carbon source such as cellulose.

Enzyme	Enzyme Function	Substrate
β-1,4-glucosidase	Catalyzes the hydrolysis of terminal 1,4 linked β-D-glucose residues from β-D-glucosides, including short chain cellulose oligomers.	4-MUB-β-D-glucoside
α-1,4-glucosidase	Principally a starch degrading enzyme that catalyzes the hydrolysis of terminal, non-reducing 1,4-linked α -D-glucose residues, releasing α-D-glucose	4-MUB-α-D-glucoside
β-D-1,4-cellobiosidase	Catalyzes the hydrolysis of 1,4-β-D-glucosidic linkages in cellulose and cellotetraose, releasing cellobiose.	4-MUB-β-D-cellobioside
β-1,4-N-acetyl-glucosaminidase	Catalyzes the hydrolysis of terminal 1,4 linked N-acetyl-beta-D-glucosaminide residues in chitooligosaccharides (chitin derived oligomers).	4-MUB-N-acetyl-β-D-glucosaminide
Leucine amino peptidase	Catalyzes the hydrolysis of leucine and other amino acid residues from the N-terminus of peptides. Amino acid amides and methyl esters are also readily hydrolyzed by this enzyme.	L-Leucine-7-amino-4-methylcoumarin
Phenol oxidase	Also known as polyphenol oxidase or laccase. Oxidizes benzenediols to semiquinones with O_2.	L-DOPA
Peroxidase	Catalyzes oxidation reactions via the reduction of H_2O_2. It is considered to be used by soil microorganisms as a lignolytic enzyme because it can degrade molecules without a precisely repeated structure	L-DOPA

Table 1: List of microbial extracellular enzymes, their functions and classification.(Adapted from ((A. S. Grandy, Neff, & Weintrau, 2007)).

The extracellular enzyme activity for the degradation of lignin and cellulose has been correlated with rates of litter and organic matter decomposition. Given the close association between microbial enzyme activity and SOC dynamics, enzymes can provide us with insight into the functional response of the microbial community to anthropogenic N addition. There are

many hypotheses regarding the specific effects of N addition on enzyme activity and decomposition rate. These hypotheses are not mutually exclusive and in any given system there may be several in play at the same time, which could help explain the complex responses of SOC to N:

I. N increases the randomization of the chemical bond structure of organic material, thereby reducing decomposition rates and stabilizing soil C (Fog, 1988).

II. Hhigh N stimulates the activity of hydrolytic enzymes which are responsible for the degradation of more labile C (Carreiro et al., 2000; Sinsabaugh et al ., 2002)

III. Suppression of the microbial oxidase enzymes due to increased availability of the inorganic N may result in reduced lignin decomposition (Fog, 1988);

IV. N addition alters the composition of the decomposer community in a way that slows down its overall degradation ability (Bardgett et al., 1999; Frey et al., 2004) and

V. N addition changes the chemical composition of litter and organic matter over time. Following is a discussion that encompasses these hypotheses:

Hydrolytic Enzymes: Cellulose is a major component of plant material and its degradation is mediated by a group of functionally similar hydrolytic enzymes collectively called *'cellulase'*. The activity of this enzyme system is known to be limited by the availability of N in nature. Therefore N is a critical element and its higher concentrations, either in litter or in the soil solution may accelerate the decay process in the early stages, when the litter has higher cellulose content (Mellilo et al., 1982; (Fog, 1988). This hypothesis has been well studied in non-

agricultural systems (Carreiro et al., 2000; Geisseler & Horwath, 2009; R. L. Sinsabaugh et al., 2002). Carreiro et al., (2000) reported an increase in the hydrolytic enzyme activity in the more labile cellulosic litter such as, flowering dogwood, red maple and red oak. Interestingly, the positive response of this enzyme often shown in response to N may decline during the course of decomposition. Although the specific mechanisms are not well known, possible explanations include:

I. High N suppresses lignin degradation which results in the accumulation of phenolics, which have an inhibitory effect on hydrolase enzymes (DeForest et al., 2004; DeForest et al., 2005; Dijkstra et al., 2004; Fog, 1988; Freeman et al., 2001)

II. Since much cellulose is bound in lingo-cellulose complexes, and the degradation of lignin is inhibited by the presence of N, carbohydrates may be protected from microbial decay (DeForest et al., 2004).

Oxidative Enzymes: The effects of N on the activity of lignin degrading enzymes have been examined primarily in forest ecosystems. Both phenol oxidase and peroxidase have been found to exhibit a reduced activity under high N availability, and phenox is shown to respond more strongly to N addition in comparison to peroxidase (DeForest et al., 2004; Gallo et al., 2004). For example, Carreiro et al. (2000) found reduced activity of phenol oxidase in litter with a high lignin content (oak leaves) and argued that although lignin degrading enzymes are produced by some bacteria, the 'white rot fungi' from the Basidiomycota and xylariaccous Ascomycota (Dix & Webster, 1995) are the only fungi known to produce phenol oxidase. Therefore, fungi are considered the principal degrader of lignin. Hammel (1997) suggested that the decreased

activity of phenox oxidase in lignified oak litter in their study could be due to either suppression of the synthesis of the enzyme by white rot fungi or to reduction in their overall abundance. This assumption regarding the abundance of the lignin degrading fungi has been well explored and many studies have reported that the reduced lignase activity was not correlated to reduction in the abundance of this fungi in response to N (DeForest et al., 2004; Galloway & Cowling, 2002).

Waldrop et al (2004) correlated phenol oxidase activity with decomposition rate and observed a high decomposition rate at sites where there was high phenol oxidase activity and low decomposition rates at sites with low phenol oxidase activity, suggesting that the response of this enzyme is crucial in determining soil C dynamics. Similarly, Carreiro et al (2000) also supports this finding and suggests that the response of this enzyme is a good indicator of changes in decomposition rates due to N addition. In contrast to the above explanation of reduced decomposition rate, another view is that microbes synthesize phenolic compounds during degradation that react with inorganic N and form *'browning precursors'* that are toxic and/or inhibitory to microbes and suppresses their activity (Couteaux et al., 1995). Regardless of what the mechanism of reduction in the oxidase enzyme activity might be, the suppressed activity of this enzyme can help accrue SOC in the long run. In relation to N effect on mechanisms, the more convincing evidence of N addition effect comes from the studies on the decomposition rates of litter and the related changes in its chemistry, partly due to differential activity of the hydrolytic and oxidative enzymes:

Litter Quality: Litter decomposition involves the mineralization and humification of plant material (hemicelluloses, cellulose, lignin) by microbes (Couteaux et al., 1995). The quality of litter, availability of nutrients and structure of the decomposer community are primary controls on litter decomposition (Berg & Matzner, 1997; Couteaux et al., 1995; Knorr et al., 2005). For example, Neely et al. (1991) measured decomposition in agricultural systems using a litter bag experiment. A wide range of litter types were used including crimson clover, hairy vetch, crabgrass, grain sorghum and wheat. They reported significant differences in the decomposition rates between the species and observed that the initial N concentration of the litter was the best predictor of decomposition rate and found a linear relation of initial N and decay rates for all the species. These results suggest that N is an extremely important driver of the differences in decomposition rates between species, and also suggest that the application of external N may be a key driver of decomposition.

However, there is considerable evidence that the quality of litter strongly influences its response to N enrichment. This is reflected in two phenomena that have frequently been reported. First, the chemistry of litter, in particular its lignin and polysaccharide contents, is often correlated with N response. Second, several studies suggest that N addition can have different effects on litter decomposition rate in its early and later stages, which is likely due in part to changes in chemistry during decomposition.

Initial stage of Decomposition: Fresh litter often contains insufficient nutrients to meet microbial needs (Aber et al., 1991), which has been shown by immobilization of N in the initial stages of litter decomposition (Berg & Matzner, 1997; Hobbie, 2000). Nutrient limitation in the

early stages of decomposition can be overcome by the addition of N. Therefore, decomposition rates often increase with the application of nutrients (Hobbie & Vitousek, 2000). In particular, studies often report a rapid disappearance of cellulose due to N applications increasing cellulose degrading enzyme activity in the initial stages of decomposition (Berg, 2000; Hobbie & Vitousek, 2000; McClaugherty et al., 1985). Similarly, Berg and Ekbohm (1991) reported that the most nutrient rich litter types exhibited rapid decomposition in the beginning of decomposition. They also observed that the initial lignin content in the litter had a negative relation with mass loss. We find similar results in other studies as well (Berg, 2000; Berg & Meentemeyer, 2002; Neely et al., 1991), indicating that the addition of N to litter with high cellulose and relatively low lignin contents can increase decomposition rates, particularly in the early stages of decomposition.

Later stage of Decomposition: A negative effect of N availability on mass loss in litter with high lignin concentration has been frequently reported. The suggested rationale behind the rate decline is that low weight molecular N compounds repress the production of lignolytic enzymes in certain fungal species (Berg & Meentemeyer, 2002). Another suggested mechanism is that as decomposition proceeds and the products of lignin degradation accumulate, they react with the available N to form compounds which are resistant to further degradation (Fog, 1988). Thus, it is expected that the suppressive effects of N will be strongest in the later stages of decomposition when lignin concentrations in litter are at their highest.

Microbial Community Structure: With the introduction of molecular based techniques in assessing microbial functional and taxonomic diversity in recent years, we have a better

understanding of the response of the microbial community to N enrichment. Many studies have reported changes in the function of the microbial community based on the changes in enzyme activity and respiration rates in response to N addition. Therefore, one might also expect a change in the microbial community structure as well, resulting from either a direct or indirect effect of N on microbial communities. A decrease and/or inhibition of enzymes, for example, can hamper a community's ability to derive energy and could change the patterns of microbial community substrate use and ultimately microbial biomass, fungal:bacterial ratio or the relative abundance of specific decomposers. Other indirect effects on communities may be mediated by changes in litter inputs or microbial biomass.

Studies suggest that N increases above ground plant biomass and litter inputs into the soil (Liu & Greaver, 2010; Sylvia, Fuhrmann, & Hartel, 1999). Based on this, we can hypothesize that with high aboveground inputs there would be high nutrient availability which in turn will affect the belowground microbial population, but this may not necessarily be true for the MBC. For example, Deforest et al. (2004) conducted a study in Northern Hardwood stands in Michigan and reported a significant decline in overall microbial biomass carbon (MBC) with no effect on fungal: bacterial ratio. Similarly, Treseder (2008) conducted a meta analysis including 82 field studies and reported a 15% decline in the overall MBC in response to added N. These results have been supported by other studies as well (Compton, Watrud, Porteous, & DeGrood, 2004; Frey et al., 2004; Liu & Greaver, 2010). For example, Kaur et al., (2008) reported a significant decline in MBC with N application under wheat-corn rotation and argued that this negative effect might be due to the effect of N on soil pH. Henriksen and Breland (1999) also observed a decline in fungal biomass with no effect on bacterial biomass in a wheat straw

decomposition experiment under increased availability of N. Frey et al., (2004) also reported a significant decline in fungal biomass after N addition and observed a significant decrease in phenol oxidase activity. Similar results have been reported by other studies as well (Allison et al., 2007; Frey et al., 2004; Nemergut et al., 2008; Treseder, 2008) .

