

ABSTRACT

LEI TIAN. Understanding Soil Organic Matter Mineralization in Agroecosystems: Soil Enzyme Perspectives. (Under the direction of Dr. Wei Shi).

Soil enzymes play vital roles in organic matter degradation and nutrient cycling. While a number of publications have revealed the relations of soil enzyme activities with organic matter dynamics in agroecosystems, the underlying mechanisms remain elusive. The objectives of this study were to (1) characterize the associations of soil enzyme activities with the chemical composition of dissolved organic matter, the intermediate product of soil organic matter degradation as well as with soil carbon and nitrogen mineralization; (2) determine primary pathways by which a soil oxidative enzyme controls the decomposition of soil organic matter; and (3) identify regulatory principles of plant litter quality on soil enzyme production.

Surface soils from 0-10 cm depth were collected from diverse farming systems and used for characterizing the interrelationships among soil enzyme activity, dissolved organic matter, and soil C and N mineralization. Among the five enzyme activities examined, only soil peroxidase activity was related to the relative abundance of reducing sugars and with soil C and N mineralization. The relative abundance of reducing sugars was also negatively associated with soil C mineralization and so was relative abundance of amino acids with soil N mineralization. These results raised a research question: How did peroxidase control soil C and N mineralization?

A working hypothesis that peroxidase could enhance soil C and N mineralization through improving the bio-accessibility of carbohydrates and proteins was tested via soil

amendments of horseradish peroxidase and/or hydrolytic enzymes. Compared to soil addition of peroxidase at a low activity unit (i.e., 0.1 units g^{-1} soil), the addition at 0.2 units g^{-1} soil generated phenolic compounds that were able to inhibit soil hydrolytic enzyme activities. However, soil reducing sugar content was increased after sterile soil addition of peroxidase. Effects of peroxidase on bioavailability of reducing sugars and amino acids were more pronounced when sterile soil was amended with peroxidase in combination with cellulase, protease, or both. These results suggested that the positive effects of peroxidase on organic matter degradation were not through the production of soluble phenolic compounds or improved soil enzyme activity, but rather the bio-accessibility of carbohydrates and proteins that were otherwise bound to soil humus.

Microbial production of extracellular enzymes represents a primary source of soil enzyme. Regulatory roles of plant litters on soil enzyme activity were examined by using grass materials, soybean residues and pine needles. Generally, grass materials had greatest impacts on microbial production of cellulase and glucosidase, whereas soybean residues exerted more influences on glucosaminidase, and pine needles on phenol oxidase. These differential effects of litters on soil enzyme activities could be explained by microbial C and N demands associated with the relative abundance of substrates and nutrients contained in plant litters.

Overall, this dissertation research provided some insight on factors regulating microbial extracellular enzyme production and principles by which soil enzyme activities determine organic matter mineralization.

Understanding Soil Organic Matter Mineralization in Agroecosystems:
Soil Enzyme Perspectives

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DEDICATON

For my parents

BIOGRAPHY

Lei Tian was born in Lanxi, China. She received her Bachelor degrees in Agricultural Resource and Environmental Science from Nanjing Agricultural University (Nanjing, China) in 2004 and then continued her study in Ecology and got her Master's degree from the same university in 2007. After that, she came to the United States and enrolled in North Carolina State University to pursue the doctoral degree in Soil Microbiology under the direction of Dr. Wei Shi.

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CHAPTER ONE

INTRODUCTION

Mineralization of soil organic matter (SOM) is an important microbially-mediated process by which carbon (C), nitrogen (N), and other nutrients are converted from organic forms into inorganic forms (Fig. 1). It is well known that soil microbes are unable to directly assimilate complex and solid SOM, but rather simple and dissolved compounds for growth and metabolisms. As a consequence, soil microbes must produce extracellular enzymes, i.e., soil enzymes, to catalyze the breakdown and depolymerization of SOM and to make readily-usable dissolved compounds. A study demonstrated that the production of dissolved organic matter (DOM) was the rate-limiting step for the overall mineralization of SOM (Jones et al., 2004). There are numerous soil enzymes to facilitate degrade SOM of different complexities. For example, β -glucosidase, a hydrolytic enzyme can act on the β -1,4 linkage of oligomers and produces low molecular weight and soluble compounds, whereas phenol oxidase, an oxidative enzyme can act on the phenolic moiety of SOM and catalyzes the release of oxygen radicals (Hammel, 1997; Claus, 2004). Via soil enzyme-catalyzed reactions, SOM is subjected to decomposition of different degrees and a portion of SOM may be completely degraded into CO₂, inorganic N, and other inorganic compounds. It should be noted that some products of decomposition may negatively affect the activities of soil enzymes due to either toxic effects on microbial production of soil enzymes or inhibitory effects on soil enzyme activity. For example, Hanif et al. (2004) reported that β -cellobiohydrolase activities could be suppressed by high concentrations of low-molecular weight compounds, e.g.,

glucose and amino acids. Although DOM production has been increasingly considered as an important control on SOM decomposition and C and N cycling, so far, little information is available on the interrelationships of the chemical composition of DOM with soil enzyme activity and SOM mineralization.

Microbial production of extracellular enzymes

Microbes produce extracellular enzymes to acquire energy, C and nutrients for growth and metabolisms. It is generally accepted that microbial extracellular enzyme-production abides by a cost-effective rule of economy, i.e., microbes will maximize the energy, C and nutrient returns by minimizing resource utilization (Allison et al., 2011). Several principles by which microbes produce extracellular enzymes have been proposed and accordingly tested. One of these principles is called product inhibition (Sinsabaugh and Moorhead, 1994; Kang and Freeman, 1998), which states that the catalytic product of an enzyme can inhibit microbial production of the enzyme. This principle appears to underlie the inverse relationship between available phosphate and phosphatase activity. For example, Kang and Freeman (1998) reported that soil phosphatase activity was decreased when soil phosphate concentration was increased. Microbial resource allocation represents another principle dictating microbial extracellular enzyme production. It states that microbes are able to allocate the resource of energy, C and nutrients to produce the enzymes for acquiring the most limited nutrition for growth. For example, soil microbes can be phosphorus (P) limited when primary production increases due to atmospheric CO₂ elevation, thereby causing increased soil input of plant litter. In this case, microbes may allocate more resource to

produce phosphatase for acquiring P from SOM rather than to produce the enzymes for acquiring C (Moorhead and Linkins, 1997). As another example, when microbial growth is limited by available N in soil, microbes may even produce oxidative enzymes, which help degrade recalcitrant organic matter and liberate occluded N (Carreiro et al., 2000; Waldrop et al., 2004).

Diverse organic materials may contain different amounts of C and nutrients. When microbes acquire C and nutrients from these materials for growth and metabolisms, they likely produce different types and amounts of extracellular enzymes based upon the biochemistry of organic materials. When grass litters were used as the major C and nutrient source for microbes, for example, Dornbush et al. (2007) found that the N content of grass litters had a positive correlation with the activity of β -glucosaminidase. Microbial enzyme production in response to the quantity and quality of crop residues needs to be better understood should crop residues be managed for sustaining long-term soil productivity.

Significance of soil enzymes and DOM

Soil enzymes play important roles in biochemical reactions in organic matter decomposition and nutrient cycling. A recent meta-analysis has shown that soil peroxidase and phenol oxidase activity accounted for 32% and 37% of variation in SOM across various ecosystems, respectively (Sinsabaugh, 2010). To date, most studied soil enzymes are those involved in the degradation of cellulose and lignin, the two major components of plant litters (Allison et al., 2007). Oxidative enzymes, i.e., phenol oxidase and peroxidase, are able to depolymerize lignin, whereas hydrolases such as cellulase can catalyze the depolymerization

of cellulose, producing simple monomers or oligomers for microbial assimilation (Coyne, 1999). Other commonly studied enzymes are those known to benefit soil nutrient availability, such as β -glucosaminidase for increasing available N from chitin and phosphatase for increasing available P from organic phosphate.

As a biological catalyst, soil enzymes depolymerize high-molecular weight organic polymers such as lignin, cellulose, and humus. Because enzymatic catalysis is of substrate specificity, changes in the relative abundance of soil enzymes may result in quantitative and qualitative variations in the degradation products of SOM, i.e., DOM. For instance, when phenol oxidase increased $20 \mu\text{mol h}^{-1} \text{g}^{-1} \text{OM}$, the soil organic C decreased by 250% ($r^2=0.38$) (Waldrop and Zak, 2004). While attention has been given to the relationship between soil enzyme activities and soil organic C content, little is known about the regulatory roles of soil enzymes on the chemical composition of dissolved organic C (DOC). One study showed that about 73% decrease of phenol oxidase activity resulted in 57% increase in phenolic compounds in sugar maple–red oak (*Acer saccharum*, *Quercus rubra*) forest ecosystems, but without significant change of DOC concentration (Waldrop and Zak, 2006). In contrast, sugar maple–basswood (*Tilia americana*) ecosystems showed 50% lower DOC production when phenol oxidase activity increased 700%. The contradictory results are perhaps related to the biochemistry of plant litters. Nevertheless, a growing body of evidence supports that correlations exist between soil enzyme activities and the concentration of DOC (Sinsabaugh et al., 2002; Waldrop and Zak, 2006).

As a substrate or an inhibitor, DOM can also affect soil enzyme activity. During SOM decomposition, numerous dissolved organic compounds will be produced and serve as the

substrates for other enzymes. For example, cellubiose produced by exoglucanase can be further decomposed to glucose by β -glucosidase. Decomposition of humic material by oxidative enzymes, such as peroxidase, could also release some occluded bioavailable DOM for further enzymatic reaction (Nadeau et al., 2007). On the other hand, the enzymes activity can be inhibited by some dissolved compounds due to toxicity (Neuhauser and Hartemstein, 1978; Freeman et al., 2004) or negative feedback inhibition (Chróst, 1991, Hanif et al., 2004).

DOM and its biodegradability in soil

DOM is defined as the organic matter in soil solution. Operationally, DOM can be obtained via numerous laboratory and field techniques, such as suction, leaching, and extraction/filtration (Zsolnay, 2003; Deforest et al., 2004). Despite technical variations, DOM is generally considered to be the organic matter in aqueous solution after filtration through 0.45 μm (Zsolnay, 2003).

Given that DOM is primarily produced from enzyme-catalyzed depolymerization of soil organic matter or plant litters, it certainly contains a spectrum of organic compounds varying in the biodegradability, defined as the degree to which microbes use organic compounds and often quantified by the disappearance of DOM or by the evolution of CO_2 over time (Kalbitz, 2003). Therefore, many studies separate DOM into two fractions based on biodegradability, i.e., labile versus stable components (Guggenberger et al., 2003; Kaiser et al., 2001; Van Hees et al., 2005). It is generally considered that labile DOM consists mainly of simple carbohydrate monomers including glucose and fructose, organic acids of low-molecular weight including citric, oxalic, and succinic acids, amino acids, amino sugars, and proteins

and peptides of low-molecular weight (Lynch, 1982; Guggenberger et al., 1994; Kaiser et al., 2001; Koivula and Hanninen, 2001). Perhaps, these labile compounds can be assimilated by microbes without the need of breakdown extracellularly. By contrast, stable DOM may contain polysaccharides and other plant or microbially derived compounds or degradation products (Lynch, 1982). Numerous lab incubation studies revealed that < 50% of DOM was biodegradable (Jandl and Sletten, 1999; Kalbitz et al., 2000; Yano et al., 2000; Sachse et al., 2001) and the biodegradability of DOM seemed to relate to its chemical compositions. Sun (1997) found that the biodegradability of DOM in river could be estimated by its bulk chemical composition, such as H:C, N:C and O:C ratio. Kalbitz (2003) also proposed that the biodegradability of DOM could be predicted from its relative abundance of aromatics, as evidenced by the correlations between CO₂ evolution and parameters of aromatics, such as UV and XAD-8 extractable C. It is reasonable to consider that the degradability of DOM is correlated with its chemical composition.

Multiple functions of dissolved soil phenolic compounds

Phenolic compounds, referred as chemicals having hydroxyl groups attached to at least one aromatic hydrocarbon ring, are important components of plant litters. They enter soil solution through microbial decomposition of lignin (Elder and Kelly, 1994) and/or rainfall leaching of plant materials (Kuiters, 1990). The concentration and chemical composition of phenolic compounds in soil solution depend on primary productivity and plant litter quality, which may vary from 1-37% of total mass of plant materials (Hattenschwiler and Vitousek, 2000; Todd et. al., 2006). Depending on soil type, vegetation and seasonal variation,

concentration of several phenolic monomers could range from 0.07 – 5 $\mu\text{g C L}^{-1}$ in soil solutions (Whitehead, 1964). It seems that these compounds could be manageable through crop rotation and crop residue management practices. Phenolic compounds can be roughly divided into two groups including low molecular weight compounds and high molecular weight compounds (Harborne, 1997). The former contains simple phenols, phenolic acids and flavonoids and exists widely in different plant species, whereas the latter, such as condensed tannin, polymers of three-ring flavanol monomer units joined by C-C bonds, is most abundant in woody plants (Hattenschwiler and Vitousek, 2000). The two groups of phenolic compounds can be reversely transformed. Phenols may react with oxygen to form reactive quinone and/or radicals that can polymerize with other phenols (Martin and Haider, 1971). Phenols can also polycondense with a variety of organic components such as nitrogen- and sulfur-bearing substances (Wang and Huang, 1987, 1991; Wang, 1995; Huang et al., 1999).

Despite low concentration, soluble phenolic compounds have recently drawn great attention due to their triple roles on soil processes as (1) binding agents to limit substrate availability for enzymatic reactions, (2) toxic compounds to inhibit enzyme activity, and (3) substrates for soil microbial growth (Freeman et al., 2004; Deforest et al., 2005; Fenner et al., 2005; Kraus et al., 2004). Phenolic compounds of low molecular weight (i.e., ferulic, vanillic, p-hydroxybenzoic and p-coumaric acids), which are smaller than 200 Da (Turner and Rice, 1975; Black and Dix, 1976; Blum and Shafer, 1988) may be subjected to microbial decomposition via ring cleavage, ring fission and then conversion to aliphatic compounds (Fenner et al., 2005). However, Neuhauser and Hartemstein (1978) hypothesized that high

concentration of phenolic compounds might restrict the activity and abundance of microbes. By inhibiting microbial respiration, phenolic compounds of high molecular weight may be toxic to soil microbes (Kanerva et al., 2006). More than 26 enzyme activities had been reported to be inhibited by tannin in forest ecosystems (Makkar et al., 1988; Nicholas-Orians, 1991; Scalbert, 1991; Field and Lettinga, 1992; Juntheikki and Julkunen-Tiitto, 2000). However, the concentration of tannin (from 0.1-2 g L⁻¹) inducing enzyme reduction depended on type of enzyme and tannin (Kraus et al., 2004). The common observation that the high concentration of phenolic compounds accompanied with the low activity of hydrolysable enzyme in the wetland and upland N-fertilized forest systems also indicated the effect of inhibition (Freeman et al., 2004; Deforest et al., 2005). Phenolic compounds may also bind to other organic compounds, thereby affecting their bioavailability or bio-accessibility to soil microbes. For example, tannins leached into soil or released during litter decomposition could combine with proteins and also other non-protein compounds to form resistant complexes in the soil (Benoit et al., 1968; Benoit and Starkey, 1968). High molecular weight tannins tended to precipitate more protein than low molecular weight tannins (Porter and Woodruffe, 1984; Asquith and Butler, 1985; Kumar and Horigome, 1986; Osborne and McNeill, 2001). Because of the resistance of these complexes to soil organisms (Butler, 1989; Muir et al, 1999), the decomposition rate was decreased by up to 50% (Lewis and Starkey, 1968; Benoit et al., 1968). Therefore, phenolic compounds, like tannins, might affect soil nutrients dynamics (Northup et al., 1995; Kraus et al., 2004).

This dissertation study was to test three hypotheses: (1) the chemical composition of soil

dissolved organic matter is correlated with soil enzyme activities involved in soil C and N cycling from differently managed farming systems; (2) peroxidase can stimulate organic matter decomposition by improving the bioavailability of carbohydrates and peptides; and (3) regulatory roles of plant litters on microbial enzyme production vary with particular enzymes and plant litters. The specific objectives were to (1) compare soil enzyme activities and chemical composition of dissolved organic matter in different farming systems; (2) determine the relationships of chemical composition of dissolved organic matter with soil enzyme activities and soil C and N mineralization; (3) evaluate the possible pathways by which peroxidase regulates soil organic matter decomposition; and (4) assess both hydrolytic and oxidative enzyme activities in response to soil additions of plant litters with different C:N ratios. This dissertation includes three main chapters, each addressing one hypothesis not previously addressed in the literature. Chapter 2 characterizes the relationships of chemical composition of DOM with soil enzyme activities and soil C and N mineralization via comparing differences in five farming systems; Chapter 3 determines the primary pathways by which peroxidase increases soil C and N mineralization rate; and Chapter 4 identifies the regulatory roles of soil amendment of plant litters on microbial production of extracellular enzymes involved in C and N dynamics. The overall goal of this dissertation research was to gain fundamental knowledge regarding regulations on microbial enzyme production and principles by which soil enzymes mediate SOM mineralization.

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CHAPTER TWO

CHEMICAL COMPOSITION OF DISSOLVED ORGANIC MATTER IN AGROECOSYSTEMS: CORRELATIONS WITH SOIL ENZYME ACTIVITY AND CARBON AND NITROGEN MINERALIZATION

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ABSTRACT

Soil enzyme-catalyzed depolymerization of organic matter results in the production of low molecular weight and other dissolved organic compounds. This fraction of soil organic matter is the immediate energy, carbon, and other nutrient substrate for microbial catabolic pathways and thus likely plays an important role in soil processes. The purpose of this study was to elucidate interrelationships among dissolved organic matter, soil enzyme activity, and soil C and N mineralization from diverse agroecosystems. These systems included a conventional cropping, organic cropping, integrated crop-livestock, plantation forestry, and succession from an abandoned agricultural field. We collected surface soil samples from 0 – 10 cm depth in early spring 2009 and measured the concentrations of soil-derived dissolved organic C and N, soluble phenolics, reducing sugars, and amino acids, the activities of β -glucosidase, exoglucanase, phenol oxidase, peroxidase, and β -glucosaminidase, and the rates of soil C and N mineralization. The integrated crop-livestock system showed the highest concentrations of dissolved soil organic C ($78 \mu\text{g C g}^{-1}$ soil) as well as phenolic compounds ($1.5 \mu\text{g C g}^{-1}$ soil), reducing sugars ($23 \mu\text{g C g}^{-1}$ soil), and amino acids ($0.76 \mu\text{g N g}^{-1}$ soil), and these components were up to 3-fold greater than for soils under the other systems. However, soil β -glucosidase activity in the integrated crop-livestock system was significantly

lower than the other systems and appeared to reflect the inhibitory role of soluble phenolics on this enzyme; this enzymatic disparity was also revealed in our preliminary study conducted in 2008. Among the five enzyme activities examined, only peroxidase activity was correlated significantly with the chemical composition of dissolved organic matter as well as soil C and N mineralization. Soil peroxidase activity was negatively related to the relative abundance of reducing sugars (i.e., reducing sugar C as a fraction of dissolved organic C, $r = -0.92$, $P < 0.05$) and positively with soil C and N mineralization ($r = 0.86$, $P < 0.1$ for C mineralization; $r = 0.85$, $P < 0.1$ for N mineralization). Furthermore, relative abundance of reducing sugars was negatively associated with soil C mineralization ($r = -0.80$, $P < 0.1$) as was relative abundance of amino acids with soil N mineralization ($r = -0.97$, $P < 0.01$). Our results suggested that diverse agroecosystems differed in the chemical composition of dissolved organic matter and the differences could be correlated with soil peroxidase activity and soil C and N mineralization.

