ORGANIC MATTER CONTENT, MICRIOBIAL BIOMASS, AND ENZYME ACTIVITIES: INTERACTION AND VARIABILITY IN SOILS UNDER LONG-TERM CROP ROTATION

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2008

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ACKNOWLEDGMENTS

The past two years at OSU have been an immensely rewarding educational and intellectual experience. Much of the credit for this must go to my advisor Dr. Shiping Deng. Interacting with Dr. Deng has developed my research and writing skills more than I could have imagined. Many thanks to Dr. Hailin Zhang and Dr. Michael Anderson for their encouragement and kind help in the conducting the research and thesis preparation. Their feedbacks have been always invaluable.

I have been very lucky to have had very pleasant, friendly and helpful coworkers. Special thanks to Dr. Eirini Katsalirou for her help in chemical analyses and data analyses; Thanks to Kevin Owen, Donna Caasi, and Samar Shawaqfeh, for their help and encouragement in my study and life, for the memorable moments we spend together.

Last but not least, I would like to thank my husband Meng Li, for his love and understanding, and for always helping me overcome the difficulties I faced in my daily life. Also special thanks to my parents Zhenguo Wu and Xiaoxiu Shang, my brother and sister, and Meng's family, for their endless love, encouragement, and for everything they have done for me.

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CHAPTER I

INTRODUCTION

Long-term productivity and sustainability of soil are receiving increasing attention due to deleterious effects of agricultural production and concerns on soil and environmental quality. Soil productivity and sustainability are directly affected by land management practices that impact soil properties and soil-crop relationships (Francis and Clegg, 1990; Studdert et al., 1997). Some agricultural activities could negatively impact soil quality, resulting in soil erosion, degradation, and acidification, which often lead to decline in crop yield as well as soil microbial population, activity and diversity. Continuous monocropping system, for example, may lead to accelerated decrease in soil organic matter content, especially in conventional tillage systems. Davidson and Ackerman (1993) showed a 30% soil organic carbon loss following 20-year continuous monoculture. Crop rotation has been practiced to overcome some of the negative impacts, and is recognized to play crucial roles in sustaining soil organic matter (Edwards et al., 1992; Wood et al., 1991), promoting crop productivity (Ball et al., 2005; Crookston et al., 1991), and conserving soil quality (Ball et al., 2005; Buman et al., 2004). Soil organic C and N contents were higher under sorghum-soybean rotations than under continuous soybean (Havlin et al., 1990). As a vital

catalyst of N cycling, urease activity was also significantly higher under 4-year corn-oats-meadow rotations than under continuous corn (Klose and Tabatabai, 2000).

Although extensive studies have been conducted to reveal the impact of agricultural practices on soil properties (Carroll et al., 1997), most research focused on the evaluation of a single soil parameter (Bowman et al., 1999; Klose and Tabatabai, 2000; Reeves, 1997). It is generally accepted that no single parameter can be used universally when studying the responses of ecosystem processes to different crop rotations (Brookes, 1995; Dick, 1992; Dick, 1994; Reeves, 1997). Moreover, different soil variables exhibit different behaviour in response to different soil management practices (Bending et al., 2004). Therefore, a combination of various parameters would provide a more accurate evaluation on changes in soil quality (Dick, 1992). Commonly used indicators of soil quality and functional capacity include soil organic matter content, microbial biomass, and enzyme activities (Bowman et al., 1999; Klose and Tabatabai, 2000; Reeves, 1997; Trasar-Cepeda et al., 2000).

Soil organic matter plays crucial roles in soil structural stability, water holding capacity and nutrient cycling, and thus has been recognized as a key component in sustainable agriculture systems and the most important indicator of soil quality and agronomic sustainability (Reeves, 1997). However, changes in soil organic C is slow (Bandick and Dick, 1999; Staddon et al., 1998), which may have led to inconsistent results on the effects of crop rotation on soil organic matter contents. Although soil organic matter contents were generally higher