Microbial Respiration: Bowden et al., (2004) conducted a study in red pine and mixed deciduous stands and observed increased soil respiration with N addition in the first year but reductions in respiration in subsequent years. They obtained similar results in lab incubations of root-free soil as well. They attributed the increase in CO_2 flux in the first year to the increased aboveground biomass in the form of litter fall, and suggested that a decrease in respiration in the later years could be due to reduction in decomposer efficiency due to either inhibition of enzyme production or reduction in the MBC. It is possible that in the field N addition initially accelerates decomposition of fresh litter due to increased enzyme activity. This, plus any initial increases in litter inputs could increase respiration rates in the short term. Although there may be short-term increases in soil respiration, most studies show long-term decreases in respiration. These declines may be due to declines in fungal abundance and biomass and the inhibition of lignin-degrading enzymes. This has been supported by Frey et al (2004), who found N reduced fungal biomass and phenol oxidase activity. In addition Al-Kaisi et al., (2008) observed inconsistent respiration rates under corn-soybean rotation and attributed it to changes in moisture and temperature, but they found a significant decrease in the lab incubations of the soil under high N. Kowalenko et al., (1978) also observed reduced CO_2 in field measurements and lab incubated soils. The decrease in soil respiration has been reported in

several other studies (Foereid et al., 2004; Wilson & Al-Kaisi, 2008) but the mechanism for the decrease in the CO_2 emissions under high N are not clear but have been correlated to reductions in MBC and enzyme activity. Soil pH has also been suggested for a decrease in MBC and CO_2 emissions (Aerts & de Caluwe, 1999; Bowden et al., 2004). Increased nitrification of ammonium in soil results in acidification, thus reducing MBC and CO_2 emissions.

Dissolved Organic Carbon: Another way that N enrichment influences SOC dynamics is through changes in dissolved organic carbon (DOC). It is a small fraction of the total soil carbon and its composition ranges from simple amino acids to complex high molecular weight molecules (Neff & Asner, 2001) containing readily available C sources for microorganisms (Sylvia et al., 1999). Studies have shown an increase in DOC with N enrichment (DeForest et al., 2004; Liu & Greaver, 2010; Pregitzer et al., 2004; Sinsabaugh et al., 2004)

It is important to understand the mechanisms for the increase in DOC because it might result in the loss of SOC through leaching from the soil profile. The increase in DOC can be explained by increases in plant biomass inputs to soil. Lebauer and Treseder (2008) found that N additions increased aboveground litter production, which increased soil C availability and resulted in higher C losses by leaching. Similarly the negative effect of N addition on lignase enzyme activity, fungal biomass and MBC has been well established and that might have a direct effect on DOC consumption and availability. Sinsabaugh et al. (2004) observed in their study that increases in DOC were parallel to the decrease in oxidase enzyme activity suggesting that there is a lower consumption of DOC due to lower microbial activity in response to high N.

In addition Pregitzer et al., (2004) also observed increases in DOC leaching from N amended plots with a simultaneous decline in respiration.

Crop Residues in Agricultural System: Along with the quantity, the quality of the crop residue is also crucial in C sequestration. Although all plants contain similar classes of compounds they differ in their proportions and thus decomposability varies with different species (Hadas, Kautsky, Goek, & Kara, 2004). The C and Nitrogen concentrations are considered important indicators of crop residue quality (Kononova, 1961) as these give us insight into the rate and extent of decomposition by microbes and hence the potential to sequester C. C/N ratios of the crop residues depend on the crop species. For example corn and wheat residues have high phenols and C/N ratios, which will result in low decomposition rate and increased SOC (Russell, Laird, Parkin, & Mallarino, 2005) in comparison to soybean (Glycine *max* L.) which has lower phenols and C/N ratio and decompose readily and hence contribute less to the SOC content. In a meta-analysis Knorr et al., (2005) conclude that low quality litter with high lignin content decomposed faster than the high quality litter with increased availability on N. However C/N ratio of the crop residue is not the only factor regarding the quality of the residue that controls decomposition rate as some researchers have reported that high concentrations of N in the SOM were not responsible for the decomposition rate (Hobbie & Vitousek, 2000).

Conclusion

Due to inconsistency in the results in the response of microbes and the associated mechanisms to N addition, it is hard to draw a general conclusion, but the above discussion can be summarized as follows:

I. Increased availability of inorganic N due to N deposition or agricultural management practices in soils may accelerate SOC losses and diminish soil quality.

II. N addition accelerates the decomposition of labile litter but it can slow the decomposition of more recalcitrant litter. Therefore, N addition in the long run might result in the accumulation of SOC in ecosystems characterized by high-lignin litter.

III. The role of lignin degrading enzymes can be critical due to their strong response to N addition. The C quality and the N content in the substrate (litter or crop residue) degraded by microbes plays primary controls over decomposition rate. If lignin content is high, we can expect an increase in SOC.

IV. The inhibition of microbial enzymes and reduction in MBC and soil respiration could result in SOC loss in the form of increased DOC leaching.

V. Land use for agricultural purposes has substantially reduced topsoil C. The SOC sequestration in agricultural systems can improve by proper N fertilization and crop

residue management in combination with reduced tillage and improved cropping system management.

CHAPTER 2

NITROGEN FERTILIZATION EFFECTS ON LITTER DECOMPOSITION DYNAMICS AND SOIL CARBON UNDER NO-TILL, CONTINUOUS CORN CROPPING SYSTEM.

INTRODUCTION

Human activities, including N fertilization, the cultivation of N fixing crops, and fossil fuel combustion, have more than doubled the global flux of biologically reactive N (Vitousek et al.,1997; Galloway et al., 2004). This increase in available N is linked to accelerated nitrous oxide flux (Bouwman et al., 2002a, 2002b; Mosier et al., 1998), ecosystem acidification (Bolan, Hedley, & White, 1991), reduced water quality (Hallberg, 1987), and changes in plant species distribution and abundance (Chung et al., 2007; Dybzinski et al., 2008; Vitousek et al., 1997). However, the effects of reactive N on decomposition dynamics have proven variable and remain difficult to predict, owing in large part to uncertainties about how soil biological communities respond to inorganic N. Our knowledge of the relationships between inorganic N, soil communities, and decomposition dynamics is particularly limited in agricultural systems. Although the majority of recent efforts to understand N effects on decomposition processes have been carried out in non-agricultural soils, N inputs to agricultural soils increased from 12 to 104 Tg/yr (FAO, 2009) globally over the last 40 years, and will continue increasing in the decades ahead as the global population approaches 10 billion people. Further, the rate of N application to most high-yielding, intensively managed cropping systems is high. In the U.S. Corn Belt, for example, the average rate of N application to corn is ~135 kg N/ha ,or more than

an order of magnitude greater than the rates of atmospheric N enrichment in most U.S. ecosystems (Russell, Cambardella, Laird, Jaynes, & Meek, 2009)

Soils contain approximately 1580 PG of C in the form of organic matter and surface litter (Schlesinger, 1997), which is more than the combined amount in vegetation and in the atmosphere. Agricultural land use, however, has reduced top soil C by ~35-55% and the restoration of this lost C is one of only a few short term, high impact options proposed to stabilize atmospheric CO_2 concentrations. (Christopher & Lal, 2007) Soil C sequestration is also attractive to many land managers and policy makers because of its positive effects on soil fertility, water dynamics, and erosion control (Smith ,2004; Bell et al., 2009), and is thus considered a 'win-win' scenario with benefits to producers and society at large. While the best methods to sequester soil C are still being established, SOM concentrations are in part a function of soil C inputs. Given the relationship between soil C inputs and SOM concentrations (Campbell et al., 1991), agronomists often attempt to increase plant productivity through expanded fertilizer use, the development of high yielding varieties and other practices such as reduced or no-tillage (VandenBygaart et al., 2003) and crop rotation (Wilson & Al-Kaisi, 2008). However, recent long-term field experiments and new insights into the priming effect call into question the links between fertilizer use, crop yields and SOM concentrations. Is it possible that N use in agricultural systems is increasing C inputs but decreasing soil organic matter decomposition via accelerated decomposition rates?

Recent work by Khan et al., (2007) suggested just this dynamic has been at play in the Morrow plots in Illinois. They examined long-term soil C data and found that the initiation of

inorganic N fertilizer use corresponded with a long-term decline in SOM concentrations, despite increases in plant productivity. However, these observed changes may have also been due to changes in climate and/or the diversity of residue inputs that coincided with the onset of inorganic N fertilizer applications. Russell et al., (2009) found that under four levels of N fertilization 0,90, 180, 270Kg/ha there was a significant increase in aboveground net primary production and thus total organic C inputs with N addition but they also observed higher decomposition rate with high N such that the high decay rates offset higher inputs and net C sequestration was nil. Other studies have shown that synthetic N application in agricultural systems can increase soil CO_2 emissions and soil C loss (Al-Kaisi et al., 2008), but results are mixed and reports show accumulation (Reay et al., 2008; Al-Kaisi et al., 2008; Poirier et al., 2009), loss (Hofmann et al., 2009; Khan et al., 2007; Mulvaney et al., 2009) or no change in soil C (Halvorson et al., 2002). Further, disentangling the effects of N fertilizer from other forms of agricultural disturbance that influence biogeochemical processes, including tillage and reduced plant community diversity, can be difficult under most experimental designs.

Our understanding is also limited by the scant number of studies in agricultural systems that have focused on how soil biological processes that control decomposition dynamics and soil C – rather than soil C, per se - respond to N. Measuring changes in soil C stocks is notoriously difficult because soil C concentrations are high and spatially variable (Conant et al., 2003; Robertson et al., 1997). Because of this, a decade or more may be needed to detect significant changes in SOM pools. Further, some long-term experiments investigating soil C responses to inorganic N are not replicated, or changes in N fertilizer use may be confounded by other changes in management. An alternative, complimentary approach is to

focus on the short term biological processes that control decomposition and their responses to N. Such an approach avoids the need to detect changes in total soil C stocks while providing fundamental insights into the processes controlling C cycling.

In forest and grassland ecosystems, studies have shown changes in soil communities, enzymatic activities, and decomposition rates following simulated N enrichment. For example, many studies have shown that N inhibits oxidase enzymes as well as the decomposition of lignin, while accelerating the activity of hydorlases and the degradation of labile C pools changing microbial communities (Carreiro et al., 2000; Sinsabaugh et al., 2002; Frey et al., 2004; Hofmann et al., 2009). Recent studies indicate that these changes in biological processes may be accompanied by shifts in the chemistry of soil organic matter (Neff et al., 2003; Gallo et al., 2005). For example, Grandy et al. (2008) found that N additions to forest ecosystems increased the ratio of lignin:nitrogen in coarse soil fractions >63 µm, and also increased the ratio of lignin:polysaccharides in soil fractions >250 µm. They also found that N increased the abundance of specific polysaccharides, but all of these effects depended on ecosystem type. The mechanisms underlying changes in litter chemistry following N fertilization are not well known, but these changes in chemistry appear to correspond with shifts in soil enzyme activity. Other studies have also demonstrated strong relationships between changes in soil communities, enzyme activities, and the chemistry of litter after decomposition (Grandy et al., 2009; Wickings et al., 2010), but none of these studies have explored such relationships in agricultural systems. Together, these studies suggest that N-induced changes in decomposer communities may influence organic matter chemistry via changes in soil decomposer communities.

In recent years, major advances have been made in understanding the environmental consequences of intensive N fertilizer use in agricultural systems, mainly N leaching and denitrification (Aber et al., 1998) but few studies have used high-resolution biological and biochemical methods to resolve decomposition responses to N in agricultural systems. While it is well established that agricultural conversion accelerates soil C cycling, (Lal, 2002) few studies have decoupled the direct effects of N fertilization from associated changes in plant chemical composition and community diversity, tillage intensity, and edaphic soil properties (e.g. pH). To better understand how inorganic N fertilizer influences decomposition in agricultural systems, I examined litter decomposition rates, decomposer communities and processes (soil enzymes, and microbial communities), and soil C pools, enzyme activities and chemistry along an agricultural N fertilizer gradient. The objective of this study is to investigate the effects of N fertilizer application on microbial activity, decomposition dynamics, and soil C storage. Given previous studies suggesting that decomposition responses to N are regulated by litter quality, I hypothesized that N fertilizer would accelerate biological processes and decomposition rates in corn litter but suppress decomposition rates in wheat litter, which has higher lignin concentrations and lower polysaccharide concentrations (Ghidey & Alberts, 1993). These differences in litter decomposition rates will be associated with changes in litter enzyme activities. I also anticipated changes in soil enzyme activities and labile, readily degradable SOM pools hence effecting soil C pools.