Introduction

Dissolved organic matter is produced by soil enzyme-catalyzed depolymerization of organic matter and is comprised of low molecular weight chemicals that are often water soluble and thus more accessible to microbial assimilation as energy, C, and nutrient sources. Microbial utilization of these soluble compounds leads to microbial immobilization or soil C and N mineralization. Laboratory incubation studies have shown that the degradation of dissolved organic matter ranges from <10% to as much as 90% due to differences in its chemical composition (Jandl and Sletten, 1999; Hongve et al., 2000; Kalbitz et al., 2000;

Yano et al., 2000; Sachse et al., 2001). UV absorbance and aromaticity of dissolved organic matter have been used to predict its biodegradability (Kalbitz et al., 2003), suggesting the intrinsic linkage between its chemistry and biodegradability.

Phenolic compounds are an important component of dissolved organic matter and may affect soil organic matter decomposition through interactions with soil enzyme activities. Freeman et al. (2001) proposed an “enzymatic latch” hypothesis to elucidate soil C decline in peatlands caused by drainage. This concept states that upon release from oxygen constraints, phenol oxidase activity will increase, thereby reducing soluble phenolics, the compounds inhibitory or toxic to hydrolytic soil enzymes. Therefore, hydrolytic enzyme activities may be enhanced and thus soil C degradation is increased. The “enzymatic latch” model seemed to partially explain the differences in soil C storage between no-till and tillage practices (Zibilske and Bradford, 2007). The coordination of soil enzyme activities, soluble phenolics, and soil C dynamics has also been observed in highly managed turfgrass systems (Yao et al., 2009). In those systems, the activities of soil hydrolytic enzymes and soil C storage could be explained by soil pH-affected differences in the amount of soluble phenolic compounds. Relationships among dissolved organic matter, soil enzyme activity, and soil C decomposition also appeared in studies on forest and grassland ecosystems (Carreiro et al., 2000; Saiya-Cork et al., 2002; Waldrop et al., 2004; Sinsabaugh et al., 2005; Stursova et al., 2006; Zeglin et al., 2007; Keeler et al., 2009). For example, Sinsabaugh et al. (2005) reported that after synthetic N application to forests, soil oxidative enzyme activities declined whereas dissolved organic C concentration increased. They found that the response of dissolved organic C to synthetic N application negatively paralleled the response of oxidative enzymes,

in particular peroxidase. However, relationships among dissolved organic matter, soil enzyme activity, and soil C decomposition seem to be inconsistent across various ecosystems. This is perhaps due to differences in chemical compositions of soil organic matter and plant litters (DeForest et al., 2005; Gallo et al., 2005; Waldrop and Zak, 2006).

Agroecosystems are characterized by great human intervention to sustain crop production and soil quality. Soil enzyme activity has been found to change with various management practices such as tillage, rotation, and fertilization, and therefore has been considered as an important indicator of soil quality and ecological stability (Dick, 1994; Bergstrom et al., 1998; Ajwa et al., 1999; Bandick and Dick, 1999; Badiane et al., 2001; Saviozzi et al., 2001; Vepsäläinen et al., 2001; Roldan et al., 2005; Lagomarsino et al., 2009). Generally, an increase in soil enzyme activity is linked to the improvement in soil organic matter content, soil microbial activity, and soil C and nutrient cycling (Tabatabai, 1994). However, the effects of organic matter content on soil enzyme activity may vary with type of the enzyme (Gil-Sotres et al., 2005; Trasar-Cepeda et al., 2008). For example, poultry litters needed to be applied at a higher rate for increasing the activities of soil enzymes involved in P and S mineralization than for enzymes for soil C and N mineralization (Acosta-Martínez et al., 2006). Even within an integrated crop and livestock system, soil enzyme activities were found to be different between the grazed and non-grazed areas (Acosta-Martínez et al., 2010). There are numerous studies showing that a management practice can lead to the contradictory behavior of soil enzyme activities. Long-term N fertilization significantly increased activities of β -glucosidase and acid phosphatase but decreased urease activity (Ajwa et al., 1999). By examining organically cultivated soils of different ages, Monokrousos

et al. (2006) found that acid phosphatase activity increased from newest to oldest organic systems whereas the activities of amidohydrolase and alkaline phosphatase were highest in the middle-aged ones. These observations suggest that complex relationships exist among soil enzyme activities, soil organic matter content, and management practices. Perhaps, these relationships can be better clarified if dissolved organic matter, the enzymatic product of soil organic matter is incorporated.

This study aimed to determine the correlations between the chemical composition of soil dissolved organic matter and soil enzyme activities involved in soil C and N cycling from differently managed farming systems. Specific objectives were (1) to compare soil enzyme activities and chemical composition of dissolved organic matter in different farming systems; and (2) to determine the relationships of chemical composition of dissolved organic matter with soil enzyme activities and soil C and N mineralization. The investigation was conducted in diverse, long-term managed farming systems presumed to differ in soil organic matter quality and thus dissolved soil organic matter, soil enzyme activity, and soil C and N mineralization (Mueller et al., 2002; Sydorovych et al., 2009).

Materials and methods

Study site and soil sampling

Our study was conducted in a farming systems-center (i.e., Center for Environmental Farming Systems, North Carolina State University), located in Goldsboro, NC. This center was established in 1999 and included five farming systems: conventional cropping system (CON), organic cropping system (ORG), integrated crop-livestock system (ICL), plantation

forestry system (PF), and successional system from abandoned agricultural field (SUCC). A completely randomized block design was used to arrange the five systems into each of three blocks, leading to 15 selected plots. Blocks were assigned based upon soil types with Tarboro loamy sand (mixed, thermic Typic Udipsamment) for one block and Wickham sandy loam (fine-loamy, mixed, semiactive, thermic Typic Hapludult) for the other two blocks. Soil bulk density ranged from 1.23 g cm⁻³ in ORG to 1.58 g cm⁻³ in ICL. Plot sizes varied from 0.66 to 3.64 hectares, being smaller for CON, ORG and PF and larger for ICL and SUCC. Crop rotations, which mainly included corn (*Zea mays*), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), wheat (*Triticum aestivum*), sweet potato (*Ipomoea batatas*) and sorghum (*Sorghum bicolor*), were performed annually for CON, ORG and crop phase of ICL. During the pasture phase of ICL, cattle were subjected to controlled rotational grazing. PF was planted with long-leaf pine (*Pinus palustris*) in one plot and with ash trees (*Fraxinus pennsylvanica var. lanceolata*) in the other two. SUCC was comprised of a mixture of invaded species of shrubs such as *Solidago virgaurea minuta*, *Baccharis articulata*, small trees such as *Pinus taeda*, and grasses such as *Andropogon*. Tillage methods also varied within a system because of crop rotation. More details on soil physical properties, plant cover, and rotation management of the farming systems have been published by Mueller et al. (2002) and Sydorovych et al. (2009). Synthetic N, P, and K fertilizers were applied as needed to CON and ICL, whereas turkey litters were used in ORG. Gypsum was used to lime soils in CON, ICL, and ORG. Herbicides, insecticides, and fungicides were applied as needed in CON, PF, and primarily the crop phase of ICL, but chemicals were not applied to ORG and SUCC.

In February 2008, a soil sampling was conducted on the five systems, at two blocks only, for a preliminary examination of soil total C and N, soil pH, microbial biomass C and N, and soil enzyme activity. This sampling showed that there were significant differences in soil properties among these systems and a more complex assessment was decided to be done with another soil sampling. Thus, in March 2009 before the growth of annual crops or perennial vegetation, we collected soil samples from the five systems at all three blocks. This sampling time allowed us to examine dissolved organic matter derived mainly from soil organic matter, rather than plant debris and root exudates, which are often environmentally short lived (van Hees et al., 2005). Twenty soil cores (2.5 cm × 10 cm) were collected randomly from each field plot and pooled to form a composite soil sample. Soil samples were placed in plastic bags, transported in a cooler back to the laboratory, sieved (< 2 mm), and stored at 4 °C. Within two weeks, soil samples were analyzed for chemical and biological properties; incubation experiments for C and N mineralization were also set up.

Soil properties and organic C and N mineralization

Soil organic C and N were determined by dry combustion method by using a Perkin-Elmer 2400 CHN analyzer. Inorganic N was extracted by 1M KCl and then determined by the colorimetric method with a Lachat flow-injection analyzer (QuikChem 8000, Lachat instruments, Mequon, MI). Soil pH was measured in water with 1:2.5 soil to water ratio. Soil microbial biomass C and N were determined by the chloroform fumigation extraction method with extraction coefficients of 0.45 and 0.54 for biomass C and N, respectively (Brookes et al., 1985; Vance et al., 1987). Mineralization of soil organic C and N was determined via a

three-week incubation. Fifteen grams of moist soil were placed into a specimen container and placed in a 1-L Mason jar which contained 5 mL of distilled water to maintain relatively high humidity and thus minimize soil water loss during incubation. The CO₂ released during incubation was trapped in 5 mL of 0.5 M NaOH contained in a scintillation vial. After incubation, the remaining NaOH was titrated with 0.2 M HCl to determine the amount of CO₂ evolution, i.e., C mineralization. Soil inorganic N before and after incubation was extracted and measured as described above. The difference between inorganic N after and before incubation was calculated as N mineralization.

Dissolved organic matter preparation and chemical and biodegradability analyses

Dissolved organic matter (DOM) was extracted from a soil slurry based upon Zsolnay's method (2003). Briefly, soils were equilibrated with distilled water at a soil (g)-to-extractant (mL) ratio of 1:4 for 1 h followed by constant shaking at 200 rev min⁻¹ for 30 min. Soil slurries were then centrifuged at 2,000 × g for 10 min. Supernatants were filtered through 0.45-µm polycarbonate filter membranes and filtrates were used for chemical and biodegradability analyses of DOM.

Several analytical methods were used to determine the chemical composition of DOM. Concentrations of organic C in DOM was measured using a TOC analyzer (TOC-5000, Shimadzu Scientific Instruments, Japan). Dissolved organic N was calculated as the difference between total N and inorganic N in DOM. Total N in DOM was oxidized to inorganic N by persulfate (Cabrera and Beare, 1993) and then the inorganic N was determined as described above. Aromaticity of DOM was determined by UV absorbance at

280 nm (Chin et al., 1994; McKnight et al., 1997). Total soluble phenolic compounds were measured using Folin-Ciocalteu reagent and calibrated against a mixture of phenolic compounds (DeForest et al., 2005). The concentration of amino acid N was determined colorimetrically with ninhydrin reagent and a standard curve of L-leucine (Hofman and Dušek, 2003). The concentration of reducing sugar C was also measured colorimetrically with a standard curve of glucose (Hofman and Dušek, 2003). These DOM chemical properties have been shown to indicate ecological functions, such as the DOM biodegradability (Kalbitz et al., 2003).

Carbon and N mineralization of DOM were examined via an incubation experiment. DOM (150 mL), inoculated with 0.25 mL of microbial inoculum solution, was placed in a 1 L Mason jar and incubated for 33 d. The inoculum solution was obtained by water extraction of a composite of all soil samples (1:10 soil to water ratio) followed by shaking for 0.5 h and standing for 3 h. During the incubation, about 20 mL of DOM was sampled at 0, 5, 12, 26, and 33 days for the analysis of organic C and inorganic N as described above. To prevent anaerobic conditions, Mason jars were air flushed for 30 min after each time sampling.

Data from the incubation experiment was characterized with first-order kinetic models to determine potential pool size and rate constant for mineralization of dissolved organic C (DOC) and dissolved organic N (DON). Carbon mineralization data was fitted to $C_t = C_0 \cdot e^{-kt}$, where C_t is DOC at time t ; C_0 represents potentially degradable DOC; and k is the rate constant of DOC degradation. Similarly, N mineralization was fitted to $N_t = N_0 \cdot (1 - e^{-kt})$, where N_t is inorganic N at time t ; N_0 is potentially degradable DON; and k is the rate constant of DON degradation.

Soil enzyme activities

Several soil enzyme activities were determined using a 96-well microplate approach (Šnajdr et al., 2008). The enzymes selected are involved in depolymerization of soil organic C and N, including oxidative enzymes such as peroxidase (EC 1.11.1.7) and phenol oxidase (EC 1.10.3.2), and hydrolytic enzymes such as exoglucanase (EC 3.2.1.4), β -glucosidase (EC 3.2.1.21), and β -glucosaminidase (EC 3.2.1.30). First, soil was mixed with 0.1 M phosphate buffer (pH 7.0) at a weight (g) to volume (mL) ratio of 1:2.5. The soil slurry was then shaken at 200 rev min⁻¹ for 1 h and filtered through a Whatman No. 42 filter paper. In general, soil enzymes are poorly recovered in soil extracts, and thus extracts may only account for a small fraction of total soil enzyme activity (Vepsäläinen, 2001). However, measurements of enzyme activity in extracts have proved useful in examining impacts of environmental change or management on soil enzyme activities (Saiya-Cork et al., 2002; Waldrop et al., 2004). For the measurements of exoglucanase, β -glucosidase, and β -glucosaminidase activities, soil filtrates (100 μ l) were pipetted into microplate wells, which contained 200 μ l of corresponding substrate solutions (i.e., 2 mM p-nitrophenyl- β -D-cellobioside, 10 mM p-nitrophenyl- β -D-glucopyranoside, and 2 mM p-nitrophenyl N-acetyl- β -D-glucosaminide, respectively, made by a 50 mM sodium acetate buffer at pH 5.0). Our preliminary kinetic experiments showed that the final concentrations of substrates were sufficient to saturate soil enzymes in the samples. After 1 h incubation at 37 °C, 50 μ l of 1 M sodium carbonate was added to terminate the reaction and allow color development. Optical density was measured at 410 nm to detect released p-nitrophenol.

Potential activity of phenol oxidase was measured according to the method of Perucci et

al. (2000). Soil filtrates (100 μ l) were pipetted into microplate wells, which contained 50 μ l of 0.24 M catechol and 50 μ l of 0.24 M proline. Both catechol and proline reagents were made in 0.1 M phosphate buffer and adjusted to pH 5.6. After 10 min. incubation at room temperature, 150 μ l of ethanol was added and mixed well to stop the reaction. Optical density was measured at 525 nm.

Peroxidase activity was measured with 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate according to the method of Johnsen and Jacobsen (2008). Soil filtrates (100 μ l) were pipetted into microplate wells, which contained 100 μ l of TMB Easy solution (Fisher Scientific Inc.), and then incubated for 10 min. at room temperature. The enzymatic reaction was terminated by adding 100 μ l of 0.3 M sulfuric acid. Optical density was measured at 450 nm.

All enzyme assays were determined in quadruplicates against two types of controls (i.e., substrate alone and soil filtrate alone). Soil enzyme activity is expressed as μ mol of products produced per hour per g of soil.

Data analysis

Analysis of variance (ANOVA) for a completely randomized block design was performed to examine significant differences of soil chemical and microbiological properties among the five farming systems. The mean values were generally compared via Waller-Duncan k-ratio T test at $P < 0.05$. We also considered the mean values marginally significant at $P < 0.10$. Linear regressions were used to test relationships among soil C and N mineralization, soil enzyme activities, and chemical composition of DOM.

Results

Selected soil properties in the five farming systems

In general, soil properties determined in 2008 and 2009 were comparable and thus only results from 2009 were reported (Table 1). Soils collected from the five farming systems varied in chemical and microbiological properties. Soil C and N ranged from about 8 to 12 mg C g⁻¹ soil and from 0.7 to 1.1 mg N g⁻¹ soil, respectively; being lower in CON and PF and higher in ICL, ORG, and SUCC. There were significant differences in soil organic C-to-N ratio and soil inorganic N content among the five farming systems. For example, ICL had lowest organic C-to-N ratio and highest soil inorganic N. Soil pH was significantly different among the five farming systems, being 1-unit lower in SUCC and PF than the other systems due to lack of liming.

Soil microbial biomass also differed among the five farming systems. Compared with SUCC, farming systems CON, ICL, and PF had lower soil microbial biomass C. However, microbial biomass N was higher in ICL than SUCC, PF, and CON. Thus, microbial biomass C-to-N ratio was lower in ICL than the other farming systems except ORG. Soil C mineralization in ORG was comparable to the SUCC and CON, but significantly higher than the ICL and PF (Table 2). Soil N mineralization in SUCC was greater than the ICL, PF, and CON, but similar to the ORG.

Chemistry and biodegradability of DOM in the five farming systems

The DOC, DON, and selected chemical components of DOM varied significantly among the five farming systems (Fig 1). Soil DOC was highest in ICL and lowest in CON, whereas

DON varied by a smaller magnitude. Variations in selected components of DOM showed similar orders of magnitude as that of DOC, different by 3 – 5 folds between the lowest and highest values. Soil under ICL contained the highest amounts of reducing sugars, amino acids, and phenolic compounds, and also had the highest degree of aromaticity as shown by optical density at 280 nm.

Relative abundance of reducing sugars and amino acids also varied significantly among the five farming systems (Fig 2). Reducing sugar C was about 30% of DOC in CON, PF, and ICL, and 15% of DOC in SUCC and ORG. Amino acid N, as a percentage of DON, varied between 4% in SUCC and 10% in ICL. However, relative abundance of phenolic C was similar among the five farming systems and accounted for < 2% of DOC. More than 80% of DOC and DON were found to be mineralizable during the one-month incubation for all the soils collected from the five farming systems (Table 3). The degradation rate varied significantly among the five farming systems, being lower in ICL and higher in PF.

Soil enzyme activities in the five farming systems

Soil enzyme activities varied significantly among the five farming systems (Fig. 3); however, changing trends in enzyme activities among farming systems were dependent on individual enzymes. In 2009, ICL showed the lowest β -glucosidase activity among the five farming systems but highest exoglucanase activity. The β -glucosaminidase activity was found to be highest in SUCC, followed by PF and ICL and lowest in CON and ORG. Phenol oxidase activities were higher in PF and SUCC than the other systems. Both SUCC and ORG showed higher peroxidase activities than PF, ICL, and CON. Compared with the changing

trends of enzyme activities in 2009, 2008 data showed some differences. For example, β -glucosidase activity in ICL was lower than SUCC, but comparable to other systems. Exoglucanase activity in ICL was higher than PF, but it was comparable to CON and SUCC and lower than ORG. Despite higher in SUCC, phenol oxidase activity in PF and peroxidase activity in ORG were comparable to other systems.

Relationships between soil properties and soil enzyme activities

Because only 2009 soils were analyzed for DOM, the 2009 data were used to examine the relationships among soil properties, soil enzyme activity and C and N mineralization. Among the basic soil properties examined, only soil pH was significantly correlated with soil β -glucosaminidase and phenol oxidase activities (Table 4). Among DOM properties, we found that DOC and DON were not correlated with soil enzyme activities, but some components of DOM showed correlations. Reducing sugar C and optical density at 280 nm were negatively correlated with β -glucosidase activity and positively with exoglucanase activity. Amino acid N and β -glucosidase activity were also negatively related, but the relation between phenolic C and exoglucanase activity was positive. Among the five enzyme activities examined, only peroxidase activity was correlated with soil DOM chemical composition as well as soil C and N mineralization. Soil peroxidase activity was negatively correlated with reducing sugar C as a fraction of DOC, and positively with soil C and N mineralization. Furthermore, relative abundances of reducing sugars and amino acids were inversely related to soil C and N mineralization, respectively (Table 5).