under crop rotation than monocropping systems (Havlin et al., 1990; Reeves, 1997), Bremer et al. (2008) did not find any detectable changes in the content of soil organic C following six to twelve years of crop rotations, including fallow-wheat, fallow-wheat-wheat and fallow-flax-wheat. Therefore, more recent studies have been directed to evaluate soil quality using soil microbiological parameters such as microbial biomass and enzyme activities (Dick, 1992; Dick, 1994; Dick et al., 1988; Trasar-Cepeda et al., 2000). Although soil microbial biomass is less than 5% of the organic matter, soil microorganisms are the driving force of nutrient cycling and represent labile fractions of soil organic matter and nutrients (Chander et al., 1997; Jenkinson and Ladd, 1981). Soil enzyme activities reflect soil functional capacity such as decomposition and synthesis of organic matter and release of inorganic nutrients (Acosta-Martinez et al., 2003). Activities of specific enzymes could also indicate specific functional capacity of the soil ecosystem. Soil management practices were shown to affect activities of amidohydrolases (Dodor and Tabatabai, 2003), arylamidase (Acosta-Martinez and Tabatabai, 2001) and glycosidase (Deng and Tabatabai, 1996). Therefore, both soil microbial biomass and enzyme activities have been used as indicators of early changes in soil properties in response to different soil and crop management practices (Brookes, 1995; Dick, 1994a; Jordan et al., 1995).

In this study, the overall objective was to determine the impact of long-term crop rotation on soil organic matter content, microbial biomass, and enzyme activity, focusing on interactions and variability of multiple variables. Soil properties include chemical, microbiological and biochemical properties. Three

cropping systems involving wheat and soybean were evaluated. We hypothesized that crop rotations affect soil microbial community, leading to contrasting impact on soil quality which may affect its long-term productivity, and sustainability. The specific objectives were (1) to assess the impact of crop rotation on soil organic matter contents, the contents of soil microbial biomass C and N, and activities of soil enzymes involved in C- and N-cycling; (2) to evaluate sensitivity of soil parameters to reveal potential changes in soils under different cropping systems; and (3) to determine drivers of ecosystem processes and functions through evaluation of the complex relationships and interactions of soil parameters using multivariate analysis. Information obtained in this study will enhance our understanding of the complex soil systems and provide guidance in developing management practices that will preserve soil quality and sustain agricultural productivity.

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CHAPTER II

LITERATURE REVIEW

Soil quality and crop rotation

Soil is a complex environment that is an intimate mixture of the living and non-living components and it varies in time and space over the scales of the depth (Burns et al., 2006). Therefore it is challenging to define soil quality (Johnson et al., 1997; Lal, 1993; Oldeman, 1994). Larson and Pierce (1991) defined soil quality as “the capacity of a soil to function within its ecosystem boundaries and interact positively with the environment external to that ecosystem”. Karlen et al. (1997) defined soil quality as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation.” Regardless of the specific definition, soil quality is defined by its capacity to function, such as crop production and nutrient cycling. In order to sustain agricultural productivity and maintain agro-ecosystem function, it is critical to evaluate, maintain, and improve soil quality (Doran and Zeiss, 2000; Pulleman et al., 2000; Reeves, 1997).

Generally speaking, soil quality includes an inherent component and a component that is affected by land management (Doran and Zeiss, 2000). Of the two components, land management is easier to control. Unfortunately, past land managements have reduced the quality of many soils, leading to soil degradation, soil loss and other environmental problems such as global climate change, atmospheric pollution and the decline in biodiversity caused by human activities (Lal, 1998). About 29.7% of all land areas that were prone to soil degradation were agricultural land (Lal, 2001).

Of land management practices used in agricultural production, continuous cropping systems were shown to lead to soil loss and erosion and decreases in soil organic matter content (Davidson and Ackerman, 1993). Crop rotation, on the other hand, has shown to maintain and improve soil quality, owing to differences in quality and quantity of crop residues (Reeves, 1997). “Rotation effect” (Bullock, 1992) include: increased nutrients and water availability (Bagayoko et al., 1992; Buman et al., 2004); conserved soil organic matter (Wood et al., 1991); helped to control weeds and reduced the number of herbicide resistant weeds (Derksen et al., 2002), and enhanced soil erosion control (Bagayoko et al., 1992; Buman et al., 2004). Baloto et al. (2004) showed that crop rotations can change soil habitat due to their differences in extracting nutrients, depth of roots, and amount of residue under different crop species. For example, corn will produce 3 to 4 tons of surface residue per acre and 1 to 2 tons of root biomass after harvested (Balota et al., 2004). Some crops such as hemp (*Cannabis sativa*) can get nutrients from deep soil due to its long roots, which is

inaccessible to cereal roots (Robson et al., 2002). Carroll (1997) showed 90% of crop stubble covered the surface of soil resulting in early summer rainfall infiltrate when wheat was harvested in September. Doublecropping, referring to growing two crops subsequently on the same land in a year, not only reduced soil erosion, but also enabled more efficient utilization of climatic resources, land, labor, and machinery, and increased net returns (Crabtree and Makonnen, 1981).