MATERIAL AND METHODS

Site Description: This study was carried out at the N Fertility Gradient Study at the W.K. Kellogg Biological Station Long-Term Ecological Research (LTER) site. This site was established in the year 2005. This site has been under corn-soybean-wheat rotation and prior to its establishment these plots were under alfalfa. The mean annual precipitation at the KBS LTER site is ~ 890 mm/yr and soils are classified as Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs (Alfisols) developed on glacial outwash (Crum and Collins 1995).

Experimental design: This study consists of a continuous corn, no-till cropping system fertilized at 9 different N levels ranging from 0 to 291 kg N/ ha. Experimental plots are 5 x 30 m, replicated four times, and arranged in a randomized complete block design. Along the N fertility gradient, we examined three treatments, 0, 134, and 291 kg N/ha, representing zero, optimum, and very high N, respectively. Nitrogen was applied during May and June of 2008. May fertilizer N was applied at 34 kg N/ha as a starter fertilizer to all plots except for control and the June fertilizer was applied at zero, 100 and 257 kg N/ha as 28 % urea-ammonium-N using subsurface, side-dress injection when corn was at a height of 10-25 cm. Our rationale for using these rates of N fertilizer was to have experimental treatments with very different levels of soil N availability, representing: 1) N limitation, in which crop N demand would exceed soil N availability; 2) relative synchrony between total crop N demand and N availability; and 3) N saturation, in which N availability exceeds crop demand through the season.

Litterbags and soil sampling: Standing dead corn plants and wheat straw were collected in fall 2007 from sites managed according to MSU best management practices. These plants were selected because they represent litter types with different initial chemistries, but also because they represent the majority of C entering field cropping systems in the upper Midwest. Studies have shown that compared to corn and wheat very little aboveground residue from soybean is incorporated into stable soil C pools. Indeed, the vast majority of aboveground soybean residue is decomposed within months (Wickings et al. 2010) at KBS. Air dried corn or wheat litter was cut into 2-4 cm pieces, homogenized, and placed into 7 x 7 cm nylon mesh litter bags. Litterbags, measuring 18 x 18 cm and with a mesh size of 1.4 mm were filled with 7 g of either corn leaves and stems or wheat straw and secured to the soil surface in June 17, 2008. Each plot received six litter bags for each type of litter, representing the six time steps.

The placement of the litterbags on the soil surface mimics the deposition of aboveground residues in no-till systems, which typically accumulate surface litter. Therefore, this litter does not receive a direct fertilizer application because the fertilizer is injected into the soil, which is also typical in no-till systems. Although our design may not maximize the potential to see a N fertilizer effect on litter decomposition dynamics, there have been many studies designed to broadly test the potential for N to influence decomposition dynamics, often under unrealistic scenarios. In our case, even the highest rate of N fertilization was carried out under otherwise realistic no-till management conditions. Given the possibility that soil-injected N may not influence aboveground litter decomposition, we also made intensive measurements of soil responses to different fertilizer rates.

At sampling, 10 soil cores were taken randomly per plot to a depth of 10 cm from all the 12 plots along with 24 litter bags (12 bags for both types of litter). Soil and litter bags were collected at approximately monthly intervals (July 08, Aug 08, Sept 08, May 09, June 09) and transported to the lab on ice where they were sub-sampled for the physical and biological analyses.

Extracellular Enzyme Activity: Subsamples of 0.5 g litter and 1 g soil were homogenized with 40 ml of 50 mM sodium acetate buffer at a pH of 6.5 and 6.0. The slurries were then used to assess the activity of five extracellular enzymes involved in carbon and nutrient cycling following the methods reported in Saiya-Cork et al. (2002) and Grandy et al. (2007). The activity of three hydrolase enzymes, β-glucosidase(BG), N-acetyl-β-D-glucosidase(NAG),and β-D-cellobiohydrolase(CBH),were assessed using black, 96-well microplates and compound-specific substrates containing the synthetic fluorescing molecule methylumbelliferone. The substrates for these enzymes were 4-MUB-β-D-glucoside for BG, 4-MUB-Nacetyl-β-D-glucosaminide for NAG, and 4-MUB-B-D-cellobiosidase for CBH. The activity of one oxidative enzyme (phenol oxidase) was also measured using clear 96-well microplates and the substrate, L-dihydroxy-phenylalanine (L-DOPA) to assess the breakdown of lignin. All plates were incubated for a time period approximate for each enzyme (Table.2). The hydrolase enzyme activity was determined using a flourometer (355 µm excitation and 460 µm filter range) and phenol oxidase activity was measured using a spectrophotometer with a 450 µm filter (Thermo Fisher Flouroskan and Multiskan).

Enzyme	Type	Substrate
β-glucosidase	Hydrolase	4-MUB-β-D-glucoside
N-acetyl-β-D-glucosidase	Hydrolase	4-MUB-Nacetyl-β-D-glucosaminide
β-D-cellobiohydrolase	Hydrolase	4-MUB-B-D-cellobiosidase
Phenol oxidase	Oxidase	L-dihydroxy-phenylalanine

TABLE.2: Soil and litter extracellular enzymes and their substrates

Mass Loss: The decomposition rate of the litter was determined by the method as described in Robertson et al. (1999). Any roots attached to the litter bags were carefully removed and air dried. The mass of the air dried litter was recorded and a 0.5 g sub-sample of the ground litter was ashed at 450 °C for 4 hrs in a muffle furnace to determine the ash content of the sample. All masses were converted to a percentage of ash-free dry mass remaining.

Soil Respiration: A laboratory incubation experiment was set up to measure CO_2 flux as described previously (A. S. Grandy & Robertson, 2007). Approximately 20 g of air dried soil was weighed into 60 ml serum vials and 5 g water was added to adjust the moisture content to 60 % of the water holding capacity. The vials were tightly capped with rubber septa. Water was added as needed during the incubation period of 125 d to keep the moisture levels constant. On each sampling date all the vials were uncapped for approximately 30 min to allow for air exchange and then capped again. A volume of 0.5 ml was drawn with a syringe from the vials at zero, thirty and sixty min interval, immediately after recapping with the septa and analyzed with an infrared gas Analyzer, (LI.820) for the CO_2 content. The process was repeated over 46 d with a greater sampling intensity in the beginning of the 125 d incubation period. The CO_2 flux was calculated as described in Robertson et al. (1999) and reported as µg CO_2-C/g soil/d

Density Fractionation: Light fraction organic matter (LF) was separated from the soil by the density fractionation method as described in Robertson et al. (1999). A sub-sample of 20 g air-dried soil was taken into centrifuge tube and mixed with 200 ml of sodium polytungstate (NaPT) solution of 1.7 g/cm^3 density. To separate the LF from the HF the suspension was dispersed by shaking with a shaker for 30 min and then centrifuged (Sorvall RC-5B) for 20 min at 5000 rev/min. A vacuum source was used to collect the suspended LF, avoiding disturbance of the heavy fraction. The LF was washed with 400 ml of DI water to remove any residual NaPT, and weighed after drying at 105°C, and ground for further analysis.

Litter Molecular Chemistry: The molecular chemistry of litter was assessed by pyrolysis gas chromatography/mass spectroscopy following the procedures outlined in Grandy et al. (2007). Briefly, pulverized litter was pulse-pyrolyzed in quartz tubes on a Pyroprobe 5150 (CDS Analytical Inc., Oxford PA) using a pyrolysis temperature of 600°C. Pyrolized samples were transferred onto a gas chromatograph (Trace GC Ultra, Thermo Scientific) where they were further separated by passing through a heated, fused silica capillary column (60m x 0.25mm i.d.). GC oven temperature was increased from 40 to 270°C at a rate of 5°C per minute with a final temperature ramp to 310°C (30°C per min). Finally, compounds were transferred to a mass spectrometer (Polaris Q, Thermo Scientific) via a 270°C heated transfer line where they were ionized by a heated source (200°C) and detected. The identification of compounds involved the analysis of total ion chromatogram on which the abundance of all ions is scaled

relative to the largest peak. Peaks were identified using Automated Mass Spectral Deconvolution and Identification (AMDIS V 2.65) as well as the National Institute of Standards and Technology library (NIST). While this method does not provide quantitative assessment of compounds it does allow for the comparison of the relative molecular composition of substrates.

Chemically labile SOM (CLOM): The chemically labile SOM was measured as described by Weil et al. (2003). A 0.2M $KMnO_4$ stalk solution was prepared and its pH was adjusted to 7.2 by adding a drop of 0.1 *N* NaOH. Four standard concentrations (0.005, 0.01, 0.015, and 0.02*M*) were also prepared from the 0.2*M* $KMnO_4$ stock solution and then diluted to working concentrations (0.00005, 0.0001, 0.00015, 0.0002*M*). A 5.0g air-dried soil sample, 18ml of deionized water and 2.0ml of 0.2*M* $KMnO_4$ were put in centrifuge tubes. A soil standard of 5.0g of known pulverized soil and 'solution standard' (i.e no soil) were also prepared and processed in similar manner. These serve as a quality control reference. All the centrifuge tubes were shaken at 240 rpm for 15 min at room temperature. The samples were then placed in a dark area and allowed to settle for ten minutes. Meanwhile, 49.5 ml of deionized water was added in another centrifuge tube along with 0.5ml of the supernatant. This solution was inverted to mix properly and was used in a 96-well plate reading spectrophotometer to read the amount of C oxidized as a function of the quantity of permanganate reduced. The CLOM values were reported as mg of oxidizable carbon per kg of soil, following equation was used to determine CLOM:

CLOM (mg kg^{-1}) =

[0.02 mol/ L - (a + b × Abs)] × (9000 mg C/ mol) × (0.02 L solution/ Z)

Where: 0.02 mol/L = initial solution concentration

a = intercept of the standard curve

b = slope of the standard curve

Z = absorbance of unknown

9000 = milligrams of carbon oxidized by 1 mole of MnO_4 changing from $Mn^{7+} \rightarrow Mn^{2+}$

0.02 L = volume of stock solution reacted

Z = weight of air-dried soil sample in k

Statistical analysis: The experimental design for this study was a randomized complete block design (RCBD). It had a single factor, N fertilizer, with three levels (0, 134 and 291Kg N/ha), replicated four times. The data analysis for soil and litter variables was conducted in PROC MIXED (SAS Institute Inc, 2002). Normality of the residuals was checked using normal probability and box plots. Data were transformed where deviations from normality were observed. The homogeneity of variances was checked using Levene's test. For soil variables (enzymes, LF, Inorganic N, CLOM) the data was analyzed with the REPEATED statement in the PROC MIXED. Variance-covariance structure for the repeated measures was selected on AIC and BIC criteria. The results are reported statistically significant at α=0.05.

RESULTS

Litter Enzymes: There was a higher enzyme activity on litter in comparison to soil (Figures.9, 10, 11 & 12). Among the two types of litter; the activity was higher on corn than wheat. Except for CBH where there was a marginal significant effect of N (P=0.05) and a significant nitrogen by time interaction (P=0.02), none of the litter enzymes namely, BG, NAG, and POX, showed a response to the N treatment (Table.6) The CBH activity was significantly different between the two types of litter (P=0.02) being higher (28%) on corn in comparison to wheat litter. The activity of BG, NAG and POX were 19.2%, 3.4%, 15.2% higher, respectively, on corn than on wheat, but none of these differences were significant between the two types of litter.

Mass Loss: There was no N effect on litter decomposition rates (Figure:4) on either type of litter but there were significant differences between the corn and wheat litters with greater mass loss (73% mass loss) in corn than in the wheat litter (63% mass loss). There was a significant litter*time effect (Table.6).

Litter Chemistry: The corn and wheat litters were analyzed two times for their chemical nature (Table.3). The first analysis was in June 2008 before the litter was placed into the experimental plots and then at the end of the experiment, in Sept 2009. I found no significant differences between the 0N and the 291N litter treatments in all the chemical compounds in the corn as well as the wheat litter. The relative abundance of lignin, lipid, and N-bearing compounds when averaged across the N treatment and compared between the two types of litter were found 56, 350 and 10 % higher respectively in wheat than in corn litter where as phenols and polysaccharides were 25 and 63% higher respectively in corn than in wheat.