Discussion

Soil enzyme activities as affected by management and type of agroecosystem

Similar to previous studies, this study found that long-term diverse farming practices resulted in divergent soil chemical and microbial properties. Soil organic C and microbial biomass C varied moderately among the five farming systems with less than 27% of coefficient of variation. By contrast, the five farming systems differed greatly in soil enzyme activities with up to 89% of coefficient of variation. Our results provided additional evidence that soil enzyme activities are very sensitive to management practices (Dick, 1994; Bandick and Dick, 1999; Acosta-Martínez et al., 2007; Sotomayor-Ramírez et al., 2009).

Differences in soil enzyme activities have been attributed to management-associated changes in soil chemical properties, such as soil organic matter content (Saviozzi et al., 2001; Gil-Sotres et al., 2005; Trasar-Cepeda et al., 2008). In our study, however, variations in soil enzyme activities could not be explained by changes in soil organic C or N. The negative correlation between soil pH and soil phenol oxidase activity in our study disagreed with a study showing that the two are often positively related (Sinsabaugh et al., 2008). Nevertheless, soil enzyme activities appeared to reflect the characteristic differences of individual farming systems. PF and SUCC were woody plant dominant ecosystems where plant litters might contain more lignin and/or lignin derivatives, the substrates of phenol oxidase and precursors of soil organic matter, than the cropping systems. Thus, the higher activity of soil phenol oxidase was expected to be found in PF and SUCC. While this was true in 2009, we found phenol oxidase activity in PF in 2008 was comparable to CON, ICL, and ORG. Perhaps soil sampling from only two blocks made 2008 data less powerful to

determine authentic differences among the farming systems. Furthermore, with little or no fertilization in PF and SUCC, soil phenol oxidase activity was expected to be greater in comparison to the other systems where this enzyme activity could be inhibited by annual intensive N fertilization (Fog, 1988). The activities of soil enzymes involved in N mineralization were also expected to be improved in the N-limited, woody plant dominant systems according to feedback inhibition on microbial production of extracellular enzymes (Dick et al., 1988). The activity of β -glucosaminidase, an important N-mineralization enzyme, was indeed found to be greater in PF and SUCC.

The β -glucosidase activity has often been reported to be very sensitive to management practices and, accordingly, its activity has been included within soil quality assessment equations (Lagomarsino et al., 2009; Paz-Ferreiro et al., 2009; Stott et al., 2010). Soil hydrolytic enzyme activities could be increased when organic and conservational management practices improved soil organic matter content and microbial biomass (Lagomarsino et al., 2009). Despite that ICL had a higher concentration of soil organic C and similar microbial biomass compared to the PF ecosystem, it showed lower soil β -glucosidase activity. On the contrary, soil exoglucanase activity was higher in ICL than the PF. It is very interesting that the activities of the two C-mineralization enzymes behaved differently in the two systems, but soil C and N mineralization were similar. Our results suggested that compounds other than polysaccharides were also important C sources for microbial activity.

Among the five enzymes evaluated, only soil peroxidase activity was associated significantly with soil C and N mineralization. Soil peroxidase plays an important role in the degradation of recalcitrant organic matter. Consequently, greater soil peroxidase activity

might accelerate the degradation of humic materials and thus lead to more CO₂ release from soil (Dec et al., 2003). Furthermore, humic materials might associate with a great portion of carbohydrate moieties and N compounds due to physiochemical interactions. Peroxidase oxidation of humic materials could make carbohydrate moieties and N compounds, such as cellulose and proteins, more accessible for soil hydrolytic enzymes and thereby leading to increases in C and N mineralization. Nadeau et al. (2007) found that the addition of both cellulase and peroxidase to a soil improved the hydrolysis of soil cellulose, due to an increase in its availability, as compared to only cellulase addition from 4-day incubation. Apparently, soil peroxidase was the proximate control for soil C and N mineralization in the agroecosystems that we examined. This observation seemed to agree with similar conclusions generated from unmanaged ecosystems that soil oxidative enzymes dictated soil organic C degradation and accumulation (Freeman et al., 2001; Waldrop et al., 2004; Sinsabaugh et al., 2008).

While activities of soil oxidative enzymes have been documented to be independent of soil C content, they are sensitive to other factors, such as soil pH and thus can be regulated by management practices (Iyyemperumal and Shi, 2008; Sinsabaugh et al., 2008; Floch et al., 2009; Yao et al., 2009). Floch et al. (2009) examined the activities of soil phenol oxidase as well as hydrolytic soil enzymes including β -glucosidase in conventional, organic, and integrated farming systems. They found that only phenol oxidase activity was not related to soil properties but pest and weed control management strategies (e.g., different types of fungicides). Their observation implied that management-associated chemical compounds such as herbicides, pesticides, and heavy metals might have pronounced impacts on oxidative

soil enzymes. Unlike CON, PF, and ICL, SUCC and ORG were not managed with chemicals and synthetic fertilizers. Perhaps no chemicals for weed and pest controls in SUCC and ORG were the major cause for different soil peroxidase activity from the other systems.

Dissolved organic matter linked to soil enzyme activity and organic mineralization

We hypothesized that the chemical composition of DOM could be correlated with the activities of soil enzymes involved in soil C and N mineralization. Specifically, we expected a negative relation between the concentration of soluble phenolics and hydrolytic enzyme activities that have been reported in several previous studies (Freeman et al., 2001, Yao et al., 2009). This supposition was partially supported by our observations in ICL where lowest β -glucosidase activity corresponded to the highest concentration of soluble phenolics. However, there were no negative correlations between soluble phenolics and activities of other hydrolytic enzymes, i.e., exoglucanase and β -glucosaminidase. In fact, exoglucanase activity was higher in ICL. Exoglucanase primarily catalyzes the release of oligosaccharides from the reducing ends of cellulose and thus contributes to the pool of reducing sugars. Although β -glucosidase catalyzes the production of glucose from oligosaccharides, the product could be rapidly consumed through microbial assimilation and metabolism, leading to a decline in reducing sugars. The high concentration of reducing sugars in ICL appeared to be the net result of high exoglucanase activity and low β -glucosidase activity. The β -glucosaminidase hydrolyzes chitooligosaccharides and results in the release of amino sugars, which could be further degraded to amino acids. High β -glucosaminidase activity could result in a low concentration of amino acids if amino acids were being immobilized. The concurrent

presence of the lowest concentration of amino acids and the highest activity of β -glucosaminidase in SUCC also supported our hypothesis that chemical composition of DOM had inherent relations with soil enzyme activities. However, these relationships did not occur in all the agroecosystems and appeared to be ecosystem specific. We did observe that variation in some components of DOM was well explained by the variation of soil peroxidase activity among the five farming systems. It is well known that soil peroxidase and phenol oxidase catalyze the oxidation of aromatic materials such as humic acids, but their activities did not correlate with soluble phenolic concentration or aromaticity as measured by UV absorbance. Instead, tight relations were found between soil peroxidase activity and the relative abundance of reducing sugars and amino acids.

Given the variations in their chemical composition, DOM in the five farming systems were expected to decompose differently. We found that the majorities of DOC and DON were biodegradable, but turnover rates varied among the five farming systems. The low turnover rate found in ICL could be caused by the inhibitory role of phenolics on β -glucosidase and microbial activity. As an easily accessible source of energy, C, and nutrients for soil microbes, DOM may contribute considerably to the overall soil C and N mineralization. Bengtson and Bengtsson (2007) reported that soil CO₂ respiration in forest ecosystems was primarily derived from DOM. However, they found that soil CO₂ respiration was not correlated to the concentration of DOC, but was tightly associated with its production rate. In a recent study, Jan et al. (2009) also documented that protein depolymerization but not amino acid mineralization primarily limited N cycling in grassland soils. These studies support that DOM production through enzyme-catalyzed depolymerization is a rate limiting step for soil

organic matter decomposition.

Rapid consumption of DOC and DON leads to the small pool size of DOM (Jones and Kielland, 2002; Roberts et al., 2007). Normally, DOC and DON are < 3% of soil organic C and N (Kalbitz et al, 2003; Rees and Parker, 2005). This relation agreed with our observation that DOC and DON were < 1% of soil organic C and N. However, DOC and DON in ICL were higher than the other systems, which was also true in other geographic locations. Ghani et al. (2007) surveyed 93 sites of New Zealand pastoral soils and found that DOC and DON were greater in pastoral soils than crop fields, native vegetation, and forestry soils.

Despite being a small pool, DOM can perhaps characterize soil C and N mineralization given that its turnover is limited by enzyme-catalyzed depolymerization. A significant correlation between the concentration of amino acids and N mineralization was observed in agricultural soils (Mengel et al., 2003). Similarly, relative abundance of amino acids in our study explained 94% of the variation in soil N mineralization among the five farming systems. Relative abundance of reducing sugars could also explain 64% of the variation in soil C mineralization but with less certainty than for amino acids and soil N mineralization. This is probably because amino acids were the dominant substrates for microbial N assimilation.

Implications

Our study is the first report regarding the relationships of DOM with soil enzyme activity and soil C and N mineralization. DOM has been widely explored for its ecological significance as a source of soil C and N loss from terrestrial lands to adjacent rivers and lakes

and as a binding agent for metals and other pollutants in soil (Stevenson, 1994; Zsolnay, 2003). However, DOM is rarely considered important for understanding soil C and N processes. There is increasing evidence that DOM is tightly related to soil organic C sequestration and nutrient cycling in wetlands, peatlands, and managed ecosystems (Freeman et al., 2001, Zibilske and Bradford, 2007, Yao et al., 2009). By examining interactions among soil enzymes, DOM, and soil C and N mineralization, our study helps to gain new insights into enzymatic controls on soil C and N dynamics in agroecosystems.

This study has extended current knowledge on relationships between DOM and soil enzymes. Negative correlations between soluble phenolics and hydrolytic enzymes such as β -glucosidase have been reported in various ecosystems and therefore an enzymatic latch mechanism has been proposed to interpret organic matter dynamics in response to environmental changes and management practices (Freeman et al., 2001; Zibilske and Bradford, 2007). This concept seemed to underlie organic matter degradation in ICL. Beta-glucosidase activity was found to be lowest in ICL where concentration of soluble phenolics and aromaticity of DOM were highest. In consequence, turnover rate of DOM was lower and so was degradation of soil organic matter. However, this relationship was not universal and it could not explain soil enzymes activities, soluble phenolic concentration, and soil C and N mineralization in the other agroecosystems. Alternatively, we found that relative abundance of reducing sugars and amino acids could reliably interpret soil C and N mineralization and these components of DOM were well correlated with soil peroxidase activity. Soil peroxidase activity appeared to have important effects on soil C and N mineralization and the chemistry of DOM in agroecosystems that we have examined.

Our results represented a snapshot in time for the diverse farming systems. Although quantity and quality of DOM may change seasonally, management or treatment effects would likely remain. Recent studies conducted in arable soils (Embacher et al., 2007, 2008) demonstrated that quality of DOM was tightly related to soil- and management-associated factors rather than seasonality. Nevertheless, the concept that dissolved organic matter chemistry is related to soil enzyme activity and soil processes needs further examinations in agroecosystems of other geographic locations and subjected to other management practices.

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Table 1. Selected soil chemical and microbiological properties of five different farming systems.

Systems [#]	Soil C	Soil N	Soil C:N	Inorganic N	Soil pH	MBC	MBN	MBC:MBN
	(mg C or N g ⁻¹ soil)			(µg g ⁻¹ soil)		(µg C or N g ⁻¹ soil)		
CON	8.6 bc	0.7 c	12.7 a	1.9 bc	6.6 b	274.3 b	21.9 c	11.2 ab
ICL	10.5 ab	1.1 a	9.6 c	3.6 a	6.7 b	258.3 b	35.2 a	6.8 c
ORG	10.5 ab	0.9 b	11.3 b	2.7 ab	6.9 a	300.8 ab	34.3 ab	8.7 bc
SUCC	11.5 a	1.0 ab	11.6 b	1.9 bc	5.6 c	385.8 a	30.1 b	12.9 a
PF	7.9 c	0.7 c	11.7 b	1.2 c	5.7 c	250.8 b	20.8 c	11.9 ab

[#] CON, Conventional cropping system subjected to best management practices; ICL, Integrated crop-livestock system; ORG,

Organic cropping system; SUCC, Successional system from abandoned agricultural field; PF, Plantation forestry system.

MBC and MBN represent microbial biomass C and N, respectively. Different letters within each column indicate significant differences of mean values for n = 3 (Waller-Duncan k-ratio T test, *P* < 0.05).

Table 2. Potential rates of soil C and N mineralization determined during a 21-d incubation of soils from five different farming systems.

Systems [#]	Carbon mineralization	Nitrogen mineralization
	(μg C or N g ⁻¹ soil d ⁻¹)	
CON	5.0 ab	0.33 c
ICL	4.7 bc	0.31 c
ORG	6.6 a	0.54 ab
SUCC	5.9 ab	0.60 a
PF	3.0 c	0.42 bc

[#] CON, Conventional cropping system subjected to best management practices; ICL, Integrated crop-livestock system; ORG, Organic cropping system; SUCC, Successional system from abandoned agricultural field; PF, Plantation forestry system. Different letters within each column indicate significant differences of mean values for n = 3 (Waller-Duncan k-ratio T test, $P < 0.05$).

Table 3. First-order model parameters derived from a 30-d laboratory incubation experiment for characterizing the biodegradability of dissolved organic carbon (DOC) and nitrogen (DON) from soils of five farming systems.

Systems [#]	Mineralizable C or N (%) [§]		Rate constant of mineralization (d ⁻¹)	
	DOC	DON	DOC	DON
CON	88.1 a	82.1 b	0.064 bc	0.051 ab
ICL	82.0 a	83.5 b	0.052 c	0.043 b
ORG	87.1 a	97.2 a	0.095 ab	0.053 ab
SUCC	87.5 a	85.0 ab	0.089 ab	0.053 ab
PF	95.7 a	91.8 ab	0.092 a	0.059 a

[#] CON, Conventional cropping system subjected to best management practices; ICL, Integrated crop-livestock system; ORG, Organic cropping system; SUCC, Successional system from abandoned agricultural field; PF, Plantation forestry system.

[§]Data were calculated by dividing potentially degradable DOC and DON derived from first-order models as described in materials and methods by total DOC and DON, respectively. Different letters within each column indicate significant differences of mean values for n = 3 (Waller-Duncan k-ratio T test, $P < 0.05$).

Table 4. Pearson's correlation coefficients of soil enzyme activities with soil and dissolved organic matter properties and soil C and N mineralization.

	β -glucosidase	Exoglucanase	β -glucosaminidase	Phenol oxidase	Peroxidase
<u>Basic soil properties[§]</u>					
Soil C	-0.023 [#]	0.54	-0.16	0.35	0.74
Soil N	-0.43	0.68	-0.18	0.24	0.51
pH	-0.62	0.71	-0.90**	-0.90**	0.1
MBC	0.71	-0.12	0.32	0.77	0.49
MBN	-0.70	0.82*	-0.40	-0.094	0.37
<u>DOM properties</u>					
DOC	-0.73	0.59	-0.12	0.055	0.35
DON	-0.56	0.68	-0.45	0.21	0.65
RS	-0.90**	0.81*	-0.41	-0.25	-0.17
AA	-0.83*	0.74	-0.56	-0.52	-0.55
Ph	-0.77	0.84*	-0.37	-0.079	0.068
OD	-0.88**	0.91**	-0.65	-0.47	-0.093
RS/DOC	-0.58	0.11	-0.11	-0.43	-0.92**
AA/DON	-0.74	0.56	-0.51	-0.61	-0.73
Ph/DOC	-0.003	-0.24	0.59	0.55	-0.67
<u>Soil C and N mineralization</u>					
C min	0.21	0.42	-0.48	-0.078	0.86*
N min	0.61	-0.38	0.43	0.62	0.85*

[#] Correlation significance is represented by *, **, *** at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively. [§] MBC: microbial biomass C; MBN: microbial biomass N; DOC: dissolved organic C; DON: dissolved organic N; RS: reducing sugars-C; AA: amino acids-N; Ph: phenolic compounds-C; RS/DOC: reducing sugar C as a fraction of dissolved organic C; AA/DON: amino acid N as a fraction of dissolved organic N; Ph/DOC: phenolic compound C as a fraction of dissolved organic N; C min: C mineralization; N: N mineralization.

Table 5. Pearson's correlation coefficients of soil C and N mineralization with relative abundance of reducing sugars-C, amino acids-N, and phenolic compounds-C.

	RS/DOC	AA/DON	Ph/DOC
C min [§]	-0.80* [#]	-0.45	-0.77
N min	-0.94**	-0.97***	0.50

[#] Correlation significance is represented by *, **, *** at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively. [§] C min: C mineralization; N min: N mineralization; RS/DOC: reducing sugar C as a fraction of soil C; AA/DON: amino acid N as a fraction of soil N; Ph/DOC: phenolic compound C as a fraction of soil C.

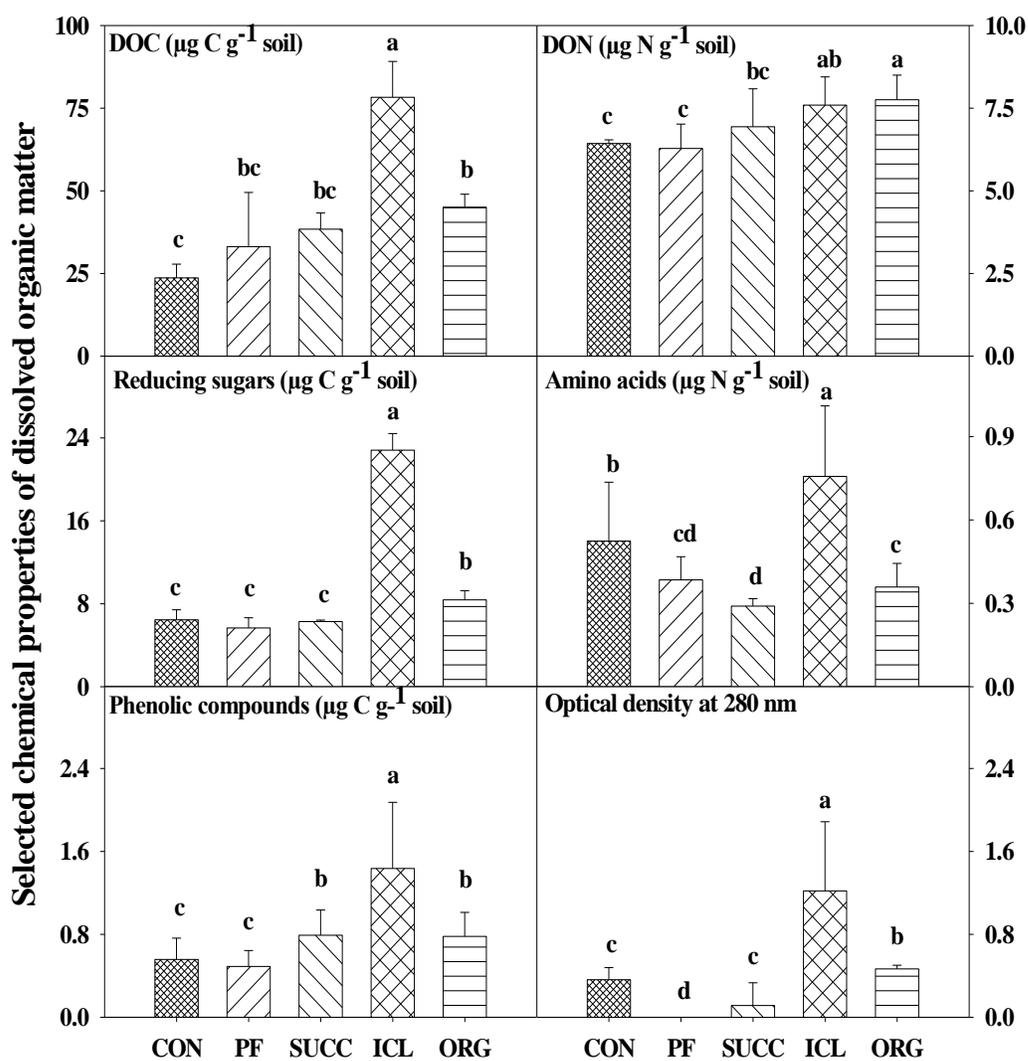


Figure 1. Total C and N concentrations and selected components of dissolved soil organic matter in five different farming systems. Different letters indicate significant differences of mean values for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. CON denotes conventional cropping system subjected to best management practices; ICL for integrated crop-livestock system; ORG for organic cropping system; SUCC for successional system from abandoned agricultural field; and PF for plantation forestry system.