Evaluation and indicators of soil quality

Measurements of soil quality can be likened to a medical examination for human health (Larson and Pierce, 1991). In a medical exam, the doctor will take some key measurements of the body system such as temperature, blood pressure and heart beat. These are considered as indicators to monitor the health system. Similarly, a set of key soil quality indicators are need. Soil quality indicators should be useful to practitioners, extension scientists, conservationists and socioeconomic scientists (Doran and Parkin, 1996). A good indicator should be: (1) correlated with the ecosystem process; (2) easy to use and interpret and be accessible; (3) sensitive to variations of management; and (4) components of an existing soil database. However, scientists often focus on the discipline that they are most familiar with, limiting studies to physical, chemical, or microbiological characteristics only. In the evaluation of a complex system, no single parameter can provide accurate evaluation of the system (Brookes, 1995).

Many researchers have attempted to develop soil quality indicators by measuring various soil characteristics and relating them to different land management, crop productivity and environmental quality (Doran and Parkin, 1996; Larson and Pierce, 1991; Stenberg, 1999). Larson and Pierce (1991) recommended some indicators including physical and chemical indicators called "Minimum Data Set" (MDS) which could be used to monitor soil quality (Doran and Parkin, 1996). A summary of soil physical and chemical properties that were included in the evaluation of soil quality is shown in Table 1.

One of the most important criterions for an indicator is that it should be prompt, sensitive, and accurate to respond to the change of a soil (Doran and Parkin, 1996; Stenberg, 1999). The physical parameters such as soil erosion and soil bulk density are not sensitive and reliable to respond to the effects of land use (Pulleman et al., 2000). Studies suggested that soil chemical properties are more sensitive to changes in soil. This could be, in part, due to their roles as nutrients that directly impact microbiological processes (Schoenholtz et al., 2000).

Of all soil parameters, soil organic matter is the most widely accepted and used indictor of soil quality. Soil organic matter (SOM) strengthens water-stability of aggregates, improves soil structure (Abiven et al., 2007; Hudson, 1994), enhances productivity (Biederbeck et al., 1994) and reduces deleterious effects such as soil erosion (Burke et al., 1995). The content of soil organic matter depends on the organic materials which are applied to the soil and lost by decomposition (Dalal *et al.*, 1991). Although agricultural activities often lead to

Table 1. Minimum Data Set of physical and chemical soil quality indicators (modified from (Schoenholtz et al., 2000; Stenberg, 1999)).

Soil property indicators	Relationship to soil condition and function
Physical	
Texture	Transport and retention of water and chemicals; soil erosion and variability estimate
Soil depth, topsoil depth	Estimate of productivity and erosion
Soil bulk density	Potential for leaching, productivity and erosion.
Water holding capacity	Transport and retention of water.
Soil loss/erosion	Available soil, water, nutrient, and root growth, environment concern
Soil strength	Root growth
Soil porosity	Water/air balance, water retention, and root growth
Chemical	
Soil organic matter	Defines soil fertility, stability and erosion extent
pH	Biological and chemical activity
Electrical conductivity	Plant and microbial activity
Extractable N, P and K	Plant available nutrients and potential for N loss
Soil N, P and K	Part of a farmer-based qualitative assessment system of agronomic soils

decline in SOM, the degree of the impact varies depending on management practices. Changes in agricultural management can increase or decrease SOM. Of different cropping systems, continuous monoculture often decreases SOM, especially in the conventional tillage system. Davidson and Ackerman (1993) showed a 30% soil organic carbon loss following 20 years of continuous monoculture. Even though there are so many advantages of adopting conservation tillage systems (Deng and Tabatabai, 1996a; Kladivko, 2001; Madejón et al., 2007), Due to the lack of equipment, low yield in no-till systems, and machinery cost, the use of conservation tillage decreased by approximately 2,430,000 ha in four years in the Midwest of the U.S.. Instead, conventional tillage is still predominant in some area (Buman et al., 2004). However, the loss of SOM in conventional tillage can be reduced through appropriate crop rotation (Studdert et al., 1997). Varvel (1994) found SOM contents were significantly higher in both 2-year and 4-year rotations under conventional tillage compared with monoculture. Under conventional tillage, inclusion of soybean in crop rotation led to loss of soil organic matter when compared with wheat and corn crop rotations (Studdert and Echeverria, 2000). Odell et al. (1984) showed that a conventional rotation of maize, oats and clover increased SOM more than continuous maize.