Light Fraction Organic matter (LF): The light fraction (mg/g soil) increased by 22% in June in the 291N plots in comparison to 0N, but decreased by 14% in Aug but both the differences being not significant (Table.4). The C concentration (mg/g of LF) of the LF organic matter increased by 8% in June and 1.6% in Aug in the high N treatment in comparison to 0N, similarly the N concentration (mg/g of LF) increased by 16% in June and by 8% in Aug under 291KgN/ha treatment in comparison to 0N (Table:4). Also, there were no significant differences in the LF-C and LF-N (mg/g soil) in soil. The only significant increase (P = 0.04) in LF (mg/g soil) was in June by 3.9% in 134N in comparison to 0N. Also the LF decreased by 9.7% in 291N plot in Jun and 17% in Aug in comparison to 134N plots but the differences being not significant. The LF in soil (mg/g soil) increased by 178% (P = <.0001) from Aug to Jun in 0N plots, 112% (P= 0.0003) in 134N plots and 94% (P = 0.0012) in 291N plots.

Soil Enzymes: The activity of the soil enzymes decreased in high N plots in comparison to 0N plots, as for BG (Figure.8), CBH (Figure.6), and NAG (Figure.5), there was 14.5, 21.8, and 17.8 % decrease in the activity respectively, but none of these differences being significant at (α =0.05) between 0 and high N. However POX activity (Figure.) in soil increased (8.8%) under high N but the differences were not significant (Table.7). The overall activity for POX peaked in the early time steps and then declined with time showing no significant differences except for the month of Oct when there was a higher (66% increase) activity (P=0.01) under the high N treatment.

Soil Respiration: The soil CO_2-C emission (Figure: 2) during the laboratory incubations showed a significant decrease (P=0.0001) in the CO_2 flux in the soils treated with high N and this trend

was persistent in all the time steps. The CO_2 flux decreased significantly by 41.2% in the high N plots in comparison to the 0N plots in June, 54.5% in July 53.8% Aug, and 37.2% in the month of September. Also there was a significant time effect (Table.7).

Chemically Labile Organic matter (CLOM): There were no significant differences in the CLOM. I did not observe any N effect between the 0 and the high N treatments also there was no time effect (Table: 7).

Inorganic N: The inorganic NH_4 and NO_2 + NO_3 (Table:5) showed a significant nitrogen and time interaction with 378.8 and 296.7% increase in the soil concentrations averaged across all time steps, under high N. All of the variables showed significant time effect.

DISCUSSION

To understand the main concept behind this experiment, I propose a conceptual model for my study (Figure:1). This model highlights the importance of litter, litter tissue chemistry and soil microbial activity in decomposition dynamics and soil C sequestration. This model summaries all the steps and events from the application of N fertilizer to the litter and/or soil, the possible changes in the soil microbial activity in the form of enzyme activity, the effects on the rates of decomposition of two chemically different litter types to the release of soil C as CO_2. This study also explores the possibility of the aforementioned changes in the above ground litter being translated into the below ground LF and eventually either its contribution in to the soil organic carbon pool, its direct effects of the added inorganic N on the soil microbial activity as well as the indirect effects on the microbial activity through changes in the above ground biomass (Figure:1).

Litter Dynamics: In general, it has been established that N fertilization increases decomposition of litter with relatively low lignin content and decreases or has no effect on mass loss of residues with high lignin content (Waldrop et al. 2004; Knorr et al. 2005). The different responses to N enrichment based on litter quality have been clearly demonstrated in the Manistee National Forest, MI. Here, Waldrop et al. (2004) showed accelerated litter decomposition in a sugar maple basswood forest with relatively low concentrations of lignin derivatives but in a black oak white oak forest higher litter lignin concentrations controlled decomposition dynamics. Multiple studies have sought to understand the biological basis for these changes (Saiya-Cork et al., 2002; Waldrop et al., 2004aa).

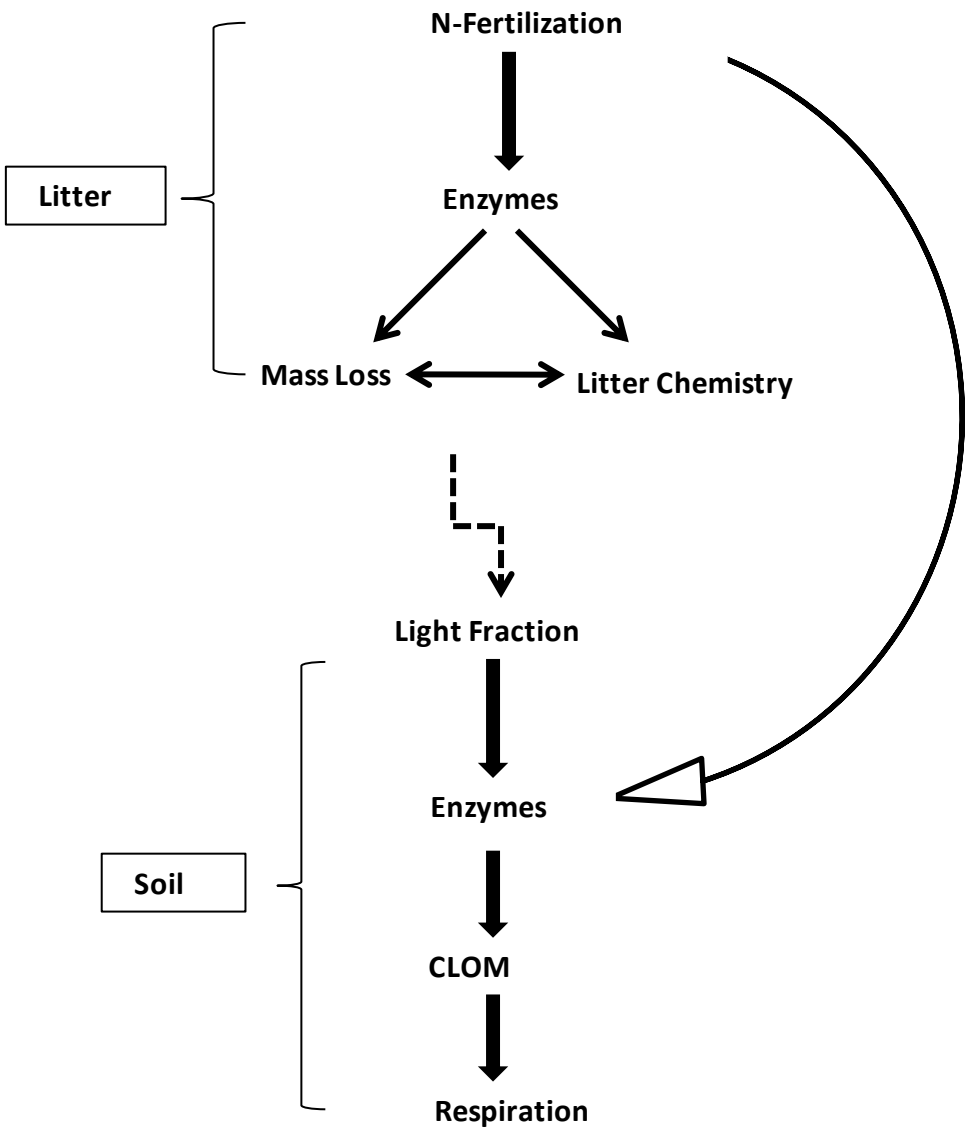

Figure: 1 A conceptual model showing direct and/or indirect effects of the inorganic fertilizer-N and the subsequent effect on litter and soil C cycling processes.
CLOM: chemically labile organic matter.

As long ago as 1927, Waksman and Tenney suggested that inorganic N accelerated the decomposition of crop residues. A handful of subsequent studies also suggested that supplemental inorganic N can stimulate the breakdown of residues with high C/N ratio (Recous et al., 1995). Waksman's studies along with those of Recous et al. (1995) show that under some conditions N fertilization increases litter decomposition rates, which will reduce soil C storage. Indeed, many no-till producers have adopted the idea that N accelerates decomposition and now use inorganic N applications to reduce their surface residues.

Evidence suggests that N alters microbial decomposition by differentially affecting degradation of polysaccharides and polyphenols, resulting in an uncoupling of these processes (DeForest et al., 2004; Sinsabaugh et al., 2003). High N levels stimulate hydrolase enzymes that break down carbohydrates, resulting in accelerated decomposition of organic materials rich in labile C compounds. In contrast, phenol oxidase and peroxidases are suppressed by high N availability, which reduces decomposition of litter or SOM with high concentrations of lignin and its derivatives and other secondary compounds (Sinsabaugh et al., 2005; Sinsabaugh et al., 2004).

The chemical composition of plant biomass impacts decomposition. For a net increase in SOC the C inputs must exceed the CO_2 flux. On a global scale climate is the best predictor of rate of decomposition where as on a climatic scale biomass chemistry is the best predictor. As mentioned above many studies have reported an inhibitive effect of lignin on decomposition rate. I used two types of litter with a different chemical composition.

Litter quality may act alone or in concert with other factors, including the form of N or N rate, to influence the transfer of plant-derived C into different SOM pools (Bradford et al. 2008). To check this assumption I measured the chemical composition of litter and the rate of mass loss and the enzyme activity on litter. Before decomposition, the relative abundance of lignin (Table: 3) was 20% in corn and 28% in wheat, which increased to 35% and 54% in corn and wheat, respectively, after decomposition, whereas the relative abundance of polysaccharides was 38% in corn and 22% in wheat which increased to 43% and 16% in corn and wheat respectively after decomposition. The relative abundance of lignin, lipid, and N-bearing compounds when compared between the two types of litter were 56, 350 and 10% higher in wheat than in corn litter, respectively, whereas phenols and polysaccharides were 25 and 63% higher in corn than in wheat respectively. There was >50% mass loss in both types of litters. (Figure.4) Thus, in this study the litter being examined had very different chemical characteristics; the wheat possessing significantly higher chemical recalcitrance than the corn. I anticipated that because of the differences in chemistry, N enrichment would suppress decomposition in wheat and have positive or no effect on decomposition in corn. However, I did not detect any effects of N on total mass loss (Figure.4) in either litter type, and litter enzymes either did not respond to N, or exhibited modest decreases in activity due to N fertilization (CBH and BG) at the June time point only (Figure.6 & 8). As anticipated that more recalcitrant litter i.e. with higher lignin content would show a decreased decomposition rate with higher N application rates, my data did not show response to the N addition with no significant correlations among litter enzymes and lignin and polysaccharides (Table.9)

Nonetheless I observed higher enzyme activity on corn than on wheat litter, and a greater mass loss in corn (73% mass loss) than in wheat (63% mass loss) litter.

Soil Processes: In this study, fertilized soils had higher LF and total C concentrations, although the differences not being significant but we see a trend (Table: 4). This is similar to the findings of Gregorich et al. (1996) who used natural abundance methods to demonstrate that the half-life of corn-derived C in a continuous corn cropping systems was the same irrespective of N fertilization rate. The authors concluded that fertilization increases crop residue production, without accelerating its decomposition rate, resulting in SOM increases. Many other studies have shown that N fertilization increases SOM content by increasing crop residue inputs (e.g Sainju et al. 2006; Christopher and Lal, 2007; Kaur et al. 2008; Banger et al. 2010). Although these studies did not measure litter decomposition, per se, they do indicate that increased residue inputs are not offset by potential increases in litter decomposition rates, resulting in increased SOM concentrations. I acknowledge that short-term, process-level studies may not always reflect long-term dynamics, and future efforts to understand N effects on decomposition and SOM dynamics need to encompass a range of temporal and spatial scales. Nonetheless, when the results of this study are interpreted along with others examinining long-term soil C responses to N fertilizer use (e.g. Gregorich et al., 1996; Banger et al., 2010), there is little evidence that N fertilizer induced changes in soil biological processes, per se, will accelerate litter decomposition in corn-based cropping systems.