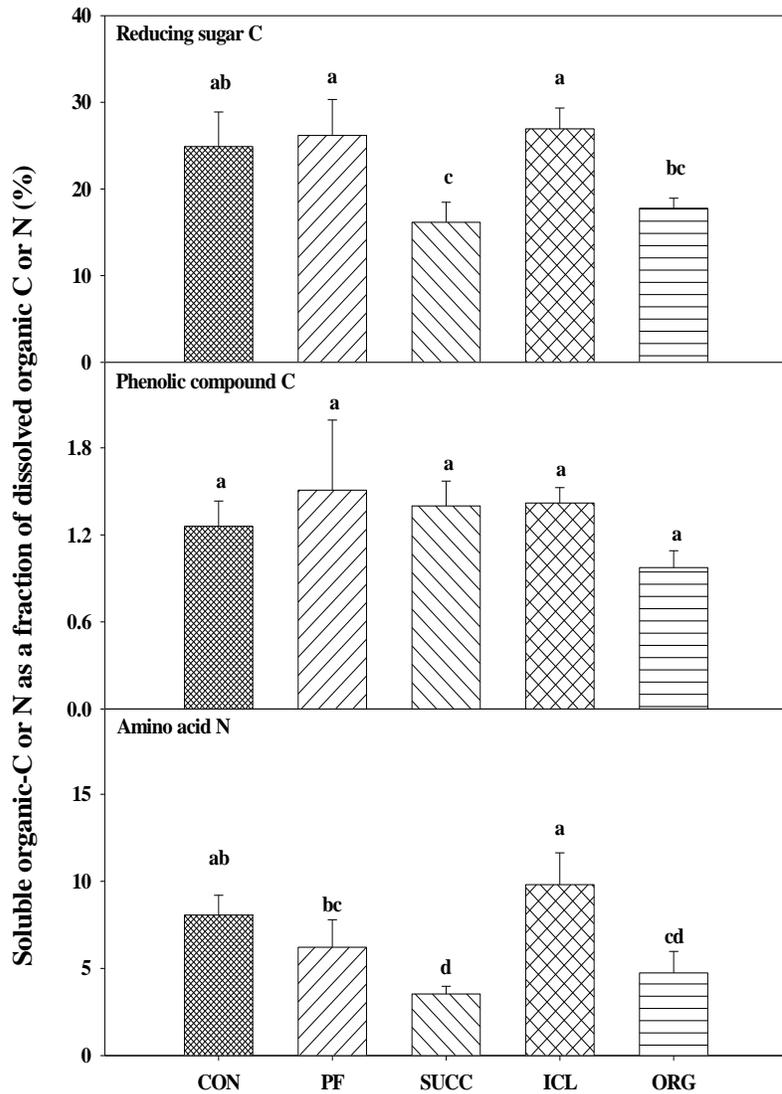
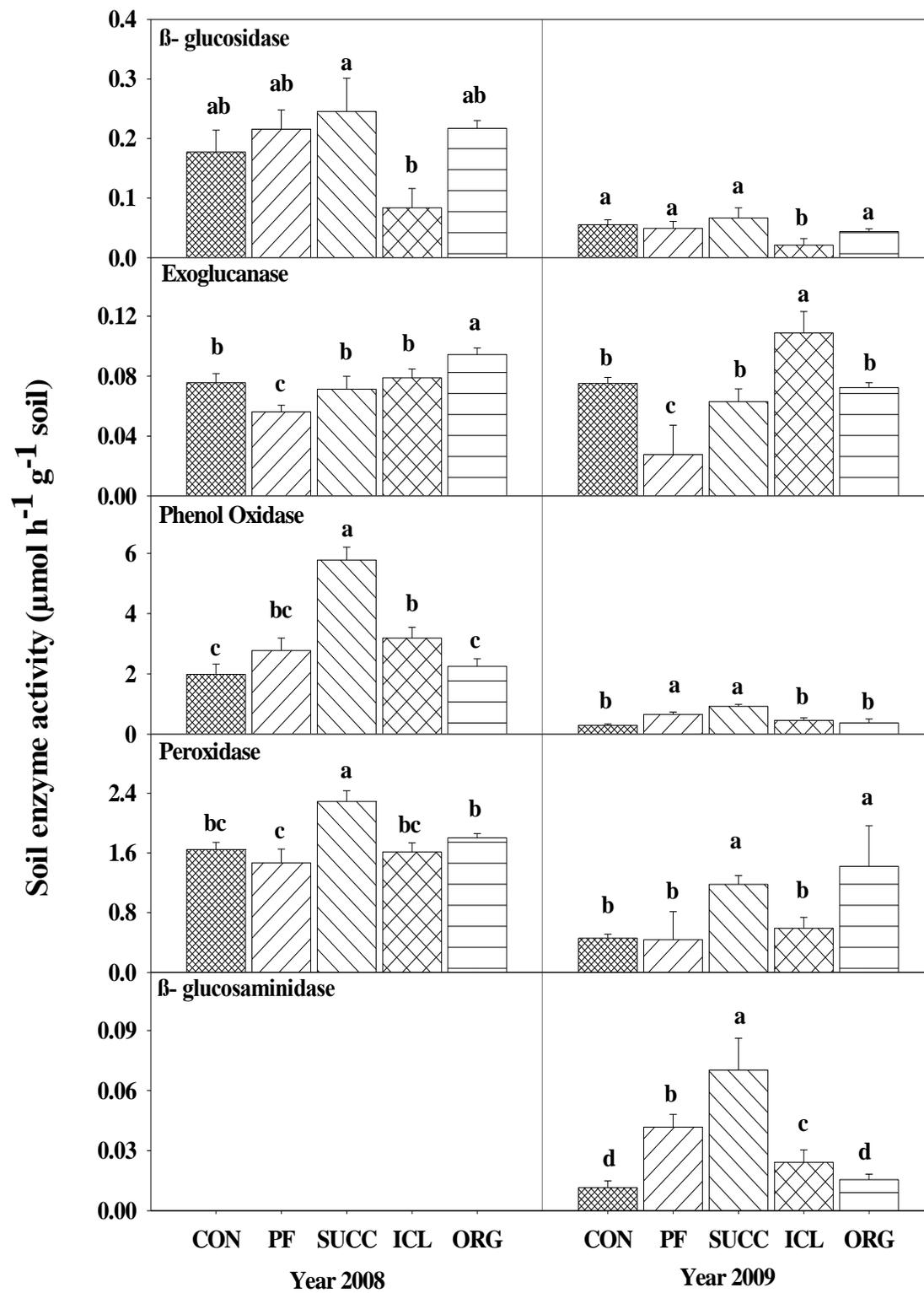


Figure 2. Relative abundance of reducing sugars, amino acids and phenolic compounds in five different farming systems. Different letters indicate significant differences of mean values for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. CON denotes conventional cropping system subjected to best management practices; ICL for integrated crop-livestock system; ORG for organic cropping system; SUCC for successional system from abandoned agricultural field; and PF for plantation forestry system.

Figure 3. Soil enzyme activities in soil samples collected in 2008 and 2009 from five different farming systems. Different letters indicate significant differences of mean values for $n = 3$ in 2009 and $n = 2$ in 2008 (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. CON denotes conventional cropping system subjected to best management practices; ICL for integrated crop-livestock system; ORG for organic cropping system; SUCC for successional system from abandoned agricultural field; and PF for plantation forestry system.



CHAPTER THREE

SOIL PEROXIDASE AFFECTS ORGANIC MATTER DECOMPOSITION THROUGH THE BIODEGRADABILITY OF REDUCING SUGARS AND AMINO ACIDS

ABSTRACT

Soil peroxidase activity can be an important parameter for predicting soil organic matter dynamics, but peroxidase control on organic matter degradation has not yet been fully understood. Using soil amendments of a horseradish peroxidase and/or hydrolytic enzymes, we tested the hypothesis that peroxidase could enhance soil C and N mineralization via improving the bioavailability of reducing sugars and amino acids. An arable soil from the top 10 cm depth was collected from an organic farm in Goldsboro, NC. First, this soil sample was amended with the horseradish peroxidase at 0, 0.1, and 0.2 activity units, and then examined for peroxidase and hydrolytic enzyme activities, water-extractable phenolic content, and potential rates of soil C and N mineralization over a 21-d incubation. Soil C mineralization was increased with the increased addition/activity of peroxidase, but it was not correlated to the concentration of water extractable phenolic compounds nor hydrolytic soil enzyme activity. Our results suggest that the positive effects of peroxidase on organic matter degradation were not through the production of soluble phenolic compounds or the enhancement of soil enzyme activity. Second, peroxidase, cellulase, protease or their combinations were added into an autoclaved soil for examining the enzyme-catalyzed production of reducing sugars and amino acids. Soil reducing sugar concentration was increased after the addition of peroxidase and the effects of peroxidase addition on the

bioavailability of reducing sugars and amino acids were more pronounced when the sterile soil was amended with both peroxidase and hydrolytic enzymes. The reducing sugars and amino acids thus produced were well correlated with the potential rates of soil C ($r = 0.65$, $P < 0.01$) and N ($r = 0.63$, $P < 0.01$) mineralization, respectively. Furthermore, the inhibitory effects of phenolic compounds of different complexity on soil hydrolytic enzyme activities were examined by the addition of monophenols, diphenols, or polyphenols. Polyphenols inhibited soil hydrolytic enzyme activities more strongly than diphenols, but monophenols had little effects on soil hydrolytic enzyme activities. Our study supports that soil peroxidase could improve the bioavailability of reducing sugars and amino acids that were otherwise inaccessible to soil hydrolytic enzymes and microbes and therefore stimulate organic matter decomposition.

Introduction

Peroxidase is an oxidative enzyme that has long been considered important in the degradation of lignin and humus and therefore can affect soil C sequestration. A meta-analysis has recently shown that soil peroxidase activity alone could account for 32% of the variation in soil organic matter content across various ecosystems (Sinsabaugh, 2010). Our previous work in agroecosystems also demonstrated that among five soil enzymes examined only peroxidase was correlated significantly with soil C and N mineralization (Tian et al., 2010). While a bulk of publications have provided substantial data to demonstrate that soil oxidative enzymes are the proximate controls of soil organic matter dynamics as reviewed by Sinsabaugh and his colleagues (2008), underlying mechanisms appear to be manifold.

A prevailing mechanism of soil organic matter accumulation in wetlands is thought to be that oxidative enzymes, such as phenol oxidase, control the activities of soil hydrolytic enzymes which in turn determine soil organic matter degradation (Freeman et al., 2001). When oxygen is limited at soil saturation, phenol oxidase activity decreased. This will cause the reduction in the oxidation of soluble phenolic compounds and therefore increase their concentration in soil solution. Due to the inhibitory effects of phenolic compounds on hydrolytic enzymes, soil organic matter degradation could be reduced, leading to the accumulation of organic matter in wetlands. Phenolic compounds of high molecular weight have been widely acknowledged as enzyme and microbial inhibitors (Freeman et al., 1990; Wetzel, 1992; Appel, 1993). In some habitats, phenolic compounds of low molecular weight, such as monomers, dimers, and trimers also have been found toxic to microbes (Fierer et al., 2001). These negative impacts of phenolics are thought to be more pronounced on bacteria than on fungi. For example, when phenolic compounds extracted from spruce or pine needles were added into bacterial or fungal growth media, bacterial activity was reduced greatly, whereas fungal activity was unchanged (Kanerva et al., 2006).

Another tactic by which oxidative enzymes control soil organic matter dynamics is through direct oxidation/depolymerization of lignin and recalcitrant substances containing phenolic moiety. This biochemical reaction can produce phenolic compounds of low molecular weight. These phenolic compounds may serve as growth substrates for soil microbes and can be further degraded via consecutive steps of a ring cleavage and subsequent conversion into aliphatic compounds for central microbial metabolism (Fenner et al., 2005). This mechanism seems to be dominant in plant litter degradation. For example, a

regression model incorporating lignocellulose-degrading enzymes, including phenol oxidase, could reliably estimate the mass loss rate of birch stick (Sinsabaugh et al., 1992). Others also reported that decomposition rates of plant litters in forests were associated with the activities of cellulase and phenol oxidase (Carreiro et al., 2000; Saiya-Cork et al., 2002; DeForest et al., 2004; Waldrop et al., 2004; Sinsabaugh et al., 2005). However, this mechanism may be less pronounced in agroecosystems than forests where abundant lignin-rich plant materials are constantly added to the grounds.

Oxidative enzymes may also regulate soil organic matter dynamics by improving microbial and enzyme accessibilities to carbohydrates and proteins, which are otherwise protected by phenolic moiety-containing substances. Although this concept was implicitly stated (Nadeau et al., 2007; Rezáčová et al., 2007), it has not yet been explicitly tested. In this study, we examined if peroxidase could enhance soil C and N mineralization by improving the bioavailability of reducing sugars and amino acids. By soil amendments of a horseradish peroxidase and hydrolytic enzymes involved in C and N mineralization, we could evaluate possible pathways by which peroxidase regulates soil organic matter decomposition and assess the relative importance of these pathways at a given soil condition.

Materials and methods

Soil sampling

Soil was sampled by coring techniques from an organic farming system, located in Goldsboro, NC where crops were rotated annually, including corn (*Zea mays* L), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), wheat (*Triticum*

aestivum), sweet potato (*Ipomoea batatas*), and sorghum (*Sorghum bicolor*). Turkey litters were applied annually, and the amounts depended on rotated crops. Besides gypsum for maintaining near neutral soil pH, no other inorganic chemicals were applied in the organic farming system. The soil was classified as Wickham sandy loam (fine-loamy, mixed, semiactive, thermic Typic Hapludult), having 10.5 mg C g⁻¹ soil, 0.9 mg N g⁻¹ soil, 301 µg microbial biomass C g⁻¹ soil, 34.3 µg microbial biomass N g⁻¹ soil, and soil pH 6.9. Detailed information on the organic farming system has been reported previously (Tian et al., 2010, see Chapter 2).

Twenty soil cores (2.5 cm dia. × 10 cm depth) were collected randomly from each of the three organic farming plots and pooled, leading to three composite soil samples. Soil samples were sieved (< 2 mm), adjusted for soil moisture content to 45% of water holding capacity, and then stored at 4 °C prior to a series of laboratory experiments.

Experimental research designs

Soil addition of horseradish peroxidase for its impacts on soil C and N mineralization

A stock solution of a horseradish peroxidase (Sigma P8250) at 3 unit mL⁻¹ was made by dissolving the lyophilized powder in sterile distilled H₂O. The stock solution was then added into soil samples, each containing ~ 13 g dry weight equivalent soil, to make three treatments with different peroxidase activities: 0 unit g⁻¹ soil as control, 0.1 units g⁻¹ soil as the low activity of peroxidase (i.e., Pox_L), and 0.2 units g⁻¹ soil as the high activity of peroxidase (i.e., Pox_H). In order to make the inputs of associated C and nutrients the same for all the treatments, the inactive horseradish peroxidase, which was made by boiling for up to 1 h

until no activity was detected, was also proportionally added to the soil, leading to a total of 1 mL addition of active and inactive peroxidase solutions. Our preliminary experiment showed that Pox_L and Pox_H treatments could immediately cause the increase of soil peroxidase activities by approximately 10 and 20 fold, respectively, as compared to the control. The incubation experiment was conducted for 21 d with three replications. Soil samples were collected and analyzed for soil enzyme activities and water extractable phenolic compounds at 6 h, 3 d, 10 d, and 21 d. Potential soil C mineralization was measured 3 d, 10 d, and 21 d after the peroxidase addition, and potential soil N mineralization was determined only at the end of the incubation. Another set of soil treatments were tested with three consecutive additions of the horseradish peroxidase every three days in a 9 d incubation, and soil C mineralization was measured three days after each new addition of the horseradish peroxidase.

Sterile soil addition of peroxidase, cellulase, and protease for elucidating enzyme effects on depolymerization of soil organic matter

Soil samples were sterilized by autoclaving three consecutive times to minimize indigenous soil microbial and enzyme activities. Subsequently, the sterile soil samples were amended with the horseradish peroxidase at 0.1 units g⁻¹ soil, cellulase (Sigma C9422) at 1 unit g⁻¹ soil, protease (Sigma P5147) at 0.1 units g⁻¹ soil, or their combinations for total seven treatments, including (1) no enzyme addition (Control), (2) peroxidase addition (Pox), (3) cellulase addition (Cel), (4) protease addition (Prt), (5) the addition of both cellulase and peroxidase (Cel+Pox), (6) the addition of both protease and peroxidase (Prt+Pox), and (7) the

addition of cellulase, protease, and peroxidase (Cel+Prt+Pox). Inactive enzymes, which were made by boiling for up to 1 h until no detectable activity, were also proportionally added into the sterile soil, making the total amount of inactive and active enzymes the same for all the treatments. For example, the control treatment contained the inactive peroxidase, cellulase, and protease, equivalent to the active dose of these enzymes in the treatment Cel+Prt+Pox. This experiment was set up as a completely randomized design with three replications. Soil samples, each containing 10 g of sterile soil and corresponding soil enzymes were incubated for 5 d and then analyzed for the activities of peroxidase, exoglucanase, β -glucosidase, and protease as well as the concentrations of water extractable reducing sugars, amino acids, and phenolic compounds periodically at 6 h, 3 d, and 5 d. After 5 d, 1 g of fresh soil was added into each enzyme-treated soil sample and then incubated for an additional 7 days to measure the potential rates of C and N mineralization.

Soil addition of phenolic compounds for their impacts on soil enzyme activities

Phenolic compounds of different molecular complexity were tested for their effects on soil enzyme activities. A monophenol group was represented by a mixture of equal moles of ferulic, p-coumaric, p-hydroxybenzoic, vanillic, and syringic acids, each being ~ 0.18 kDa; a diphenol group was composed of equal moles of catechol, resorcinol, and hydroquinone, each being ~ 0.11 kDa; and a polyphenol group was represented by the tannic acid of 1.7 kDa. A stock solution at $24 \mu\text{mol mL}^{-1}$ was made with 50 mM acetate buffer, pH 5.0 for each phenolic group.