Although evaluation of soil organic matter has enhanced our understanding of soil quality, soil organic matter content, however, changes slowly with time and might not sensitively reflect early changes in soil due to management practices. Soil microbial properties have been used as more

sensitive indicators of soil quality (Dick, 1992; Dick, 1994). Soil microorganisms play crucial roles in many ecosystem processes such as organic matter decomposition and the cycling of energy and nutrient (Chander et al., 1997; Schutter et al., 2001), Soil microorganisms influence aboveground ecosystems by contributing to plant nutrition as well as soil structure and fertility (Buenemann et al., 2006; Kirk et al., 2004). There are three reasons for using soil microbiological properties as indicator of soil quality (Stenberg, 1999): (1) They have key functions in the degradation of organic matter and nutrient cycling; (2) They respond promptly to changes in the soil environment; and (3) The microbial activity in soil reflects the sum of all factors regulating the degradation and transformation of nutrients.

It has been well documented that agricultural management impacts different microbiological properties of soils. Crop rotations can stimulate soil biodiversity and biological activity over monoculture. Meanwhile, the quantitative and qualitative changes in soil microorganisms can reflect changes of soil quality and productivity. Ryszkowski et al. (1998) reported that fungal biomass was higher in continuous cropping of rye than under crop rotation. Larkin (2003), comparing different rotation crops, found the populations of culturable bacteria were higher in the soybean–barley–potato rotation than barley–clover–potato rotation, soybean–corn–potato rotation and continuous potato rotations. Populations of actinomycetes and fluorescent pseudomonad were generally highest in the barley rotations and were lowest in the soybean rotation and continuous potato. These patterns indicate the strong influence of different plant

species on soil microbial organisms and activity.

Microbiological changes in response to different cropping systems can be assessed by evaluating microbiological parameters such as microbial biomass, enzyme activities, and DNA fingerprints (Brookes, 1995; Giller et al., 1997). Microbial biomass mediates nutrient cycling and serves as mobile nutrient pool as well. In fact, microbial biomass is considered the most labile C and N pools in soils (Jenkinson and Ladd, 1981), and is frequently used as an early indicator of early changes in soil properties in response to different soil and crop management practices (Brookes, 1995; Jordan et al., 1995; Kaiser et al., 1995; Powlson et al., 1987). Gupta and Germida (1988) found that fungal biomass plays an important role in the formation of macroaggregates. Microbial biomass is commonly determined by the chloroform fumigation incubation method (Jenkinson and Powlson, 1976) or the chloroform fumigation extraction (Vance et al., 1987).

Contradictory results about the effect of cropping systems on soil microbial biomass content have been reported in the literature. When compared with monocultural systems, Balota et al. (2003) found that crop rotations led to an increase in microbial biomass C (C_{mic}) and N (N_{mic}). On the contrary, Collins et al. (1992) reported that crop rotation led to a decrease in microbial biomass when compared with monoculture cropping systems. On the other hand, Franchini et al. (2007) found that amounts of C_{mic} and N_{mic} were not affected by tillage practices following a five-year crop rotation under both no-tillage and conventional tillage management.

Soil enzymes are primarily microbial origin and their activities are expected to be even more sensitive to reflect changes in soil. Enzymes are proteins that can catalyze chemical reactions to proceed at faster rates (Dick, 1994). Sources of accumulated enzymes are from all living organisms, including microorganisms, plant roots and fauna (Stenberg, 1999). Enzymes accumulated in soil may be free enzymes, such as exoenzymes that are released from living cells, endoenzymes that are released to the soil environment by cell lysis and enzymes bound to cell constituents (Kiss et al., 1975). Enzyme activities are important to soil functions such as organic matter decomposition and synthesis, nutrient cycling, and decomposition of xenobiotics (Acosta-Martinez et al., 2003). There are four categories which are based on the nutrient cycling that enzyme are involved (Dick, 1994): (1) C-transforming enzymes: amylase, cellulase, lipase, glucosidases and invertase; (2) N-transforming enzymes: proteases, amidases, ureases and deaminases; (3) P-transforming enzymes: phosphatases; and (4) S-transforming enzymes: arylsulphatases. Due to their close relationships to soil biology and microbial communities, soil enzyme activities provide a unique integrative biological indicator and have been used to assess the effect of soil cultivation on the functioning capacity of soil ecosystems (Bandick and Dick, 1999; Dick, 1994; Jordan et al., 1995). In particular, suggestions have been made that extracellular enzymes can be used to assess the protective properties of a soil (Stenberg, 1999). Hence, enzyme activities are widely recommended in soil ecology studies. A summary of major soil enzymes and their ecological functions were presented in Table 2.