The highest rate of N fertilization reduced CO_2 flux in an incubation assay, (Figure.2) by 46.9% (P = 0.0001) compared to the unfertilized treatment. Incubations are widely thought to

provide insight into the labile or 'active' soil C pool that is readily available to soil microbes and has a short turnover time. In my study I evaluated other indicators of labile SOM pools, notably light fraction carbon, and permanganate oxidizable carbon pool. In contrast to the laboratory-assessed respiration rates, I did not observe any decrease in light fraction or chemically oxidizable carbon with nitrogen fertilization. In contrast to CO_2 mineralization response, N fertilization was associated with increased LF-C concentrations and LF-N content (Table.4) compared to the unfertilized treatment, and there were no effects of fertilization on CLOM (Figure.3). Thus, the consistent declines in soil respiration that we measured may be due to changes in soil properties other than available C, including differences in decomposer communities or microbial biomass resulting from N fertilization.

In recent years, laboratory incubations such as the ones I conducted here have come under scrutiny for ignoring differences in the structure and function of decomposer communities. That is, differences in short-term laboratory CO_2 flux may be a function of the resident microbial communities, their metabolic capabilities, and substrate preferences, as well as organic matter quality and quantity. It is thus possible that microbial-level differences among N treatments explain the observed variation in potential respiration rates. Other studies have shown that high levels of inorganic N can suppress microbial activity (Al-Kaisi et al., 2008; Bowden et al., 2004; Clark et al., 2009; Fog, 1988; Wilson & Al-Kaisi, 2008). These changes in microbial activity may be related to declines in microbial biomass with N fertilization, which have been frequently reported. For example, in a meta analysis including 82 studies, Treseder et al. (2008) reported that microbial biomass declined by an average of 15%

following N fertilization, and declines in soil respiration rates were correlated with these changes in soil microbial biomass. In another synthesis, Liu and Greaver (2010) found that N addition reduced microbial biomass C by 20% and microbial respiration rates by 8%.

Changes in decomposer community composition could also help explain the observed effects of N fertilizer on soil respiration rates. Recent research suggests that decomposer community composition could regulate decomposition dynamics because of variation in substrate preferences among microbial communities (Balser and Firestone 2005; Osler and Sommerkorn 2007; Valaskova et al. 2007; Strickland et al. 2009). However, soil decomposer communities could have an additional – albeit indirect – effect on soil respiration rates by altering the chemistry of SOM. Soil organic matter biochemistry is related to its recalcitrance and decomposition rate and also to its ability to be protected from microbial attack by interactions with soil minerals (Grandy & Neff, 2008). The importance of organic matter chemistry in decomposition has been shown in empirical studies (Grandy, Strickland, et al., 2009) and is widely acknowledged in quantitative and conceptual models of the process (Grandy & Neff, 2008; Sollins et al., 1996). However, decomposition models currently focus on initial litter chemistry and do not consider the possibility that situ changes in litter chemistry during decomposition could substantially change decomposition dynamics. Further, most conceptual models describing changes in litter and SOM chemistry during decomposition suggest consistent, predictable changes in chemistry based on the extent of mass loss and do not explicitly consider the possibility that chemical changes in SOM during decomposition might follow multiple trajectories depending on decomposer community characteristics. However, Wickings et al. (2009) demonstrated that decomposer communities can strongly influence

changes in SOM chemistry during decomposition. Thus, an alternative explanation for the differences in SOM chemistry we observed is that N influenced soil communities and thereby altered the chemistry of SOM and its decomposition rate. However, we saw few changes in the chemistry of litter or LF pools No effect of N was observed in terms of litter biochemical composition at the end of the decomposition time period.

Thus, either N had no effect on litter chemistry, or effects were limited to pools that we did not measure. Although more effort is needed to understand the declines in soil respiration that are reported here and elsewhere (e.g.Treseder et al. 2007; Martinez et al. 2010), we found little evidence that these declines were associated with changes in SOM pool sizes or tissue chemistry. More likely, changes in microbial biomass, perhaps coupled with changes in microbial community structure, altered respiration rates following N fertilization.

CONCLUSION

Following are several alternative explanations for the results that could not be addressed:

a) However, N fertilizer rate may have effects on decomposition dynamics and long-term soil C balance that we did not consider. First, N fertilization rate has been shown to influence the C/N ratio of crop residues (Lee & Bray, 1949). Litter C/N ratio is one of the primary drivers of initial litter decomposition rates (Aber & Melillo, 1982), and declines in C/N ratio with increasing N availability may accelerate decomposition dynamics. Because our study focused on soil biological processes as a driver of decomposition, we used only one type of corn litter and one type of wheat litter, which allowed us to test differences in mass loss of a common species due to differences in decomposer communities, but not among litter types containing different C/N ratios.

b) Second, N fertilization might influence the allocation of resources above and belowground (Johnson, 1985; Liu & Greaver, 2010). Recent evidence points to the selective preservation of root structures in soil (Bird & Torn, 2006; Bontti et al., 2009). This is likely because of their unique chemistry as well as their intimate association with minerals. Liu and Greaver (2010) found that N addition increased aboveground litter inputs by ~ 20% but did not increase fine root inputs. Although evidence for such an effect is inconclusive, N fertilization could influence soil C dynamics through altering root to shoot ratios.

c) Third, N can have an effect on stable SOM pools and long-term SOM dynamics that short-term litter decomposition studies will not identify. Studies have suggested that N fertilization may influence the complex biochemical reactions that lead to chemical

recalcitrance of SOM (Neff et al., 2002). Plant biomass, the primary source of bulk soil organic matter, consists of heterogeneous, primarily polymeric molecules with a wide range of turnover times (Trinsoutrot et al., 2000). These compounds undergo microbial degradation and transformation, catalyzed by multiple enzyme systems, and many participate in secondary reactions, e.g. Maillard product formation (Jokic et al., 2004). N may influence these transformations by directly influencing chemical reactions involving SOM. N is an important component of humic substances and may directly catalyze or at least participate in humification reactions and play an important role in the formation of precursors involved in stabilization reactions (Haider et al., 2004). Additionally, it has been demonstrated that lignin materials and tannins in the presence of N can undergo oxidation and form heterocyclic N-containing compounds that are inhibitory to microorganisms (Skene et al., 1997).

		Lignin	Lipid	Phenols	Polysaccharide	N-bearing	Unknowns	L:N	L:P
	KgN/ha				*(Relative Abundance)*				
	na	0.2	0.01	0.1	0.38	0.03	0.27	6.4	0.51
Corn	**0**	0.33(0.067)	0.01(0.005)	0.02(0.101)	0.422(0.04)	0.052(0.002)	0.142(0.016)	6.75 (1.46)	0.86 (0.24)
	291	0.36(0.067)	0.01(0.005)	0.02 (0.101)	0.43(0.04)	0.05 (0.002)	0.13 (0.016)	7.16 (1.46)	0.91 (0.24)
	na	0.28	0.16	0.07	0.22	0.09	0.18	3.2	1.27
Wheat	**0**	0.59(0.62)	0.036(0.02)	0.01(0.03)	0.15(0.03)	0.05(2.73)	0.16(0.03)	12.18(2.6)	4.1(0.86)
	291	0.49(0.06)	0.05(0.01)	0.016(0.02)	0.17(0.03)	0.06(2.47)	0.2(0.02)	8.24(2.6)	3.13(0.80)
ANOVA									
	N	0.44	0.314	0.65	0.76	0.1	0.65	0.16	0.28
	Ltr	**0.0001**	**0.005**	**0.0001**	**0.001**	**0.0001**	**0.0001**	**0.0001**	**0.0002**
	N*Ltr	**0.02**	0.08	0.46	0.58	0.12	**0.04**	**0.001**	0.19

Table 3: Litter Chemistry**:** Relative abundance of chemical compounds in Corn and wheat litters, measured in the beginning of the experiment (June, 2008) and at the end (June, 2009). Where *N* = effect of Nitrogen, *Ltr* = effect of Litter and *N*Ltr* = Nitrogen and Litter effect, *na* =the initial litter chemistry not subject to the three (0,134,294 Kg/ha) N treatments.

	KgN/ha	LF mg/g soil	C mg/g LF	N mg/g LF	LF-C mg/g soil	LF-N mg/g soil	C:N ratio
Jun	**0**	1.66(0.12)	235(13.47)	12(0.70)	0.39(0.02)	0.02(0.00)	19(0.01)
	134	2.26(0.38)	254(17.35)	13(0.70)	0.57(0.10)	0.03(0.00)	18(0.96)
	291	2.04(0.07)	254(7.89)	14(0.40)	0.55(0.01)	0.03(0.00)	18(0.00)
Aug	**0**	4.62(0.53)	242(12.11)	12(1.10)	1.12(0.16)	0.06(0.00)	20(1.74)
	134	4.80(0.19)	208(4.062)	11(1.10)	1.00(0.04)	0.05(0.00)	18(0.17)
	291	3.96(0.08)	246(11.06)	13(0.23)	0.97(0.05)	0.05(0.00)	18(0.77)
ANOVA	**N**	0.16	**0.056**	0.069	0.325	0.194	0.438
	T	**<.0001**	**0.026**	0.07	**<.0001**	**<.0001**	0.989
	N*T	0.14	0.083	0.211	0.074	0.126	0.918

Table 4: Light Fraction organic matter and C, N content per grams of soil and C: N ratios, measured in Jun and Aug 2008, where *N* = effect of Nitrogen, and *T*= effect of time and N*T= effect of Nitrogen by Time.

		Jun	Jul	Aug	Sept	Oct	Nov
				(µg/g)			
	KgN/ha						
NH_4	**0**	3.14(0.34)	2.02(0.01)	3.61(0.53)	3.9(0.56)	4.04(0.55)	4.06(0.22)
	134	5.01(0.93)	24.03(8.01)	2.7(0.26)	3.67(0.06)	3.94(0.78)	3.24(0.38)
	291	5.01(0.97)	79.6(5.72)	5.72(1.46)	3.28(0.03)	3.72(0.33)	2.99(0.45)
(NO_3+NO_2)	**0**	7.04(1.41)	2.22(0.44)	3.33(0.31)	2.73(2.9)	2.9(0.07)	4.07(0.13)
	134	16.73(4.34)	16.81(3.75)	3.24(0.14)	3.07(3.11)	3.11(0.22)	4.44(0.19)
	291	18.61(3.36)	24.94(2.96)	24.76(7.91)	10.14(0.43)	3.55(0.21)	6.43(0.65)

Table 5: Inorganic NH_4 and NO_3+NO_2. ANOVA results consistent with significant Nitrogen and Time interaction.

		CBH	BG	NAG	POX	AFDM
	KgN/ha		(µmol/h/g)			(%)
Corn	**0**	0.57(0.08)	1.95(0.32)	0.94(0.22)	1.19(0.29)	0.54(0.04)
	134	0.97(0.21)	3.17(0.78)	1.23(0.31)	1.36(0.28)	0.53(0.02)
	291	0.85(0.13)	2.79(0.56)	1.14(0.22)	1.66(0.31)	0.53(0.03)
Wheat	**0**	0.4(0.19)	1.67(0.28)	0.85(0.12)	1.2(0.21)	0.630.04)
	134	0.64(0.17)	2.16(0.64)	1.16(0.32)	1.1(0.26)	0.65(0.02)
	291	0.68(0.003)	2.47(0.5)	1.18(0.32)	1.36(0.32)	0.64(0.05)
ANOVA						
	N	**0.05**	0.07	0.15	0.24	0.95
	Ltr	**0.02**	0.06	0.81	0.16	**<0.0001**
	N*Ltr	0.89	0.46	0.91	0.64	0.062
	Time	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	N*Time	**0.02**	**0.05**	0.36	0.14	0.23
	Ltr*Time	0.71	0.83	0.23	0.98	**<0.0001**
	N*Ltr*Time	0.47	0.65	0.26	0.13	0.89

Table 6: ANOVA of corn and wheat litter enzymes and Mass loss rates. Where *CBH* = β-D-cellobiohydrolase, *BG* = β-glucosidase, *NAG* = N-acetyl-β-D-glucosidase and *POX* = Phenol Oxidase. *AFDM*= Ash free dry mass, *N* = effect of Nitrogen treatment, *Ltr* = effect due to litter type.