To minimize soil sorption of phenolic compounds, their effects on soil enzyme activities

were tested in soil extracts, which were made by shaking soil with 50 mM acetate buffer, pH 5.0 in a weight-to-volume ratio 1:2.5 for 1 h and then passing through a # 42 Whatman filter. The experiment was set up in a completely randomized design with three replications for eight treatments, including (1) no addition of any phenolic compound (C), (2) the addition of the monophenolic group (M), (3) the addition of the diphenolic group (D), (4) the addition of the polyphenolic group (P), (5) the addition of equal moles of the monophenolic and diphenolic groups (M+D), (6) the addition of equal moles of the monophenolic and polyphenolic groups (M+P), (7) the addition of equal moles of the diphenolic and polyphenolic groups (D+P), and (8) the addition of equal moles of monophenolic, diphenolic, and polyphenolic groups (M+D+P). For each treatment besides the control, the total concentration of added phenolic compounds was $1.2 \mu\text{mol g}^{-1}$ soil. After the amendments with different phenolic compounds, soil extracts were incubated for 18 h in the dark at 25°C and then analyzed for enzyme activities and phenolic concentrations periodically at 0, 6, and 18 h.

Measurements of soil C and N mineralization

A base trap method was used to determine the potential rates of soil C mineralization for various treatments. Briefly, a soil sample contained in a specimen container and 5 mL 0.5 M NaOH in a scintillation vial were placed into a 1 L Mason jar for incubation. After the incubation, the remaining NaOH was titrated with 0.2 M HCl to determine the amount of CO_2 evolution, i.e., C mineralization. Meanwhile, soil inorganic N before and after incubation was extracted and measured colorimetrically using a Lachat flow-injection

analyzer (QuikChem 8000, Lachat instruments, Mequon, MI). The difference between inorganic N after and before the incubation was calculated as N mineralization.

Analyses of dissolved organic C, N and chemical compounds

Dissolved soil organic matter was water extracted based upon Zsolnay's method (2003) and then was analyzed for the concentrations of total C and N, phenolic compounds, amino acids, and reducing sugars according to the referenced methods as described in our previous work (Tian et al., 2010).

Determinations of soil enzyme activities

The activities of soil enzymes, including peroxidase (EC 1.11.1.7), exoglucanase (EC 3.2.1.4), β -glucosidase (EC 3.2.1.21), β -xylosidase (EC 3.2.1.37), β -glucosaminidase (EC 3.2.1.30), and protease (EC 3.4.24.31) were determined by the rates of product appearance according to the methods as described in our previous work (Tian et al., 2010).

Soil samples were mixed with 50 mM acetate buffer (pH 5.0) at a weight (g) to volume (mL) ratio of 1:2.5 and then shaken at 200 rev min⁻¹ for 1 h. For the measurements of exoglucanase, β -glucosidase, and β -glucosaminidase activities, soil slurry samples (0.8 mL) were pipetted into 2-mL Eppendorf tubes, which contained 0.2 mL of corresponding substrate solutions (i.e., 2 mM p-nitrophenyl- β -D-cellobioside, 10 mM p-nitrophenyl- β -D-glucopyranoside, 2 mM p-nitrophenyl- β -xylopyranoside, and 2 mM p-nitrophenyl N-acetyl- β -D-glucosaminide, respectively, made with 50 mM sodium acetate buffer at pH 5.0). After 1 h of incubation at 37 °C, except 2 h for exoglucanase, 0.2 mL of 0.5 M CaCl₂ and 0.8 mL of

0.1 M Tris buffer (pH 12.0) were added to stop the reaction and allow color development. After centrifugation at $11,000 \text{ rev min}^{-1}$ for 4 min., optical density of the suspension was measured at 410 nm to detect the released p-nitrophenol.

Peroxidase activity was measured with 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate according to the method of Johnsen and Jacobsen (2008). Soil slurry samples (0.2 mL) as described above were pipetted into Eppendorf tubes that contained 0.4 mL of TMB Easy solution (Fisher Scientific Inc.), and then incubated for 10 min. at room temperature. The enzymatic reaction was terminated by adding 0.8 mL of 0.3 M sulfuric acid. After centrifugation at $11,000 \text{ rev min}^{-1}$ for 4 min., optical density of the suspension was measured at 450 nm.

Protease activity was assayed using the method described by Ladd and Butler (1972). Briefly, 1 g of soil sample was weighed into a glass vial and then 2.5 mL of 0.2M Tris buffer (pH 8.0) and 2.5 mL of a 2% Na-caseinate solution were added. The capped vials were incubated in a water bath at $50 \text{ }^{\circ}\text{C}$ for 2 h. Then, the remaining casein was precipitated with 5 mL of 10% trichloroacetic acid. In a 2 mL Eppendorf tube, 0.5 mL of the solution was mixed with 1 mL of 1.4 M Na_2CO_3 and 0.2 mL of three-fold diluted Folin-Ciocalteu reagent. After 5 min. incubation, the tube was centrifuged at $13,000 \text{ rev min}^{-1}$ for 1 min. and the tyrosine concentration was measured colorimetrically at 680 nm.

All enzyme assays were determined in quadruplicates against two types of controls (i.e., substrate alone and soil slurry alone). Soil enzyme activity is expressed as μmol of products produced per h per g of soil.

Data analysis

Analysis of variance (ANOVA) was used to test the significant differences of all the measurements among soil treatments. The mean values were compared via Waller-Duncan k-ratio T test at $P < 0.05$. Linear regressions were also used to correlate soil C and N mineralization with peroxidase activity and the concentrations of reducing sugars and amino acids.

Results

Soil C and N mineralization as affected by the addition of horseradish peroxidase

Soil peroxidase activity was greatly enhanced by the addition of horseradish peroxidase and was positively related to the activity units of horseradish peroxidase addition (Fig. 1). However, this enhancement only lasted for less than 10 d (Fig. 1). We found that added horseradish peroxidase lost more than 60% of its activity at day 3 and almost all the activity at day 10. Thus, soil peroxidase activities measured 10 d later were comparable to the control regardless of peroxidase treatments.

Parallel with the change in soil peroxidase activity, soil C mineralization increased 3 d after the addition of horseradish peroxidase, whereas N mineralization decreased (Table 1). Carbon mineralization in soil with the addition of horseradish peroxidase at a high dose, 0.2 units g^{-1} , was 23% greater than that in the control. Soil C mineralization measured for the first 3 d was significantly and positively correlated with soil peroxidase activity ($r = 0.80$, $P < 0.01$). Thereafter, no treatment effect was found on soil C mineralization. Repeated soil additions of horseradish peroxidase continuously enhanced soil C mineralization. After the

amendment of peroxidase at 3 d or 6 d, soil C mineralization determined during the period of 3-6 d or 6-9 d were correlated to peroxidase activities determined at 3 d ($r = 0.89$, $P < 0.01$) or 6 d ($r = 0.70$, $P < 0.05$), respectively.

Effects of horseradish peroxidase addition on hydrolytic enzyme activities appeared to depend on the dose values (Fig. 2). The β -glucosidase activities measured at 6 h, 3 d, and 21d after peroxidase addition were greater in the Pox_L treatment than in Pox_H or control treatments, but no treatment effects were found at 10 d. Exoglucanase activity was also higher in Pox_L than in Pox_H when measurements were conducted at 6 h and 3 d after peroxidase addition. However, at 10 d and 21d, exoglucanase activity was similar between Pox_L and Pox_H. Compared with the control treatment, protease activity in Pox_L and Pox_H fluctuated with measurement times, being decreased at 3 d, increased at 10 d and similar at 6 h and 21 d.

Effect of horseradish peroxidase addition on water extractable phenolic concentration only lasted for 3 d, and afterwards no treatment effect was found (Fig. 3). However, this effect was inconsistent among the treatments; the highest phenolic concentration was found in the Pox_H treatment at 6 h, but in the Pox_L at 3 d.

Associations of soil C and N mineralization with reducing sugars and amino Acids

Five d after the addition of peroxidase and hydrolytic enzymes to sterile soil samples, cellulase and protease retained more than 70% of their activities, but peroxidase lost about 40% of its activity (data not shown). Cellulase addition (Cel) increased the amount of water extractable reducing sugars by ~ 110% as compared to the control treatment (Fig. 4). This

effect was enhanced by the addition of horseradish peroxidase; the concentration of reducing sugars in Cel+Pox treatment measured at 5 d was ~ 21% greater than that in Cel treatment and 156% greater than that in the control. Peroxidase addition (i.e., Pox treatment) also increased the amount of reducing sugars by ~ 23% at the end of the 5 d incubation than that of the control treatment. Similarly, Protease addition (i.e., Prt treatment) significantly increased the amount of water extractable amino acids as compared to the control treatment (Fig. 4). The concentrations of amino acids in Prt and Prt+Pox treatments were about 25% and 68%, respectively, greater than the control treatment at the end of 5 d incubation. However, Pox treatment did not affect the concentration of amino acids.

Soil C and N mineralization were determined via an additional 7-d incubation after enzyme-treated sterile soils were inoculated. Carbon mineralization in Cel and Cel+Pox was 20% and 43% greater than that in the control treatment, respectively (Table 2). Nitrogen mineralization in Prt and Prt+Pox was 44% and 133% greater than that in the control treatment, respectively. Carbon and N mineralization after soil inoculation were significantly correlated with the concentrations of water extractable reducing sugars ($r = 0.65$, $P < 0.01$) and amino acids ($r = 0.63$, $P < 0.01$) produced after 5 d enzyme treatments in the sterile soil, respectively. No correlation was found between extractable phenolic concentration (data not shown) and C mineralization.

Soil enzyme activities as affected by different types of phenolic compounds

Soil enzyme activities responded differently to the additions of phenolic compounds of different complexities (Fig. 5). β -glucosidase activity was suppressed after the addition of

simple as well as complex phenolic compounds. The addition of polyphenol (P) decreased this enzyme activity by 92%, followed by diphenol (D) by 45% and monophenol (M) by ~6%. The enzyme activity was also decreased after the combined addition of phenolic compounds with 74% reductions in M+P and M+D+P treatments, 55% in D+P, and 37% in M+D. A significant correlation was found between the polyphenol concentration and β -glucosidase activity ($r = -0.80$, $P < 0.01$).

Exoglucanase activity was inhibited by the di- and poly-phenolic compounds but not mono-phenolic compounds. Interestingly, we found that the suppressive effects of di- and poly-phenolic compounds on exoglucanase activity were alleviated by a combined addition with mono-phenolic compounds. Exoglucanase activity was even increased by the addition of M+P and D+P as compared to the control treatment.

β -xylosidase activity was also reduced by the addition of D, P and their combinations with M. The polyphenol suppressed the enzyme activity to a non-detectable level and diphenol decreased the enzyme activity by 80%. Again, the enzyme activity was reduced greatly in any combined addition having poly-phenolic compounds.

Mono-, di-, and poly-phenols all imposed negative effects on the activity of soil peroxidase. Phenolic treatments suppressed the enzyme activity by at least 70% and up to non-detectable levels.

Discussion

Soil addition of peroxidase stimulates organic matter degradation

Soil peroxidase is a required enzyme for the degradation of biochemically recalcitrant

and phenolic moiety-containing substances such as lignin and humus. Intuitively, soil peroxidase mediates the degradation of soil organic matter through its controls on the release of phenolic compounds. Then, simple phenolic compounds, such as mono-phenols, can be utilized and mineralized by soil microbes (Sparling et al., 1981; Blum and Shafer, 1988; Blum, 1998). However, phenolics of high molecular weight may still be recalcitrant and need further depolymerization prior to microbial use and complete degradation.

We postulated that peroxidase might also regulate the mineralization of soil organic matter through its controls on the production of bioavailable compounds, such as carbohydrates and proteins, other than simple phenolics. It has been generally believed that soil organic matter mainly contains high molecular weight and dispersive polymers, i.e., phenolic moiety-based structures associated covalently with carbohydrates and proteins (Stevenson, 1982). However, soil organic matter has recently been considered as supramolecular substances, i.e., molecular aggregates of various compounds via non-covalent bonds (Wershaw, 1999; Piccolo, 2001). Phenolic compounds are thought to have important roles in the formation of supramolecular substances because of their amphiphilic characters for interactions with carbohydrates and peptides (Haslam, 1998). Nevertheless, both humic-polymer and supramolecular models support that soil organic matter represents some interactions of phenolic moieties with carbohydrates and proteins. Thus, when soil peroxidase oxidizes phenolic moieties or humic acids (Mangler and Tate, 1982; Dari et al., 1995; Gramss et al., 1999; Rezáčová et al., 2007), carbohydrates and proteins may be released and become available for hydrolytic enzymes and soil microbes. This role of peroxidase was documented in a desert soil where the combined addition of peroxidase and

cellulase increased the concentration of soil reducing sugars more than the addition of cellulase alone (Nadeau et al., 2007). Their observation provided the evidence that by oxidizing phenolic compounds, peroxidase helped remove the protection for bioavailable compounds and thus make them accessible to hydrolytic enzymes and soil microbes.

Given the protein nature, soil enzymes can also interact with humic acids or phenolic moieties of soil organic matter, making them more thermally stable and resistant to proteolysis in the soil system (Burns, 1982; Gianfreda and Bollag, 1996; Allison, 2006). However, this interaction may reduce soil enzyme activity and/or the affinity of soil enzymes for specific substrates (Ruggiero and Radogna, 1988; Gianfreda and Bollag, 1996; Allison, 2006; Ceccanti et al., 2008). As evidenced in the present study, peroxidase activity decreased by over 60%, cellulase activity by ~20% and protease activity by ~30% (data not shown) a few days after soil addition. By oxidizing phenolic compounds, peroxidase may free humic-bound hydrolytic enzymes. Consequently, soil hydrolytic enzyme activity can be enhanced and mineralization of soil organic matter would be increased.

By improving soluble phenolic concentration, bioavailable reducing sugars and amino acids, and/or hydrolytic enzyme activity, horseradish peroxidase could enhance organic matter degradation. When peroxidase activity in soil was increased after the addition of horseradish peroxidase at different activity units, soil C mineralization was increased proportionally and its correlation with soil peroxidase activity was significant. Furthermore, repeated soil addition of horseradish peroxidase continuously enhanced soil C mineralization. These results support that an increase in peroxidase activity could increase soil C mineralization.

The possible pathway underlying peroxidase controls on soil C and N mineralization

If peroxidase stimulated soil C and N mineralization mainly through the release of simple phenolic compounds, we expected to see an increase in the soluble phenolic concentration as well as the positive correlations among soil peroxidase activity, soluble phenolic concentration, and soil C mineralization after the addition of horseradish peroxidase. However, soluble phenolic concentrations were comparable between the control and the treatment of peroxidase addition at the low dose and soil C mineralization rates between the two treatments were significantly different. Thus, lack of the correlation of soluble phenolic concentration with soil peroxidase activity and soil C mineralization excludes the pathway that the peroxidase regulated soil C mineralization through the release of simple phenolic compounds. Furthermore, phenolic compounds produced by the addition of horseradish peroxidase at the high dose appeared to inhibit soil hydrolytic enzyme activities and thus might reduce the degradation of soil organic matter. It is well known that complex phenolic compounds can be detrimental to microbial growth due possibly to their roles of inhibiting enzyme activity and stripping organic substrates and trace elements needed for microbial metabolisms (Fierer et al., 2001; Joannis et al., 2007; Rosas et al., 2008). We examined the inhibitory effects of phenolic compounds of different complexities on soil enzyme activities and found that the polyphenol most significantly inhibited soil enzyme activities, followed by diphenols, and then monophenols. Apparently, the negative effects of phenolic compounds on soil enzymes depended on their complexity and concentration. When soil was amended with horseradish peroxidase, a mixture of mono-, di-, and poly-phenolic compounds were

likely produced. For the treatment of peroxidase addition at the high dose, however, the concentration of phenolic compounds was high enough to inhibit soil enzyme activities. Thus, our results disputed the argument that peroxidase stimulated soil C mineralization by releasing humic-bound enzymes and therefore increased soil enzyme activities.

As the substrates for microbial metabolisms, simple organic compounds such as sugars and amino acids can be present in soil solution as well as in association with clay minerals and humic substances (Mayer et al., 1995; Knicker and Hatcher, 1997; Krull et al., 2001). For example, Hayes and Malcolm (2001) reported that easily-degradable compounds and humic materials were tightly associated and could not be easily separated out. These associations can protect the easily-degradable compounds from biodegradation (Lichtfouse et al., 1998; Zang et al., 2000; Fan et al., 2004). When oxidative enzymes such as peroxidase degraded humic substances, the associations between easily-degradable compounds and humic substances can be disrupted, making otherwise protected compounds available for soil enzymes and soil microbes. We did find that the concentrations of soil reducing sugars and amino acids were increased by the addition of horseradish peroxidase in sterile soils, in particular by the addition of horseradish peroxidase in combination with cellulase, protease, or both. These reducing sugars and amino acids could be rapidly mineralized as shown by significant correlations with soil C and N mineralization, respectively, after enzyme-treated sterile soils were inoculated with microbes. Although soil addition of peroxidase at the high dose decreased soil hydrolytic enzyme activities due to the production of phenolic compounds, such negative effects on soil C and N mineralization might be offset by the simultaneous production of abundant reducing sugars or amino acids. Thus, soil C

mineralization was still greater in the treatment of peroxidase addition at the high dose when soil enzyme activities were considerably inhibited than the treatment at the low dose. Our results appear to support that peroxidase regulated organic matter degradation through controls on releasing carbohydrates and/or proteins that were otherwise bound to humic substances.

We observed that soil addition of peroxidase stimulated soil C mineralization but not soil N mineralization, suggesting that peroxidase addition helped liberate more bioavailable C compounds than N compounds. When microbes assimilated organic C for biosynthesis, they also incorporated into the biomass of available N, which likely exceeded the supply of peroxidase-liberated bioavailable N. As a result, soil inorganic N was additionally assimilated into microbial biomass, causing net soil N immobilization as seen in our experiment with soil addition of peroxidase at different doses and other studies (Burger and Jackson, 2002; Jones et al., 2004). It was not surprising that net N immobilization also occurred for the treatment of sterile soil addition with cellulase. However, net N immobilization did not occur after peroxidase addition to the sterile soil due perhaps to changes in the chemical compositions of dissolved organic matter caused by soil autoclaving (Wolf and Skipper, 1994; Berns et al., 2008). After autoclaving, the content of dissolved organic C ($200\text{-}350 \mu\text{g C g}^{-1}$) increased by > 500% and dissolved organic N ($55\text{-}63 \mu\text{g N g}^{-1}$) by > 700%. Because the concentration of bioavailable N was greater than that of bioavailable C, when microbes metabolized organic C, net N immobilization did not take place.

Practical considerations

Agricultural land is intensively managed and often cropped with annual herbaceous plants rather than perennial woody plants. As a result, it may not be a favorable niche for phenol oxidase-producing microbes, such as white-rot fungi. Perhaps, soil phenol oxidase in agricultural land is not as important to organic matter degradation or soil C sequestration as in unmanaged ecosystems, e.g., forests. Compared to phenol oxidase, however, peroxidase can be produced by a broad array of microbial genera and species (Bergbaue and Newell, 1992; Howard et al., 2003; Sánchez, 2009) and thus likely serves as the primary oxidative enzyme for the degradation of lignin and humus. The significance of soil peroxidase in agroecosystems has been reported in our previous study (Tian et al., 2010).