	CBH	BG	NAG	POX	CO_2	CLOM
KgN/ha	(µmol/h/g)				(µg CO_2-C/g/d)	(mg/Kg)
0	0.06(0.01)	0.2(0.03)	0.06(0.01)	1.33(0.53)	252.2(126.1)	494.51(27.53)
134	0.06(0.005)	0.19(0.03)	0.06(0.01)	1.22(0.51)	176.2(88.1)	488.52(9.67)
291	0.05(0.003)	0.17(0.01)	0.05(0.01)	1.45(0.48)	178(89.1)	455.70(18.24)
ANOVA	T	T	T	T	N,T	*NS*

Table 7: ANOVA of soil variables with averages and standard errors in parentheses: Where *CBH* = β-D-cellobiohydrolase, *BG* = β-glucosidase, *NAG* = N-acetyl-β-D-glucosidase and *POX* = Phenol Oxidase. CO_2 = Cumulative CO_2 flux from soil lab incubations for 125 days. *N* = significant effect of Nitrogen, *T* = significant effect of time, *N*T* = interaction effect of Nitrogen and time, *NS* = Non significant.

	Pearson Correlation Coeffcients			
	NAG	**CBH**	**BG**	**POX**
		(Soil)		
Corn				
N-acetyl-β-D-glucosidase	**0.38**	0.17	0.16	0.29
β-D-cellobiohydrolase	0.17	-0.01	-0.03	0.02
β-glucosidase	**0.65**	0.04	0.02	0
Phenol oxidase	-0.003	-0.16	0.16	0.12
Wheat				
N-acetyl-β-D-glucosidase	**0.42**	0.13	0.15	**0.55**
β-D-cellobiohydrolase	0.17	0.03	0.02	0.1
β-glucosidase	0.22	0.07	0.07	0.05
Phenol oxidase	-0.01	-0.12	-0.17	0.06

Table 8: Correlations between soil and corn and wheat litter enzymes. Correlation coefficients in bold are significant (P<=0.05). *Abbreviations:* NAG (N-acetyl-β-D-glucosidase), CBH (β-D-cellobiohydrolase), BG (β-glucosidase),POX (Phenol Oxidase).

	Pearson Correlation Coeffcients							
	Lignin	**Poly**	**Phenols**	**Lipids**	**N-bearing**	**Unknown**	**L:N**	**L:P**
Corn				*Relative Abundance*				
β-glucosidase	-0.64	0.67	0.71	0.16	**0.74**	0.5	-0.68	-0.66
Phenol oxidase	0.22	-0.28	-0.32	-0.1	-0.44	-0.2	0.25	0.17
β-D-cellobiohydrolase	-0.64	0.65	**0.81**	0.36	**0.8**	0.53	**-0.69**	-0.62
N-acetyl-β-D-glucosidase	0.14	-0.14	-0.15	-0.5	0.015	-0.24	0.12	0.08
Wheat								
β-glucosidase	-0.67	0.67	0.67	**0.71**	0.64	0.62	-0.58	-0.64
phenol oxidase	0.41	-0.12	-0.56	-0.54	-0.51	-0.26	0.38	0.17
β-D-cellobiohydrolase	-0.29	0.46	0.26	0.32	0.26	0.3	-0.19	-0.39
N-acetyl-β-D-glucosidase	-0.63	0.42	**0.8**	**0.87**	**0.75**	0.36	-0.56	-0.44

Table 9: Correlations between litter enzymes and the relative abundance of chemical classes in litter. Correlation coefficient in bold are significant (P = 0.05).*Compound abbreviations:* Lip (Lipids), Poly(Polysaccharides), N-bearing (Nitrogen bearing compounds).

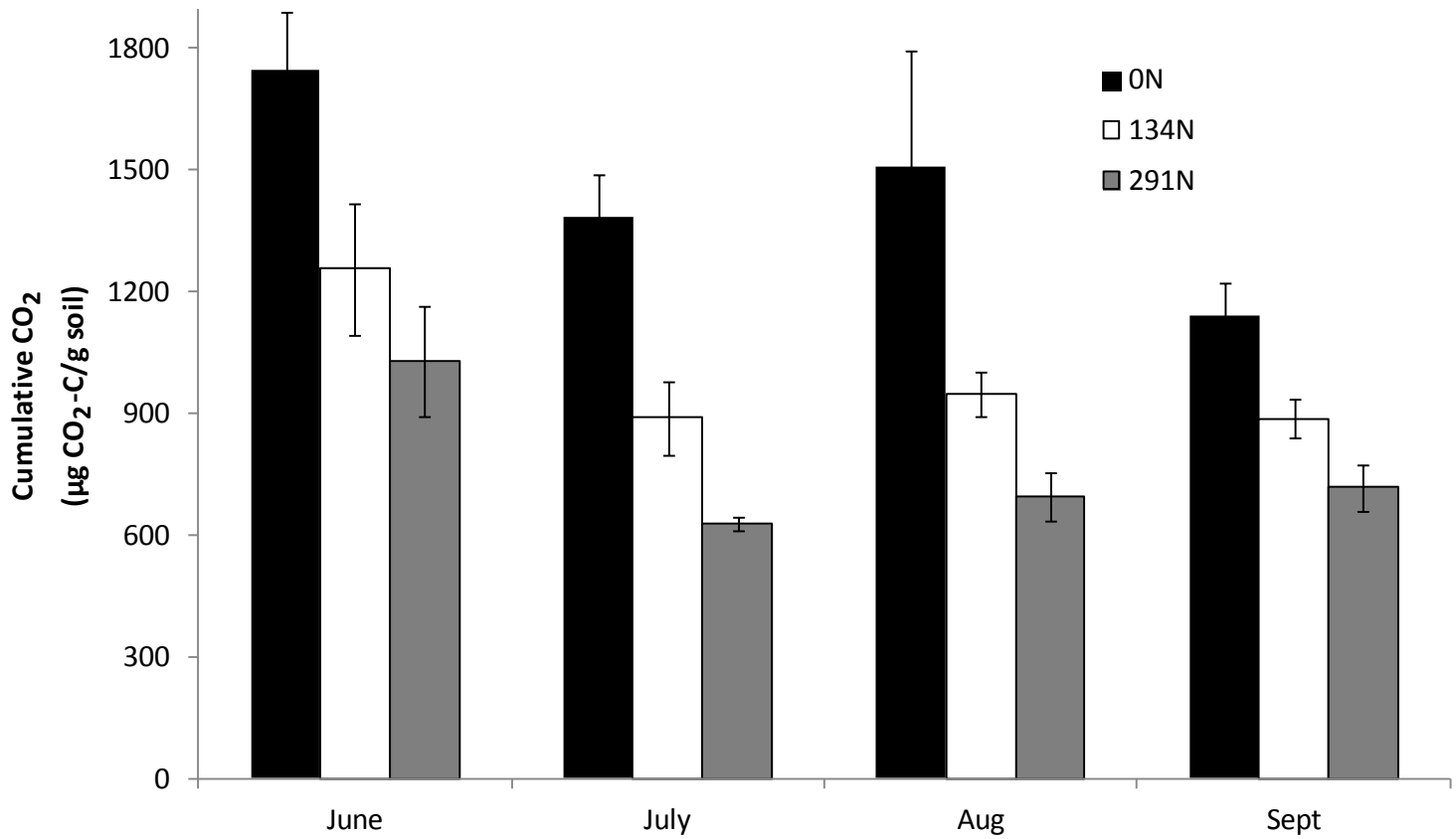

Figure 2: Carbon mineralization monitored in a field study of corn nitrogen response at the W.K. Kellogg Biological Station, Hickory corners, MI. Reported here as cumulative soil CO_2 emission from laboratory incubation of soil samples over 125 days. Samples were collected on June 17, July 09, August 05 and September 3rd of 2008. Analysis of variance (ANOVA) indicated a significant N rate effect (P = 0.0001).

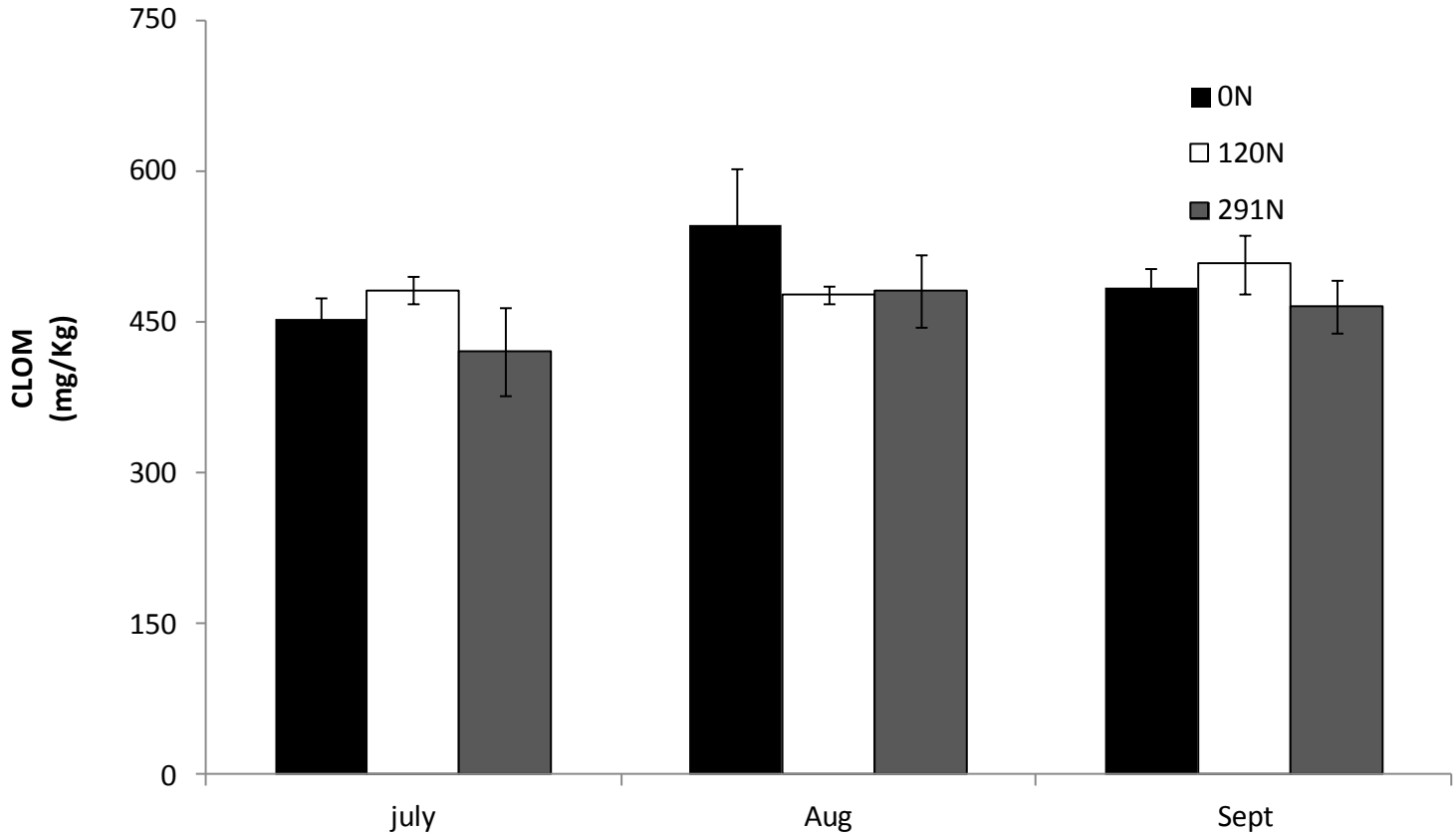

Figure 3: Chemically labile organic matter (CLOM) monitored over the summer of (2008) in a field study of nitrogen response at the W.K. Kellogg Biological Station, Hickory Corners, MI. Three levels of nitrogen fertilizer were compared, 0, 134 kg N/ha and 291 kg N/ha.