This study improves our understandings on peroxidase-mediated soil organic matter degradation. When peroxidase oxidizes the phenolic moieties of humus and lignin, thereby producing simple phenolic compounds for microbial assimilation, it can simultaneously produce bioavailable compounds such as sugars and proteins that may be otherwise bound to soil humus. Our results support that peroxidase mediated soil C mineralization through controls on the availability of reducing sugars and amino acids. Peroxidase can also produce phenolic compounds that are able to inhibit the activities of soil hydrolytic enzymes and thus perhaps exert negative effects on soil organic matter degradation. The negative effects are expected to dependent on the type and concentration of phenolic compounds, which can be regulated by soil peroxidase activity. So far, it is unclear how to regulate the positive and negative effects of peroxidase on soil organic matter degradation. We have little information on the impacts of agricultural management practices such as fertilization and pest control

programs on the production and activity of soil peroxidase.

Apparently, it is difficult to maintain soil enzyme activities when extracellular enzymes are produced and added into soils. Other studies have shown that the turnover rates of soil enzymes are rapid and therefore enzyme activities may be unstable (Allison, 2006; Sinsabaugh, 2008). If this is the case for soil peroxidase produced by microbes in agricultural soils, more information on spatial and temporal variations of soil peroxidase activity is needed.

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Table 1. The potential rates of soil C and N mineralization, which were determined periodically during a 21-d incubation for soils amended with different amounts of horseradish peroxidase.

	Carbon mineralization			Nitrogen mineralization
	(µg C or N g ⁻¹ soil d ⁻¹)			
	0-3 days	3-10 days	10-21 days	0-21 days
Control [#]	11.0c	3.5a	2.3a	0.13a
Pox_L	13.1b	3.6a	2.5a	0.11b
Pox_H	13.9a	3.6a	2.3a	0.11b

[#]Control: soil with the addition of deactivated peroxidase at 0.2 units g⁻¹ soil; Pox_L: soil with the addition of a low amount of peroxidase at 0.1 units g⁻¹ soil; Pox_H: soil with the addition of a high amount of peroxidase at 0.2 units g⁻¹ soil.

Different letters within each column indicate significant differences of mean values between treatments for n = 3 (Waller-Duncan k-ratio T test, *P* < 0.05).

Table 2. The potential rates of soil C and N mineralization determined in a 7-d incubation by inoculating sterile soils after they were amended with cellulase (Cel), protease (Prt), horseradish peroxidase (Pox) and their combinations for five days.

	Carbon mineralization	Nitrogen mineralization
	(μg C or N g ⁻¹ soil d ⁻¹)	
Control [#]	13.4c	0.09c
Cel	16.1b	0.04d
Prt	13.7c	0.13b
Pox	13.7c	0.12bc
Cel+Pox	19.1a	0.12bc
Prt+Pox	16.6ab	0.21a
Cel+Prt+Pox	15.9b	0.15b

[#]Control: soil with the addition of deactivated cellulase, protease and horseradish peroxidase at 1, 0.1, and 0.1 units g⁻¹ soil, respectively; Cel: cellulase addition at 1 unit g⁻¹ soil; Prt: protease addition at 0.1 units g⁻¹ soil; Pox: horseradish peroxidase addition at 0.1 units g⁻¹ soil; Cel+Pox: both cellulase and horseradish peroxidase addition; Prt+Pox: both protease and horseradish peroxidase addition; Cel+Prt+Pox: soil with the addition of cellulase, protease and horseradish peroxidase. The deactivated enzymes were also disproportionally added into soil, making sum of the amount of inactive and active enzymes same for all the treatments. Different letters within each column indicate significant differences of mean values between treatments for n = 3 (Waller-Duncan k-ratio T test, *P* < 0.05).

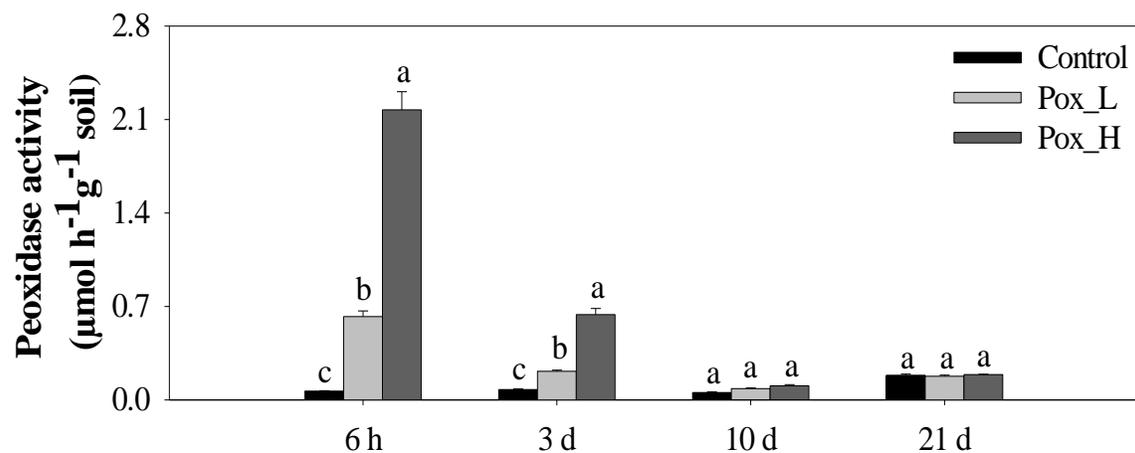


Figure 1. Soil peroxidase activity during a 21-d incubation after peroxidase addition at different activity units. Different letters indicate significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. Control represents soil with the addition of deactivated peroxidase at 0.2 units g^{-1} soil; Pox_L for soil with the addition of horseradish peroxidase at 0.1 units g^{-1} soil; and Pox_H for soil with the addition of horseradish peroxidase at 0.2 units g^{-1} soil.

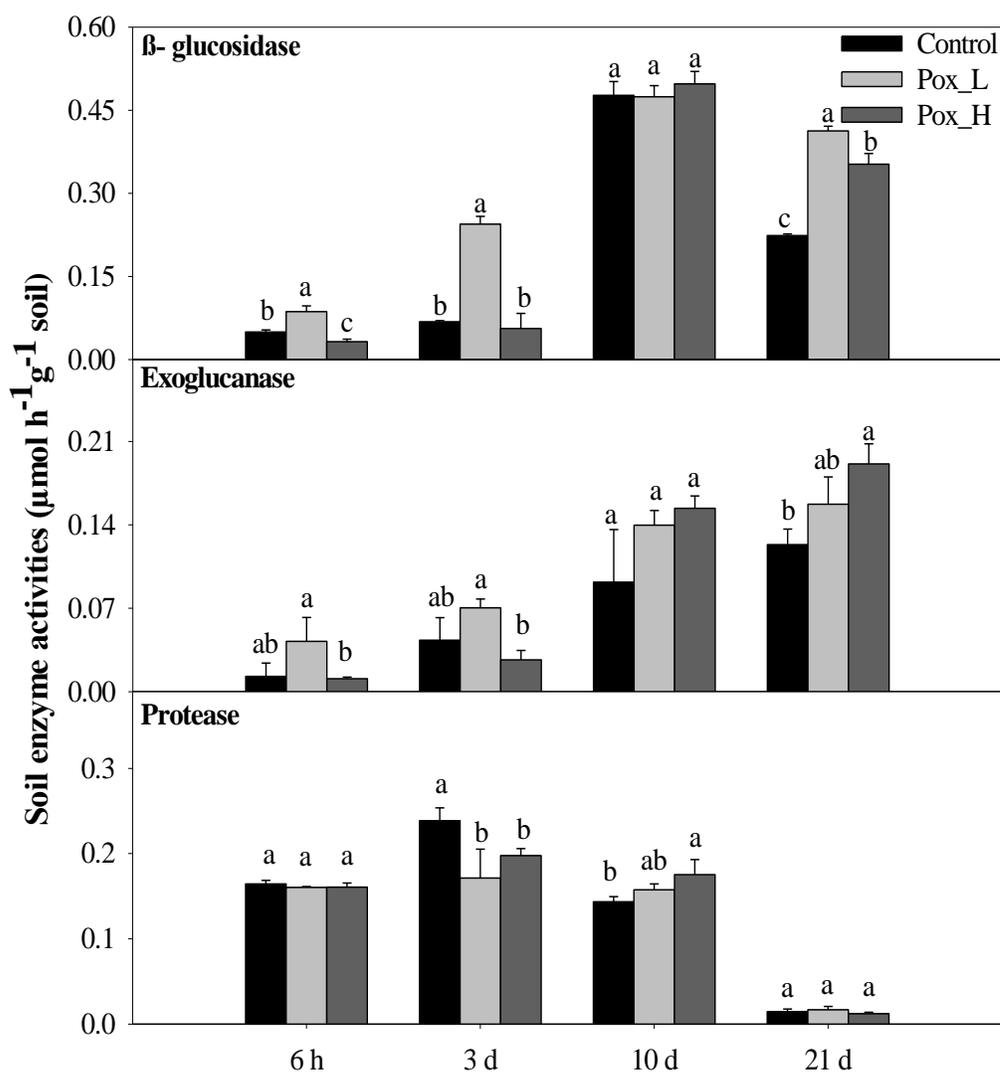


Figure 2. Hydrolytic enzyme activities in soils treated with different activity units of horseradish peroxidase. Different letters indicate significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. See Figure 1 for labels for treatments.

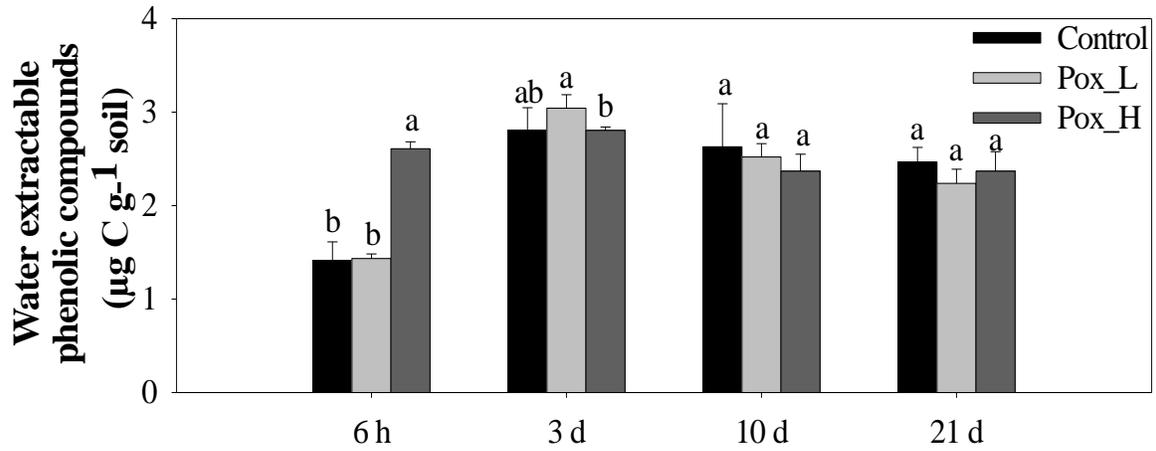


Figure 3. The concentrations of water extractable phenolic compounds in soils treated with different activity units of horseradish peroxidase. Different letters indicate significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. See Figure 1 for labels for treatments.

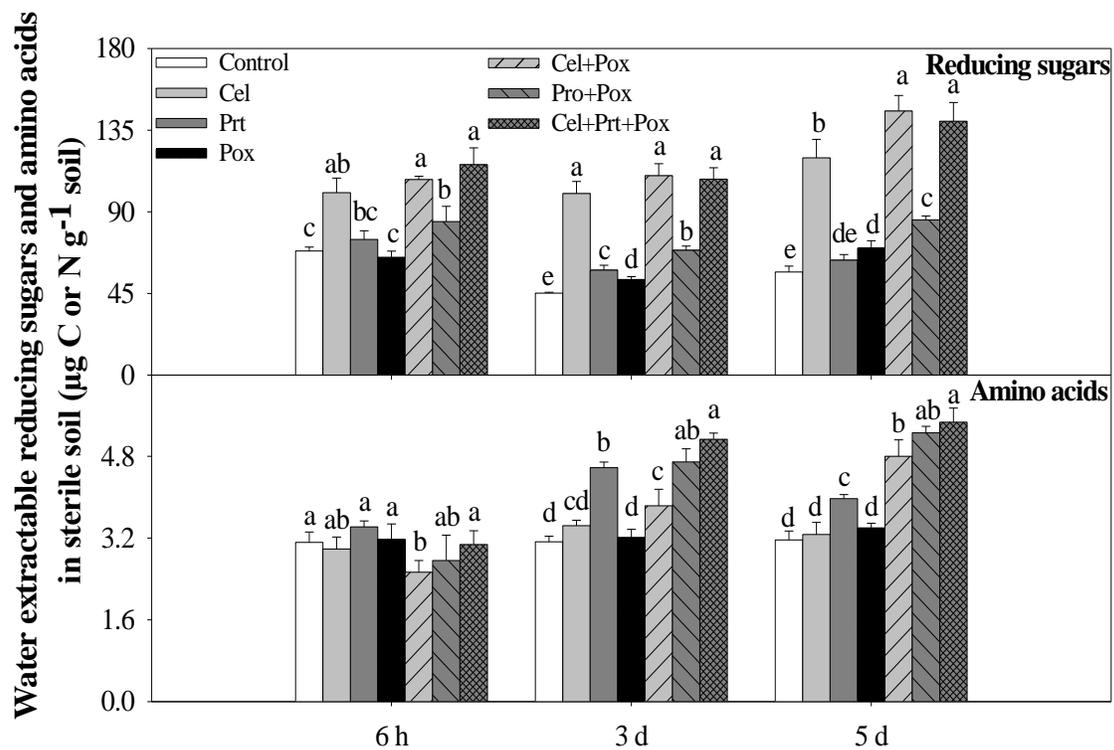
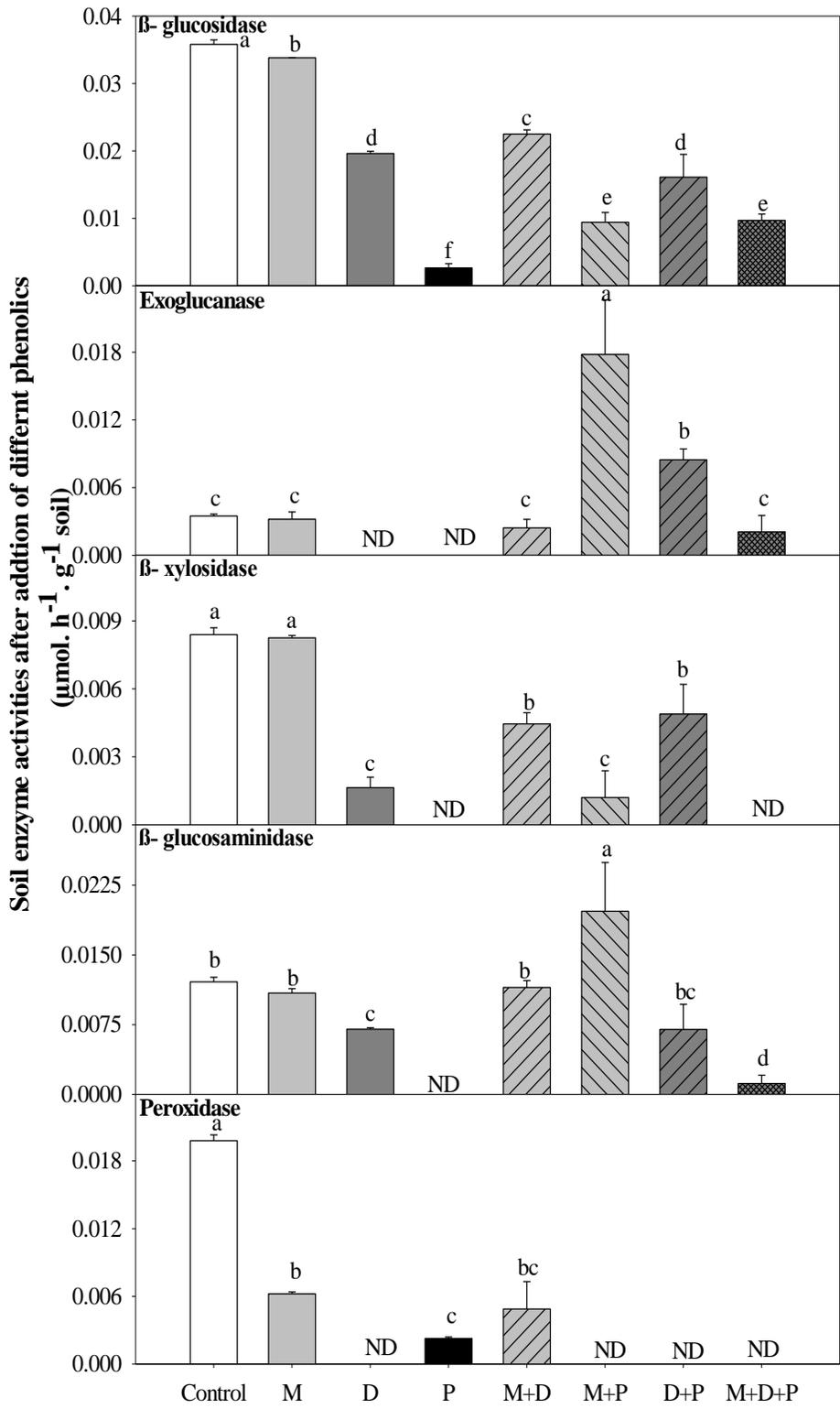


Figure 4. The concentrations of water extractable reducing sugars and amino acids in sterile soils amended with cellulase (Cel), Protease (Prt), Horseradish peroxidase (Pox), and their combination. Different letters indicate significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. See Table 2 for labels for treatments.

Figure 5. Effects of different types of phenolic compounds on soil enzyme activities in a 18-h incubation. Different letters indicate significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$). Bars represent standard errors. Control: no amendment; M: addition of monophenols only; D: addition of diphenols only; P: addition of polyphenol only; M+D: addition of both monophenols and diphenols; M+P: addition of both monophenols and polyphenol; D+P: addition of both diphenols and polyphenol; M+D+P: addition of monophenols, diphenols, polyphenol addition. The total concentration of phenolic compounds was $1.2 \mu\text{mol g}^{-1}\text{soil}$ in each treatment except control. ND: non-detected.



CHAPTER FOUR

MICROBIAL PRODUCTION OF EXTRACELLULAR ENZYMES AS AFFECTED BY PLANT LITTER QUALITY

ABSTRACT

Plant litters have been considered as an important factor regulating microbial production of extracellular enzymes. However, the degree of litter impacts appears to depend on particular enzymes as well as litter type. This study aimed to examine both hydrolytic and oxidative enzyme activities in response to soil additions of plant litters with different C:N ratios. In addition, the stability of soil enzymes was evaluated against soil acidity and alkalinity. Two soil samples with the same texture were collected from conventional and organic farms, respectively. They were amended with long-leaf pine (*Pinus palustris*) needles, soybean (*Glycine max*) residues, or a mixture of switchgrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), indiangrass (*Sorghastrum nutans*), and big bluestem (*Andropogon gerardii*) at 2 mg C g⁻¹ soil. The C:N ratios were 139, 50, and 9 for pine needles, grass materials, and soybean residues, respectively. We observed that the activities of soil enzymes for catalyzing labile C, such as β -glucosidase were enhanced quickly, peaked a few weeks after litter additions, and then declined back to their original levels. This dynamic pattern was independent of litter quality and probably induced by substrate availability. The activity of β -glucosaminidase, which helps microbes obtain labile N as well as C compounds, increased rapidly after litter additions and then remained relatively high throughout the incubation period. The magnitude of increase in this enzyme activity was greater than that of β -glucosidase. Although the dynamic pattern of this enzyme activity was

similar for the additions of different litters, the underlying mechanisms were likely different. Microbial production of this enzyme could be stimulated by substrate availability for soil addition of soybean residues and microbial N demand for soil addition of pine needles. Microbial production of polymer-degrading enzymes such as exoglucanase and phenol oxidase showed different patterns among plant litters. Only soybean residues and grass materials stimulated microbial exoglucanase production a few weeks after litter additions. While pine needles affected little on microbial exoglucanase production during the time scale of our study, they greatly stimulated microbial phenol oxidase production, in particular at the late period of the incubation. Among three litters, grass materials generally had greatest impacts on microbial production of exoglucanase and β -glucosidase, whereas soybean residues exerted more influences on β -glucosaminidase, and pine needles on phenol oxidase. These differential effects of litters on soil enzyme activities could be explained by microbial C and N demands associated with the relative abundance of substrates and nutrients contained in plant litters. However, these impacts appeared to be temporal because extracellular enzymes could lose their activities due to physical and chemical interactions with soil minerals as well as organic matter. However, results from soil samples with amendment of different enzymes showed that these enzymes may be more stable and active at high soil pH.