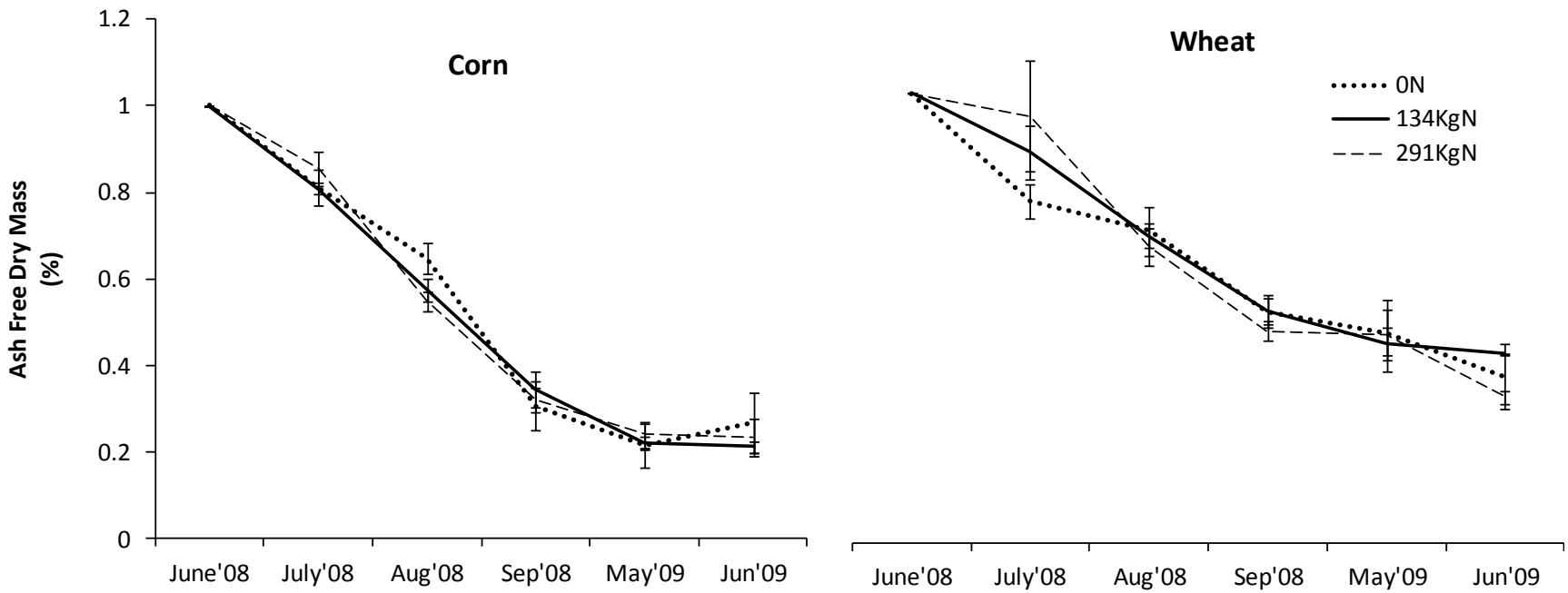

Figure 4: Effect of 0, 134 and 291kgN/ha on decomposition rates of wheat litter at the W.K. Kellogg Biological Station, Hickory corners, MI. ANOVA suggested significant differences (*P* = <0.0001) between the litter types with higher mass remaining in wheat than in Corn litter.

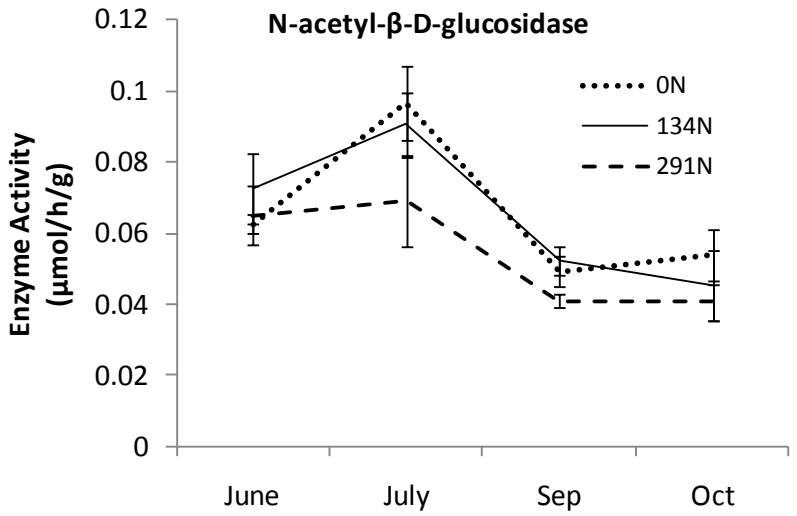

Figure 5: Activity of N-acetyl-β-D-glucosidase (NAG) in soil, showing significant time effect only under three levels (0, 134 and 291 KgN/ha) of N fertilizer treatment measured from Jun'08 to Oct'08 at W.K. Kellogg Biological Station, Hickory corners, MI.

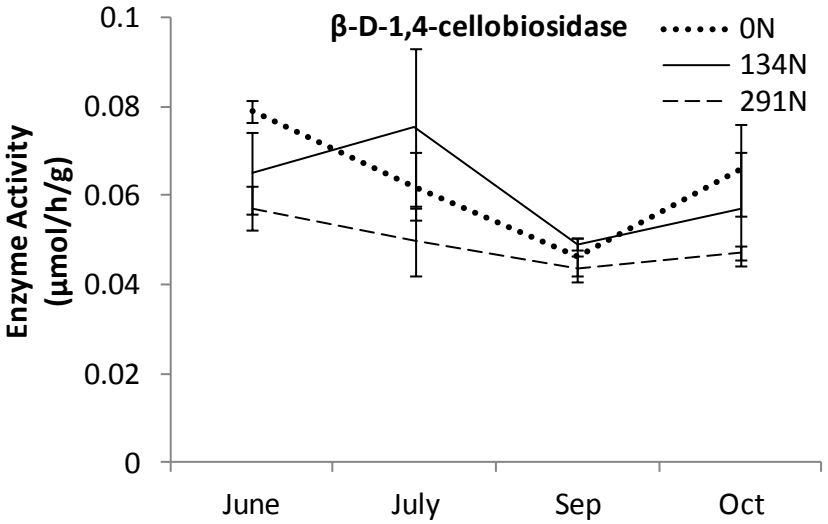

Figure 6: Activity of β-D-1,4-cellobiosidase (CBH) in soil under three levels (0, 134 and 291KgN/ha) of N fertilizer rate measured from Jun'08 to Oct'08 at W.K. Kellogg Biological Station, Hickory corners, MI, with significant time effect only.

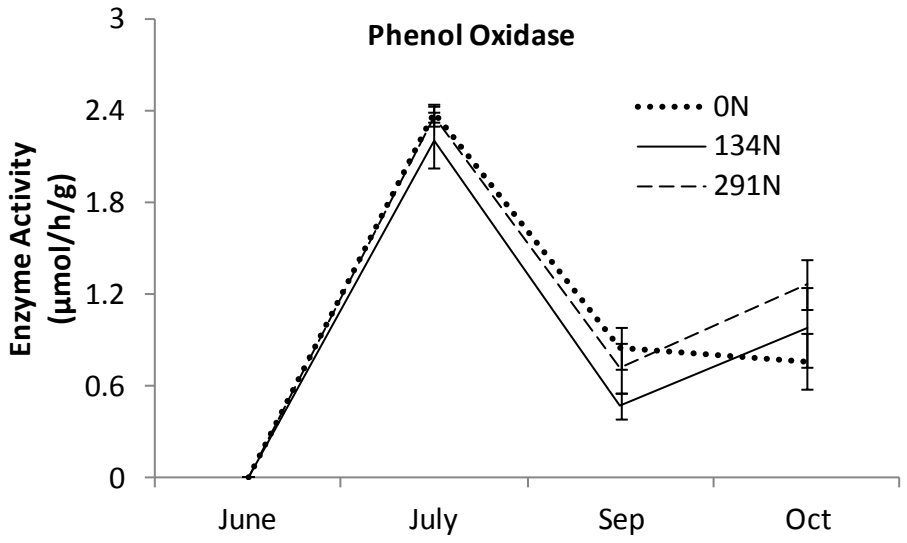

Figure 7: Activity of Phenol Oxidase (POX) in soil under three levels (0, 134 and 291KgN/ha) of N fertilizer rate measured from Jun'08 to Oct'08 at W.K. Kellogg Biological Station, Hickory corners, MI,with significant time effect only.

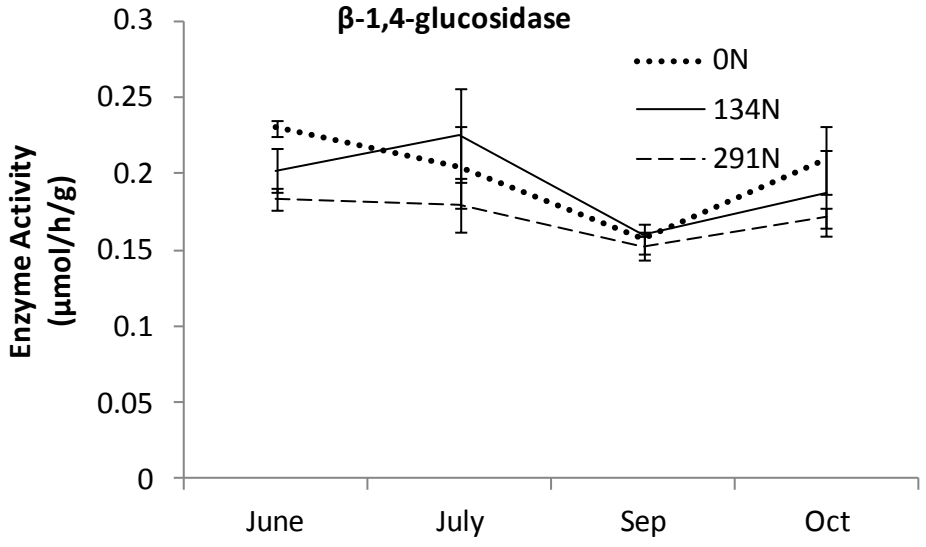

Figure 8: Activity of β-1,4-glucosidase (BG)in soil under three levels (0, 134 and 291KgN/ha) of N fertilizer rate measured from Jun'08 to Oct'08 at W.K. Kellogg Biological Station, Hickory corners, MI with significant time effect only.

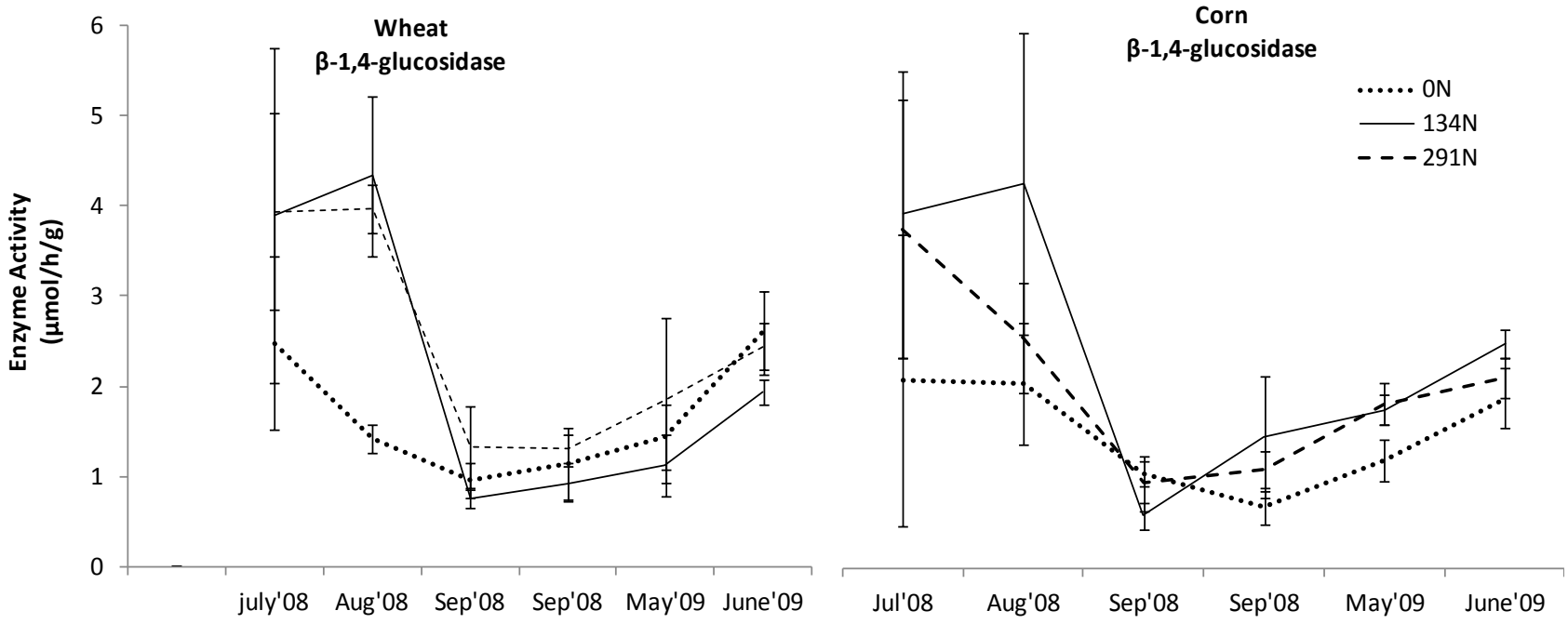

Figure 9: Activity of β-1,4-glucosidase(BG) on wheat and Corn litters under three levels of Inorganic Nitrogen Fertilizer (0, 134, 291Kg/ha), measured over a period of one year from Jun'08 to Jun'09 W.K. Kellogg Biological Station, Hickory corners, MI. ANOVA suggested a significant N by time interaction (P = 0.05).

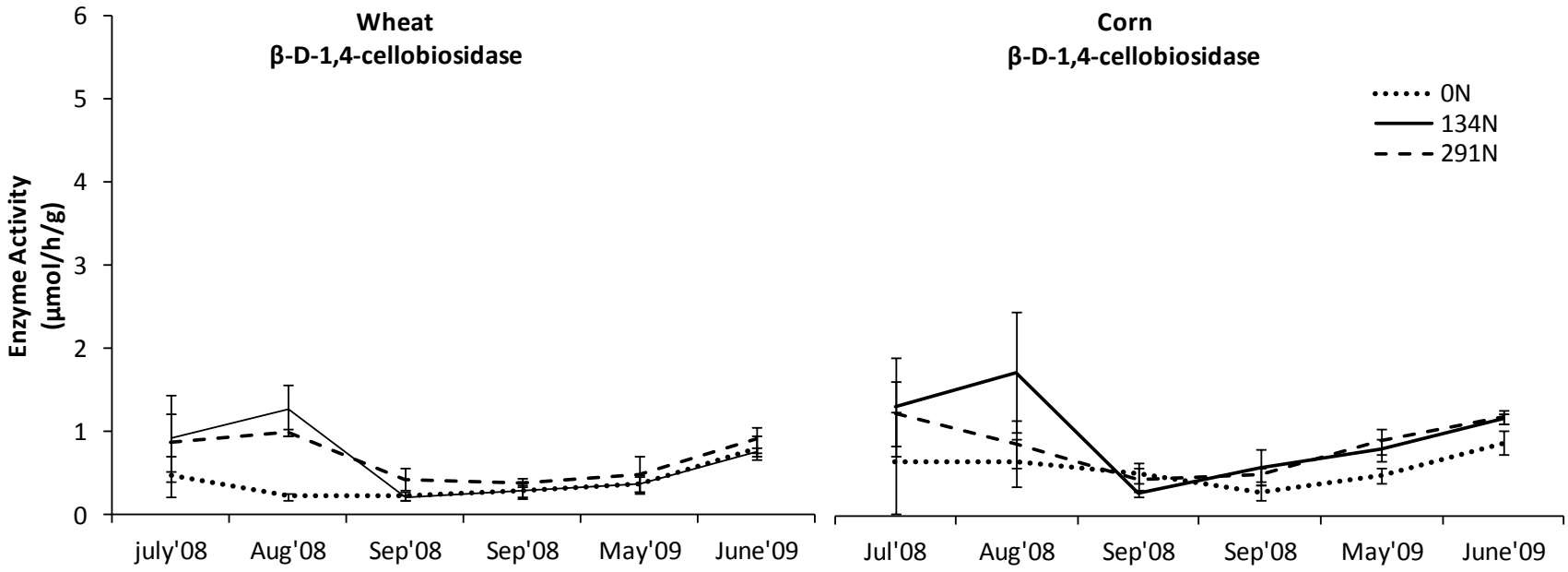

Figure 10: Activity of β-D-1, 4-cellobiosidase(CBH) on wheat and Corn litters under three levels of Inorganic Nitrogen Fertilizer (0, 134, 291Kg/ha), measured over a period of one year from Jun,08 to Jun'09 at W.K. Kellogg Biological Station, Hickory corners, MI.

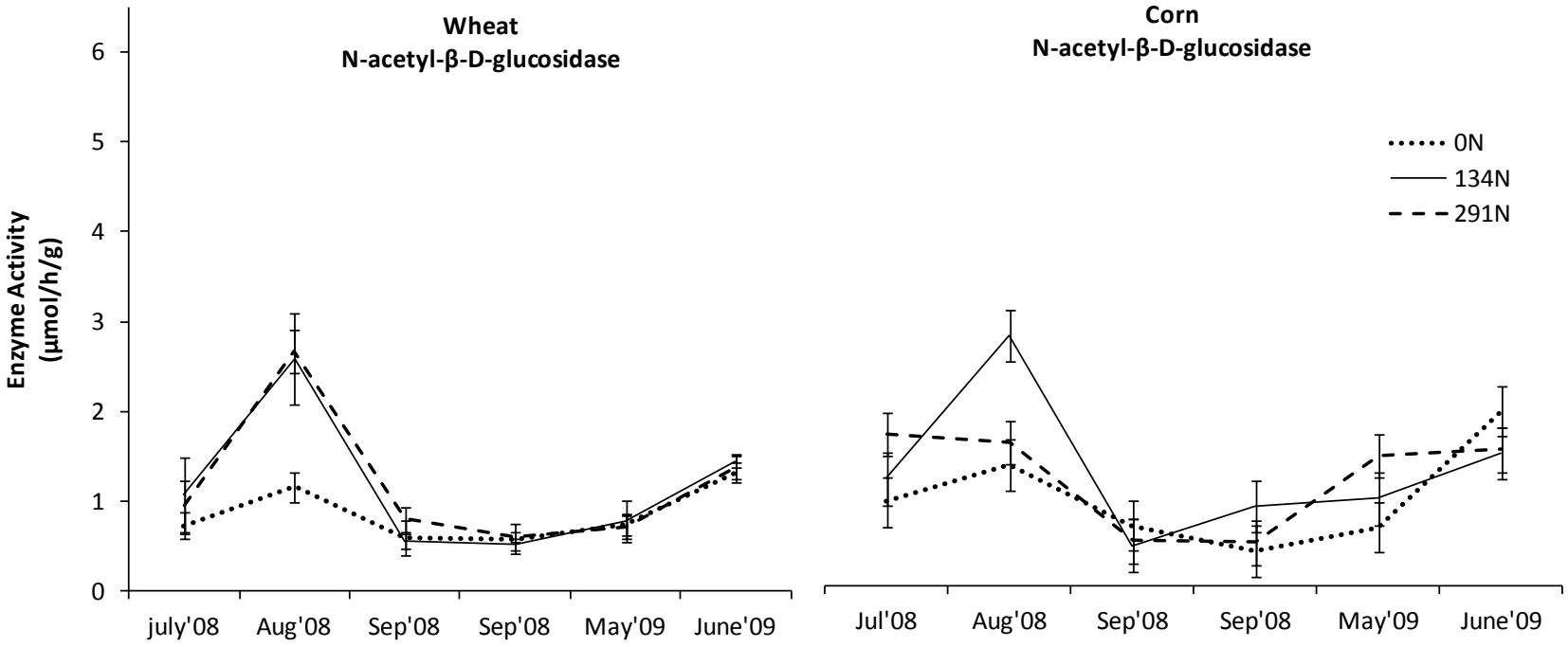

Figure 11: Activity of N-acetyl-β-D-glucosidase on wheat and Corn litters under the effect of N fertilizer treatment (0, 134, 291Kg/ha), measured over a period of one Year from Jun'08 to Jun'09 at W.K. Kellogg Biological Station, Hickory Corners, MI.

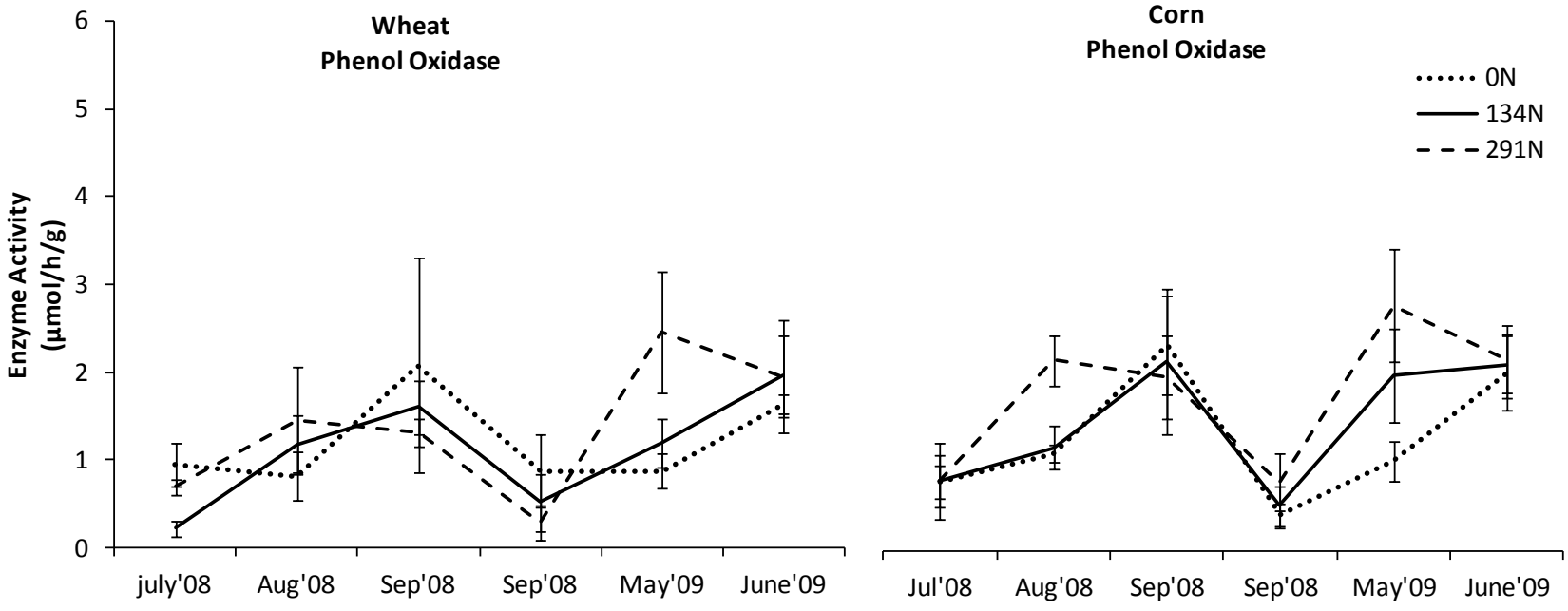

Figure 12: Activity of phenol oxidase on wheat and corn litters measured over a period of one year from Jun'08 to Jun'09 under the influence of N fertilizer treatment (0, 134, 291Kg N/ha) at W.K. Kellogg Biological Station, Hickory Corners, MI

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