Introduction

Microbes produce extracellular enzymes to acquire C and nutrients from the environment for growth and metabolism. These extracellular enzymes in soil, referred as soil

enzymes, play important roles in soil organic matter decomposition and nutrient transformations. It is considered that microbes might abide by an economic principle, i.e., achieving maximal C and nutrient returns from minimal energy cost, to produce extracellular enzymes (Allison et al., 2011). So far, several mechanisms had been documented to explain microbial production of extracellular enzymes under various environmental conditions, including substrate induction, end-product inhibition, resource allocation, and nitrogen mining (Chróst, 1991; Sinsabaugh and Moorhead, 1994; Moorhead and Sinsabaugh, 2006; Craine et al., 2007). The concept of substrate induction states that microbes will produce extracellular enzymes to break down complex substrates when they are abundant in the environment (Chróst, 1991). By contrast, the end-product theory suggests that microbes will cease the production of extracellular enzymes when enzyme-catalyzed reaction products are sufficient in the environment. Furthermore, if one nutrient becomes unlimited, microbes may reallocate resource from the production of this nutrient-acquiring extracellular enzyme to enzymes for facilitating the acquisition of other nutrients (Sinsabaugh and Moorhead, 1994). Recently, available soil N has been considered as an important factor regulating microbial production of oxidative enzymes such as phenol oxidase. It is presumed that low N availability could stimulate phenol oxidase activity, which in turn degrades recalcitrant organic matter, thereby helping the release of occluded N compounds (Carreiro et al., 2000; Waldrop et al., 2004).

Plant litters represent a primary control for microbial production of extracellular enzymes (Tscherko, 2004; Dornbush, 2007). Because plant litters provide a suite of complex substrates for soil microbes, it is not surprising that soil enzyme activities increase after soil

input of plant litters. Dornbush (2007) reported that plant litter input caused 2- to 15-fold increases in the activities of β -glucosidase, β -glucosaminidase, and acid phosphatase. A number of laboratory and field observations also demonstrated that the activities of soil enzymes, such as cellulase could be enhanced when organic materials were amended into soils (Deng and Tabatabai, 1996a, b; Bandick and Dick, 1999; Deboisz et al., 1999, Yao et al., 2009).

It begins to be realized that plant litter type or chemistry can greatly affect the relative abundance and activities of soil enzymes (Luxhøj et al., 2002; Allison and Vitousek, 2004; Hu et al., 2006; Hernández and Hobbie, 2010). For example, plant litters of low C:N ratio were found to stimulate the activities of hydrolytic enzymes, such as urease and invertase, whereas litter of high C:N ratio enhanced the activities of oxidative enzymes such as phenol oxidase (Hu et al., 2006). Plant litter chemistry, such as the percentage of N content, can also be used to predict microbial enzyme production in response to litter addition and soil N availability (Henriksen and Breland, 1999; Dornbush, 2007). When litter N was below 1.2% of dry mass, microbial production of hydrolytic enzymes for degrading cellulolytic compounds appeared to be stimulated by soil addition of mineral N (Henriksen and Breland, 1999). Dornbush (2007) also showed that differences in litter N (%) were related to differences in β -glucosaminidase. Despite increasing information on litter type effects, the degree to which plant litters regulate microbial enzyme production appears to depend on litter quality as well as particular enzymes (Allison and Vitousek, 2004; Hernández and Hobbie, 2010).

Once microbial extracellular enzymes are produced, their stability in the environment

becomes a major concern for their ecological functions. Soil enzymes can be physically and chemically associated with soil minerals as well as organic matter (Ensminger and Gieseck, 1942; Nannipieri et al., 1996; Sinsabaugh, 1994). Certainly, these associations help prevent microbial access to and subsequent degradation of extracellular enzymes, thereby stabilizing soil enzymes and keeping them active for a long time (Ladd 1978; Burns, 1982; Nannipieri et al., 1996). However, these associations may cause enzymes to lose part or all of their activities (Allison, 2006). Soil pH is an important factor regulating the associations of enzymes with soil minerals (Quiquampoix et al., 1993; Leprince and Quiquampoix, 1996) and also can affect enzyme activities via controls on the availability of substrates and enzyme co-factors (Tabatabai, 1994).

Incorporation of plant residues and liming are two components of soil management implemented often in agricultural production systems, but their effects on soil enzyme activities have received inadequate attention. The objectives of this study were to assess the degree to which plant litter quality affected the activities of soil enzymes involved in C and N transformations, and to determine the pH effects on the stabilization of these soil enzymes.

Materials and methods

Soil sampling

Two soil samples were collected by coring technique from organic and conventional farms, respectively. The farms were located in Goldsboro, NC where crops were rotated annually, including corn (*Zea mays* L), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), wheat (*Triticum aestivum*), sweet potato (*Ipomoea*

batatas), and sorghum (*Sorghum bicolor*). The conventional farm was applied annually with synthetic N, P, and K fertilizers and with herbicides, insecticides, and fungicides as needed. The organic farm was applied with turkey litters and the fertilization rate depended on rotated crops. Gypsum was used to lime soils at both farms. The soil was classified as Wickham sandy loam (fine-loamy, mixed, semiactive, thermic Typic Hapludult). Detailed information on the two farms has been reported previously (Tian et al., 2010, see Chapter 2).

Twenty soil cores (2.5 cm × 10 cm) were collected randomly from each of the three plots at each farm and pooled, leading to three composite soil samples. Soil samples were sieved (< 2 mm), adjusted for soil moisture content to 45% of water holding capacity, and then stored at 4 °C prior to a series of laboratory experiments. Selected soil properties were given in Table 1.

Plant litters

Plant litters of different C:N ratios were collected from the ground covers of senescent vegetation materials after exposure to sunlight for several months, including long-leaf pine (*Pinus palustris*) needles; a mixture of grass materials of switchgrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), indiangrass (*Sorghastrum nutans*), and big bluestem (*Andropogon gerardii*); and soybean (*Glycine max*) residues.

Plant litters were dried at 80 °C for ~ 8 h and then ground to pass 0.5 mm to minimize structural differences of plant litters. Carbon and N content of plant litters were determined by dry combustion using a Perkin-Elmer 2400 CHN analyzer (Perkin Elmer, Norwalk, CT). The C contents were similar among the plant litters, i.e., 471, 438, and 414 mg C g⁻¹ plant dry

mass, respectively, whereas N content was 3.4, 8.7, and 49.2 mg N g⁻¹ plant dry mass, respectively for pine needles, grass materials, and soybean residues. As a result, their C:N ratios varied greatly were 139, 50, and 9, respectively, for pine needles, grass materials, and soybean residues.

Experimental design

Effects of plant litters on microbial production of extracellular enzymes were determined in a 90-d incubation experiment of three replicates. Four treatments included soil without the addition of plant litters (control) and the soils with the addition of pine needles, grass materials, or soybean residues. Plant litters were amended into ~ 50-g soil subsamples at 2 mg C g⁻¹ soil. Then the soils were incubated at 25 °C in the dark for three months. The activities of phenol oxidase, exoglucanase, β-glucosidase, and β-glucosaminidase were analyzed periodically at 6 h, 14, 21, 28, 52, 73, and 90 days after the start of incubation.

Effects of soil pH on the stabilization of microbial extracellular enzymes were examined in a 42-d incubation experiment of three replicates. To minimize the confounding impacts of microbial production of extracellular enzymes, microbial activities were minimized with the application of cycloheximide at 15 mg g⁻¹ soil as a fungicide and streptomycin sulfate at 3 mg g⁻¹ soil as a bactericide. Six treatments were designed as a 2 × 3 factorial design, including 2 enzyme-amendment levels and 3 pH levels with three replications. Soil pH values were originally 6.1 and 6.6 for conventional and organic farming systems, respectively, and were adjusted to near-neutral and alkaline, respectively, with the 5M KOH solution and the amount of K added is equal between two systems at each pH level. The amount of KOH

solution that brought the soils to desired pH values were calculated based upon forward and backward titration curves made with Titration Manager (model TIM 856, Radiometer Analytical, France). Soil alkalinity was reliably adjusted as shown by the pH measurements (Table 2). Enzymes were added to soil samples as a mixture of horseradish peroxidase (Sigma P8250), cellulase (Sigma C9422), laccase (Sigma 53739), tyrosinase (Sigma T3824), and included a control with no enzyme addition. The stock solutions of these enzymes were added into 20-g soils to reach final concentrations of 1 unit g⁻¹ soil for horseradish peroxidase, 10 units g⁻¹ soil for cellulase, 5 units g⁻¹ soil for laccase, and 5 units g⁻¹ soil for tyrosinase. The treated soil samples were incubated at 25 °C in dark for 42 d, then analyzed for the activities of peroxidase, exoglucanase, β-glucosidase, and phenol oxidase at 6 h and 7, 25 and 42 d after incubation started.

Soil enzyme activities

The activities of soil enzymes, including peroxidase (EC 1.11.1.7), phenol oxidase (EC 1.10.3.2), exoglucanase (EC 3.2.1.4), β-glucosidase (EC 3.2.1.21), and β-glucosaminidase (EC 3.2.1.30) were determined according to the methods described previously (Tian et al., 2010). Briefly, soil samples were mixed with 50 mM acetate buffer (pH 5.0) at a weight (g) to volume (mL) ratio of 1:2.5 and then shaken at 200 rev min⁻¹ for 1 h. For the measurements of exoglucanase, β-glucosidase, and β-glucosaminidase activities, soil slurry (0.8 mL) were pipetted into a 2-mL Eppendorf tube, which contained 0.2 mL of corresponding substrate solutions (i.e., 2 mM p-nitrophenyl-β-D-cellobioside, 10 mM p-nitrophenyl-β-D-glucopyranoside, and 2 mM p-nitrophenyl N-acetyl-β-D-glucosaminide, respectively, made

by 50 mM sodium acetate buffer at pH 5.0). After 1 h incubation at 37 °C, except 2 h for exoglucanase, 0.2 mL of 0.5 M CaCl₂ and 0.8 mL of 0.5 M NaOH were added to stop the reaction and allow color development. After centrifugation at 11,000 rev min⁻¹ for 4 min., optical density of the suspension was measured at 410 nm to detect the released p-nitrophenol.

Peroxidase activity was measured with 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate according to the method of Johnsen and Jacobsen (2008). Soil slurry (0.2 mL) as described above was pipetted into Eppendorf tube, which contained 0.4 ml of TMB Easy solution (Fisher Scientific Inc.), and then incubated for 10 min. at room temperature. The enzymatic reaction was terminated by adding 0.8 mL of 0.3 M sulfuric acid. After centrifugation at 11,000 rev min⁻¹ for 4 min., optical density of the suspension was measured at 450 nm.

Phenol oxidase was measured according to the method of Saiya-Cork et al. (2002). Soil slurry (0.8 mL) was pipetted into Eppendorf tube, which contained 0.8 mL of 5mM DOPA (made by 50 mM sodium acetate buffer at pH 5.0). After 1 h incubation in dark under room temperature, soil mixtures were centrifuged at 11,000 rev min⁻¹ for 4 min., optical density of the suspension was measured at 460 nm.

All the enzyme assays were determined in quadruplicates against two types of controls (i.e., substrate alone and soil filtrate alone). For soils amended with plant litters, another control (i.e., plant litters only) was also applied for excluding litter interference. Soil enzyme activity is expressed as µmol of products produced per h per g of soil.

Data analysis

To test the effect of plant litter, and soil sites in the plant litter addition experiment and effect of pH, and soil sites on enzyme stability experiment, repeated-measures analysis of variance (ANOVA) was used for significant effects ($P < 0.05$). The mean values were compared via Waller-Duncan k-ratio T test at $P < 0.05$.

Results

Effects of plant litters on soil enzyme production

Soil enzyme activities increased after the addition of plant litters (Fig. 1), but the magnitude of increase as well as the dynamic patterns varied with the types of plant litters and particular enzymes. The activity of soil β -glucosidase increased over time and reached the highest value 21 to 28 d after litter additions, being \sim two-fold greater than the control treatment. Thereafter, its activity declined and was only about 10 – 20% greater than the control 90 d after litter additions. In general, the increase in β -glucosidase activity was greatest for grass materials, followed by soybean residues, and the least by pine needles.

Compared with the control treatment, increases in soil exoglucanase activity was the greatest at 45-100% from the addition of grass materials, followed by soybean residues (30-50%) and pine needles (10-25%). After the addition of grass materials or soybean residues, exoglucanase activity increased over time, peaked at \sim 3 or 4 weeks, and then declined. The exoglucanase activity increased slightly right after the addition of pine needles and then remained relatively stable over 3 months of the incubation.

Unlike β -glucosidase and exoglucanase, the β -glucosaminidase activity was increased

most significantly by the addition of soybean residues rather than the addition of grass materials. Three months after litter additions, soil β -glucosaminidase activity was still ~75% greater than the control.

After the addition of pine needles, soil phenol oxidase activity increased continuously and peaked near the end of the incubation. Generally, the stimulation effects were more pronounced for the pine needles or grass materials than for the soybean residues.

The two soil samples also showed some differences in the increase of soil enzyme activity after litter additions. For example, phenol oxidase activities were similar between the two soil samples (Table 1), but the increase after litter additions appeared to be less in soils collected from the organic farm than from the conventional farm. The β -glucosaminidase activity was lower in soils collected from the organic farm, and its increase after litter addition was also lower than the soils collected from the conventional farms.

Stability of soil enzymes as affected by soil pH

Addition of commercial enzymes immediately increased the activities of these enzymes in soils (Table 3). Thereafter, enzyme activities decreased over time (Fig. 2). The degree of reduction appeared to depend on particular enzymes as well as source of the soil samples. For example, exoglucanase activity decreased more in soils collected from the organic farm than from the conventional farm, whereas the reductions in β -glucosaminidase activity were comparable between the two soils. Added peroxidase retained ~80% of its activity in soils collected from the conventional farm at the end of incubation, but only ~40% in soils collected from the organic farm.

Soil pH was adjusted to about 7 and 8 at the beginning of enzyme additions, but it reduced over time (Table 2). Nonetheless, pH effects on the enzyme activities were apparent 7 days after enzyme additions and consistent between the two soils (Fig. 2). In general, soil enzymes were more stable under neutral and alkaline soil condition than in the acidic soils. Compared with the acidic control, β -glucosidase activity was ~ 30-50% or 70-140% greater in neutral and alkaline soils, respectively, for soils collected from the conventional or organic farms. Again, exoglucanase activity was two-fold greater in alkaline soils than in the acidic controls at the end of incubation. Although phenol oxidase activity was greater in the acidic controls during the first 7 days, it was greater in the alkaline soils at the end of incubation. Soil peroxidase also appeared to be more active over a long time at alkaline pH.

Discussion

Microbial enzyme production induced by substrate availability

Plant litters are comprised of a suite of organic compounds, including simple monomers, labile oligomers, and recalcitrant polymers. When plant litters are amended into soils, microbes can directly assimilate readily-available monomers for growth and metabolisms. Perhaps, readily-available monomers are the substrates for which microbial investment is most cost-efficient. Apparently, extracellular enzymes are unnecessary during this phase of plant litter decomposition. Thus, we observed little change in the activities of extracellular enzymes shortly after the soil addition of pine needles, grass materials, or soybean residues.

When simple monomers are consumed, labile oligomers are likely the next choice of available substrates for the energy investment of microbes. These labile compounds, however,

need to be depolymerized prior to microbial assimilation. In response, microbes may produce extracellular enzymes to catalyze the breakdown of these labile substances. The substrate-induced enzyme production represents a major physiological response of microbes to the environment and has been reliably examined by substrate-addition experiments (Smucker and Kim, 1987; Chróst, 1991; Shackle et al. 2000; Hernández and Hobbie, 2010). Our results were consistent with this concept of microbial production of β -glucosidase and its activity. It appeared that substrates that induced microbial production of β -glucosidase existed in all litters of different type, and therefore, soil β -glucosidase activity was enhanced shortly after litter additions, irrespective of litter type or quality. However, the degree to which soil β -glucosidase activity increased in response to litter addition differed among litters, being most pronounced for grass materials. The different increases in β -glucosidase activity suggested that the relative abundance of the substrates that β -glucosidase degrades might be greater in grass materials. A recent study also reported that microbial production of β -glucosidase could also be stimulated by the products, i.e., glucose, this enzyme produced (Hernández, and Hobbie, 2010). However, we could not determine which one, the substrates β -glucosidase degraded or produced, play stimulatory roles in microbial production of this enzyme.

Beta-glucosaminidase represents another enzyme for catalyzing the degradation of oligomers. Microbial production of this enzyme is also likely induced by the substrates it degrades. This induction appears to be predicted by litter quality. Dornbush (2007) reported that β -glucosaminidase activity was positively related to litter N content. Among three litters, soybean residues had the lowest C:N ratio and thus contained a higher concentration of N. As a result, we observed that soil β -glucosaminidase activity was greatest for soil addition of

soybean residues.

The C:N ratios of both grass materials and pine needles were very high, suggesting that N-containing substrates for microbial inductive production of β -glucosaminidase might be low. Therefore, we predicted less dynamic changes in β -glucosaminidase activity after soil addition of grass materials or pine needles. However, the results contradicted our hypothesis. Indeed, we found that soil β -glucosaminidase activity was very dynamic after soil addition of both litters and remained at high levels throughout the incubation, similar to the soybean residues treatment. Although soil addition of plant litters affected soil β -glucosaminidase similarly, the underlying mechanisms for its production might vary with plant litter type or quality.

Microbial enzyme production for limited nutrients

Microbial production of enzymes for acquiring relatively-limited nutrients has been attributed to microbial resource reallocation (Sinsabaugh and Moorhead, 1994). They demonstrated that when mineral N and P were applied sufficiently, C availability became a limiting factor for microbial growth. This could make microbes shift resource from producing enzymes for acquiring N and P to enzymes for acquiring C. This resource allocation theory appears to be supported by many observations as reviewed by Fog (1988) that N addition could stimulate cellulase activity and subsequently accelerated the decomposition of organic matter.

The C:N ratios of both grass materials and pine needles were well above the threshold value ~ 25 (Paul and Clark, 1996), indicating that microbial growth was limited by N supply from the decomposed litters. Therefore, microbes needed to acquire additional N from soil

environment. It was possible that readily-available soil inorganic N was insufficient and thus microbes had to increase investment in extracellular enzymes to acquire N.

The magnitude of increase in soil β -glucosaminidase activity was different in two soils and appeared to be related to differences in soil N availability. Although soil inorganic N was not statistically different at the beginning of incubation, our previous work showed that soils collected from organic farms contained more available N due to greater rate of soil N mineralization (Tian et al., 2010). As a consequence, N limitation for microbial growth might be less severe in organic soils and thus less amount of β -glucosaminidase was produced by microbes.

Microbial production of enzymes for degrading polymers

Cellulose and lignin are primary components of plant litters. However, their relative abundance varies with litter type and therefore can be used to indicate litter quality. The lignocellulose index (LCI), defined as the ratio of lignin to sum of lignin and cellulose, is one of the most-often used parameters for litter quality. In general, plant litters with high C:N ratios also have high LCI values. Linkins et al., (1990) reported that cellulase activity could be stimulated by plant litters of low LCI values. Among three litters, pine needles had the highest C:N ratio and were thought to contain the higher amount of lignin (Johansson, 1995; Campbell and Sederoff, 1996; Berg and McClaugherty, 2003). By contrast, grass materials and soybean residues have lower amounts of lignin (Mungai and Motavalli, 2006). Compared to soils with the addition of grass materials and soybean residues, cellulase activities were expected to be relatively lower in soils with the addition of pine needles. Indeed, we

observed that exoglucanase activity was increased after soil addition of grass materials and soybean residues, whereas it changed little in soils amended with pine needles over the incubation period.

Increase in exoglucanase activity in soils with grass materials and soybean residues could be attributed to microbial substrate-inductive production. When readily-available and labile C compounds were consumed shortly after litter additions, cellulose became preferred substrates for microbes to maximize growth and activity via minimal resource investment. However, cellulose contents of plant litters could decline over time (Linkins et al., 1990), leading to dynamic changes in the activity of exoglucanase. The apparent associations between exoglucanase activity and presumed change in cellulose content could further support that cellulase was produced through the induction of substrates it degraded.

However, it seemed to be a question as to why soil addition of pine needles did not stimulate microbial production of cellulase. Certainly, pine needles contained cellulose although its concentration was lower as compared to grass materials and soybean residues. We considered two suppositions to interpret this phenomenon. First, microbial cellulase production might be inhibited by a lack of nutrients for enzyme synthesis. As mentioned previously, available N in pine needles was extremely low. This could lead to more microbial resource being allocated to the production of N-acquiring enzymes, such as β -glucosaminidase. This strategy was considered to benefit microbes for adequately assimilating readily-available and labile C compounds. Allison and Vitousek (2005) also documented that cellulose induction on microbial cellulase might not occur under some conditions such as lack of a small amount of available nutrient that is required to activate

inducible enzyme synthesis. Second, cellulose might be tightly bound to lignin in pine needles and thus was unlikely accessible for microbes. Accordingly, microbial cellulase production could not be stimulated without the apparent presence of substrates.

Lignin is recalcitrant to microbial degradation. While a consortium of microbes may be required to work cohesively, the foremost microbes are those producing oxidative enzymes, such as phenol oxidase and peroxidase, which oxidize lignin and in turn making lignin more susceptible to further degradation (reviewed by Cullen and Kersten, 2004). It is widely accepted that microbial community gain little energy for degrading lignin (Zak et al. 2000). However, we still found that phenol oxidase activity was significantly increased at the late stage of decomposition of grass materials or pine needles.

We considered that microbial substrate-inductive production played little role in phenol oxidase production, because it appeared to be against the energy economic principle of microbial survival. Instead, microbes might produce phenol oxidase to acquire N concealed within recalcitrant organic substances, a so-call microbial N mining hypothesis (Moorhead and Sinsabaugh, 2006; Craine et al., 2007). This might help explain the observations that phenol oxidase activity could be induced or suppressed at low or high N availability, respectively (Kirk and Farrell 1987; Fog 1988). According to the present study, soybean residues might provide sufficient N for microbial growth and metabolism using cellulolytic compounds. Thus, microbes did not need to “mine” N from soil organic matter. In contrast, N supply from pine needles and grass materials was less than microbial N needs. When decomposing pine needles and grass materials, microbes have to “mine” N from soil organic matter, in particular at late stage of decomposition.

Persistence and stability of soil enzymes

Soil pH may greatly affect the persistence, structure, stability, and thus the activity of soil enzymes via controls on ionization and solubility of enzymes as well as the availability of substrates and enzyme co-factors (Dick et al., 1988; Tabatabai, 1994). The optimal pH varies with particular enzymes, but often within the range of pH 4-8 (Frankenberger and Johanson, 1982). Some enzymes prefer alkalinity, whereas others function better at acidic conditions (Frankenberger and Johanson, 1982; Sinsabaugh et al., 2008). However, the optimal pH derived from simple solution and laboratory conditions might be shifted after enzymes are exposed to complex conditions, such as soils (McLaren, 1957; Leprince and Quiquampoix, 1996). Although some hydrolytic enzymes, such as cellulase, prefer pH < 6 (Johnson et al., 1982), our study showed that all the enzymes tested were more stable and active at higher soil pH.

Conclusions

This study provided a comprehensive understanding of microbial production of extracellular enzymes critical to soil C and N processes. The underlying mechanisms for enzyme production appeared to vary with particular enzymes and litter quality. In general, β -glucosidase, an enzyme for degrading labile C compounds, was produced by substrate induction after litter additions. However, more than one mechanism was involved in microbial production of β -glucosaminidase. β -glucosaminidase was likely produced via substrate induction for litters of low C:N ratios and via microbial resource allocation for litters of high C:N ratios. While microbial exoglucanase could be produced via substrate

induction for litters of low C:N ratios, it could be suppressed for litters of high C:N ratios due to N limitation. We considered that phenol oxidase was significantly produced for litters of high C:N ratios for acquiring N from soil environment.

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Table 1. Selected soil chemical and microbiological properties in conventional and organic farming systems, respectively.

Soil properties	Conventional farming	Organic farming
Total soil C (mg g ⁻¹ soil)	9.0 a [#]	10.2 a
Soil C:N ratio	11.4 a	11.5 a
Microbial biomass C (µg g ⁻¹ soil)	329.6 a	326.3 a
Biomass C:N ratio	10.9 a	9.5 a
Soil inorganic N (µg g ⁻¹ soil)	6.4 a	7.8 a
Enzyme activity (µmol h ⁻¹ g ⁻¹ soil)		
β-glucosidase	0.46 a	0.35 a
Exoglucanase	0.10 b	0.12 a
β-glucosaminidase	0.24 a	0.19 b
Phenol oxidase	0.08 a	0.09a

[#] Different letters within each row indicate significant differences of mean values for n = 3

(Waller-Duncan k-ratio T test, *P* < 0.05).

Table 2. Originally-acidic soil pH and the pH values adjusted by KOH solution to near-neutral and alkaline values at the beginning and 42 days after the enzyme amendments.

Soil pH	0	42 d
Convention farming		
Original	6.1c [#]	6.0c
Near-neutral	7.1b	6.6b
Alkaline	8.0a	7.1a
Organic farming		
Original	6.6c	6.7c
Near-neutral	7.5b	7.1b
Alkaline	8.2a	7.5a

[§] Different letters within each column within farming practice indicate significant differences of mean values for n = 3 (Waller-Duncan k-ratio T test, $P < 0.05$).

Table 3. Enzyme activities of biocide-treated soils with and without the amendment of a mixture of commercially-available cellulase, horseradish peroxidase, laccase, and tyrosinase.

Soil treatments	Soil enzyme activity ($\mu\text{mol h}^{-1} \text{g}^{-1}$ soil)			
	β -glucosidase	Exoglucanase	Phenol oxidase	Peroxidase
Conventional farming				
Without enzymes	0.4b [#]	0.1b	0.02b	0.14b
With enzymes	8.1a	2.5a	0.16a	3.13a
Organic farming				
Without enzymes	0.4b	0.1b	0.07b	0.25b
With enzymes	7.8a	2.8a	0.17a	0.59a

[#] Different letters within each column within farming practice indicate significant differences of mean values for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$).

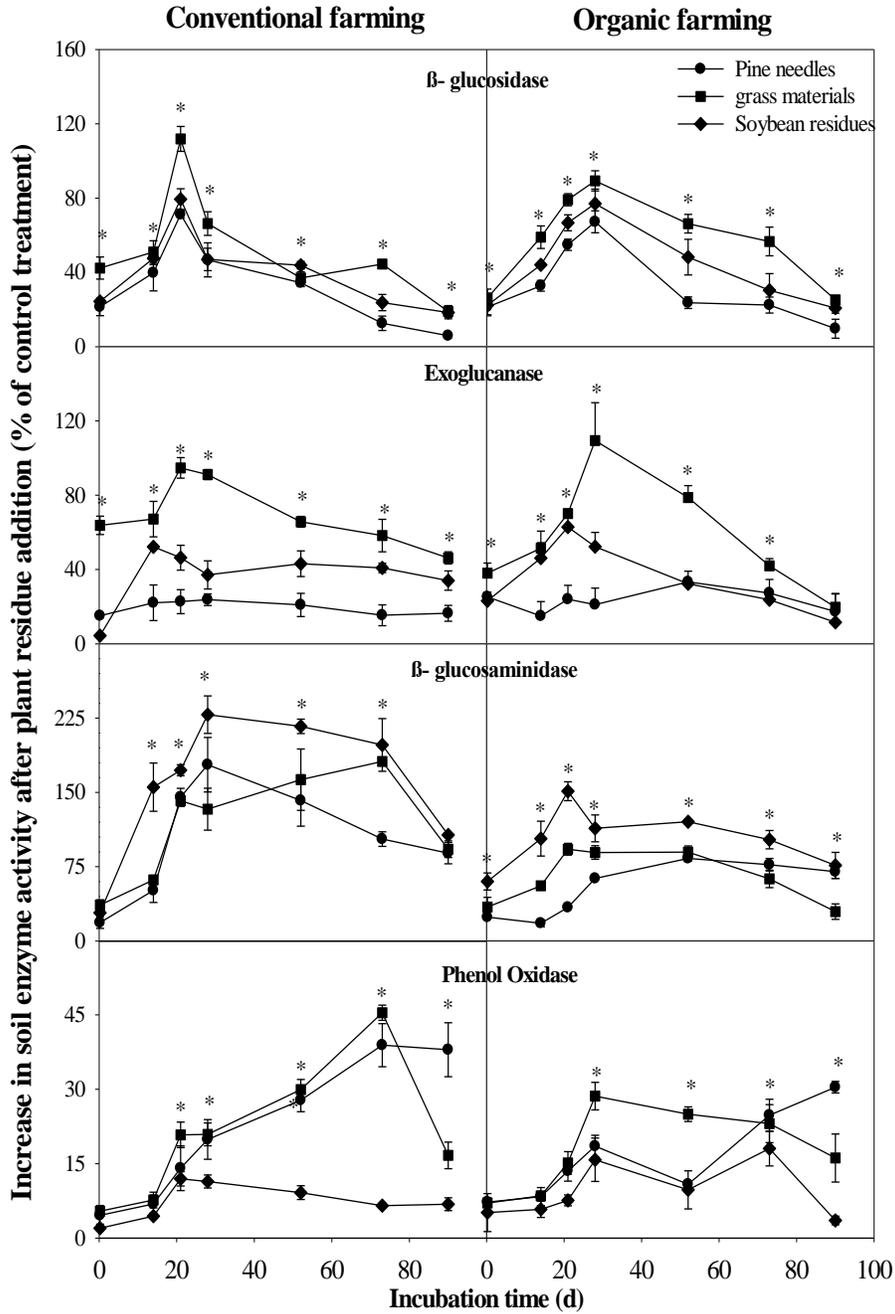


Figure 1. Microbial production of extracellular enzymes after the addition of plant residues in relation to the control treatment. The symbol * indicates significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$).

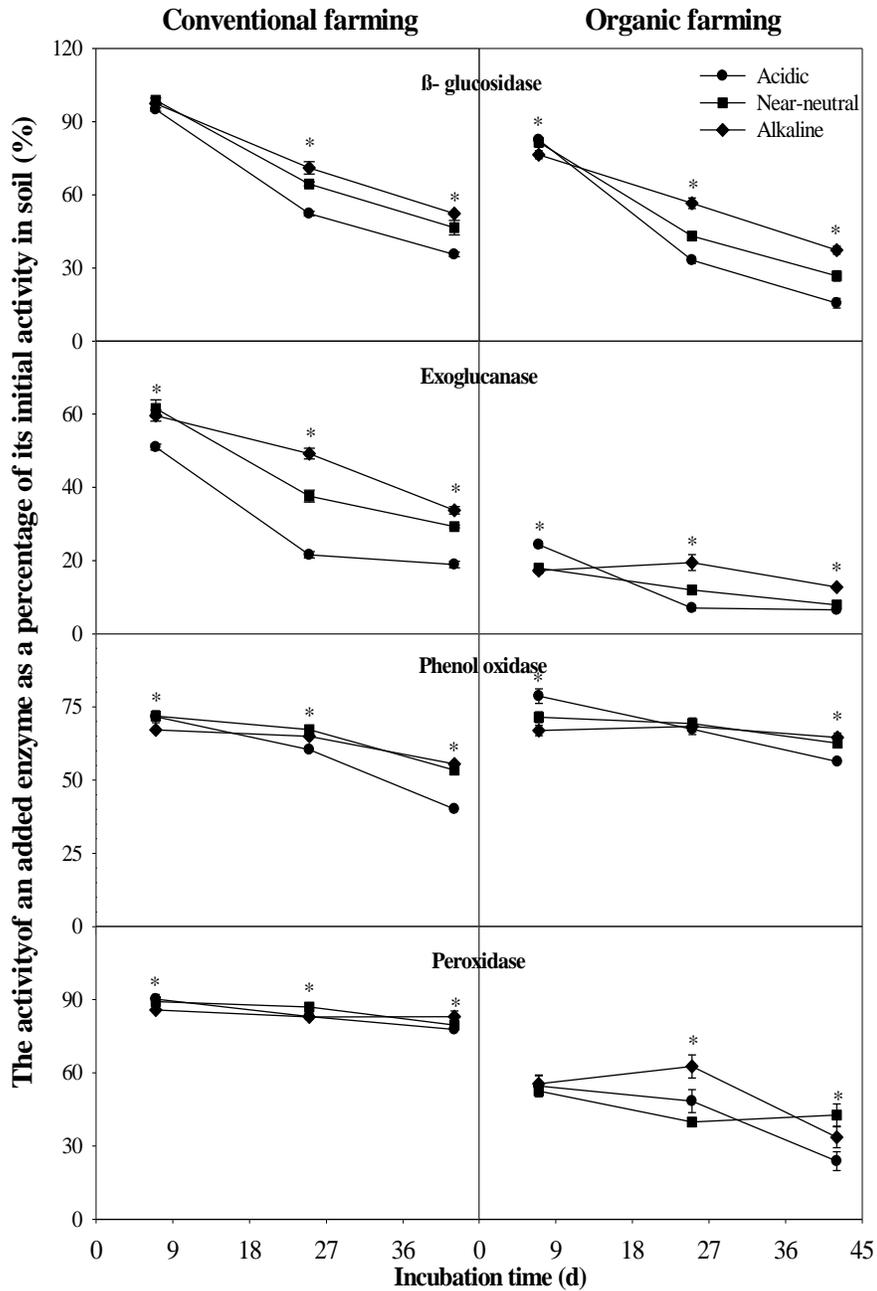


Figure 2. Persistence of added enzyme activity in soils as affected by soil pH. The symbol * indicates significant differences of mean values between treatments for $n = 3$ (Waller-Duncan k-ratio T test, $P < 0.05$).

CHAPTER FIVE

CONCLUSIONS AND FUTURE DIRECTION

This dissertation research focused on the underlying mechanisms of soil enzymes in organic matter mineralization. It is the first report regarding the relationships of DOM with soil enzyme activity as well as soil C and N mineralization. By examining relationships among soil enzymes, DOM, and soil C and N mineralization, new insight has been gained into enzymatic controls on soil organic matter mineralization. The major components of DOM, reducing sugars and amino acids could reliably interpret the differences in soil C and N mineralization among the diverse agroecosystems examined. Reducing sugars and amino acids were found to be well correlated with soil peroxidase activity but not the activities of hydrolytic enzymes. Accordingly, I considered that soil peroxidase activity might represent a proximate control on soil organic matter mineralization as well as the chemistry of DOM in agroecosystems that we have examined.

Peroxidase is one of the oxidative enzymes important in the degradation of lignin and humus. When peroxidase oxidized the phenolic moieties of humus and lignin, thereby producing simple phenolic compounds for microbial assimilation, it could simultaneously produce bioavailable compounds such as sugars and peptides that were otherwise bound to soil humus. Indeed, my results supported that peroxidase mediated soil C mineralization through controls on the availability of carbohydrates and peptides. Although peroxidase did produce phenolic compounds, I found that these phenolic compounds inhibited the activities of soil hydrolytic enzymes and thus exerted negative effects on soil organic matter degradation.

Given the significant roles of soil enzymes in the processes of soil C and nutrient cycling, it is imperative to understand the mechanisms for the production of microbial extracellular enzymes. Plant litters are one of the major energy, C and nutrient resources for microorganisms and thus soil amendment of plant litters likely induced the production of microbial extracellular enzymes. My results showed that microbial production of extracellular enzymes for degrading labile C compounds, such as β -glucosidase, was induced by the litter additions. However, microbial production of polymer-degrading cellulase was only induced by the litters with low C:N ratios. Although microbial productions of β -glucosaminidase and phenol oxidase were enhanced in response to soil addition of plant litters with high C:N ratios, substrate-induction did not play roles, but rather microbial resource allocation or “microbial N mining” for acquiring nutrients bound to recalcitrant organic matter. It should be noted that the stability of microbial extracellular enzymes is the major concern for the performance of enzymes in nutrient cycling. Soil enzymes appeared to be more stable and active at the alkaline pH.

Several issues need to be considered for further investigations. Because dissolved organic matter represented a very small but dynamic pool of soil C and N, its quality and quantity may change greatly with management practices as well as environmental conditions. Hence, the correlations derived from my study site among soil enzyme activities, chemistry of dissolved organic matter, and soil organic matter mineralization need to be vigorously tested either at the same site with multiple-year or different-season samplings or at agroecosystems of other geographic locations.

Intuitively, soil peroxidase may positively control soil organic matter mineralization

through its catalytic products, phenolic compounds. However, my results showed that phenolic compounds exerted some negative effects on soil hydrolytic enzymes and the degree of these effects depended on the complexity and concentration of phenolic compounds. Instead, the major pathway of peroxidase controls on mineralization appeared to be through the bioavailability or bio-accessibility of labile carbohydrates and proteins for soil microbes. My results add to an existing body of observations on positive and negative effects of phenolic compounds on organic matter mineralization. The issue is that ecology of phenolic compounds needs to be better understood prior to informed decisions on managing their presence and abundance in soil. Future research may be also required to focus on the issues of peroxidase controls on organic matter decomposition, in particular pertaining to the type of peroxidase and gene-level regulations.

Given that soil peroxidase might be a proximate control on organic matter degradation in agroecosystems, factors and agricultural management practices controlling microbial production of this enzyme may represent another focus of future research. While the stability of microbial extracellular enzymes can be affected by numerous soil chemical, physical and biological properties, the relative importance of these properties need to be better understood so that key soil enzymes can be identified at given environmental conditions or management practices for predicting soil organic matter degradation.