Table 2. Major assayed soil enzymes and their ecological function (Dick, 1997).

CE	Enzymes	Functions
Oxidoreductases		
1.1	Dehydrogenase	Exists as integral part of intact cell and reflects total oxidative activities of soil microflora
	Glucose Oxidase	Oxidizes glucose
1.11	Catalase, Peroxidase	Release oxygen from hydrogen peroxide
Transferase		
2.4	Dextransucrase	Hydrolyses sucrose, releasing glucose and fructose
2.8	Rhodanese	Performs the step in oxidation of S
Hydrolases		
3.1	Phosphatase	Release plant available PO_4 from organic matter
	Sulphatase	Release plant available SO_4 from organic matter
3.2	Amylase	Hydrolyses starch into maltose
	Cellulase	Endohydrolysis of 1,4-β-D glucosidic linkage in cellulose, component of wood and plant fibers
	α- and β-glucosidases	Release glucose, an important energy source for microbial activity
	α- and β-Galactosidase	Hydrolysis of melibiose and lactose, respectively
	Invertases, Saccharase	Hydrolyses sucrose to glucose and frutose
3.5	Asparginase, Glutaminase	Act on C-N bonds on respective amino acids releasing NH_3
	Urease	Act on C-N bonds of urea, a fertilizer source
Lyases		
4.1	Glutamate Decarboxylase	Hydrolyses aspartic acid
4.3	Tyrosine Decarboxylase	Hydrolyses tyrosine, involved in N mineralization

Management practices such as green manures (Dick et al., 1988; Elfstrand et al., 2007), tillage and residue treatments (Deng and Tabatabai, 1996a; Deng and Tabatabai, 1996b; Deng and Tabatabai, 1997), municipal refuse (Perucci, 1992) and fertilizer application have significant effects on soil enzymes and microbial activities (Tabatabai, 1977). Different crop rotations have shown their impact on the activities of soil enzymes and microbial biomass because organic matter from plant residues modifies microbial community which produce different enzymes (Ajwa et al., 1999; Browman and Tabatabai, 1978). Enzyme activities such as β-glucosidase and amidase have been shown to be significantly higher in soils under traditional vegetable rotation than in alternative legume vegetable rotation (Miller and Dick, 1995). Microbial biomass and enzyme activity are closely related because it is through the biomass that the transformations of organic elements C, N, and P occur (Frankenberger and Dick, 1983). Many studies have reported significant correlations between soil enzyme activities and microbial biomass (Balota et al., 2004). Content of C_{mic} and enzyme activities were increased by planting a green manure crop (*Sesbania aculeate*) in a six-year crop rotation study (Chander et al., 1997). When compared with monoculture systems, crop rotation generally led to an increase in soil enzyme activities and microbial biomass (Deng and Tabatabai, 2000; Klose and Tabatabai, 2000).

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CHAPTER III

MICROBIAL BIOMASSS AND CARBON-TRANSFORMING ENZYME ACTIVITIES IN SOILS UNDER CROP ROTATION

Abstract

Understanding the effect of cropping systems on microbial biomass and activity in soil may help the development of management practices to govern soil organic matter and maintain productivity and sustainability of agro-ecosystems. The objective of this study was to evaluate the effects of long-term crop rotations on soil chemical and microbiological parameters; to evaluate sensitivity of soil parameters in revealing potential changes in soils under different cropping systems; and to reveal the complex relationships and drivers of ecosystem processes and functions through multivariate analysis. Three crop rotations, including monocropped soybean (MS) (*Glycine max (L.) Merr.*), soybean-wheat (SW) (*Triticum aestivum L.*), and modified soybean-wheat (MSW), were evaluated. MSW involved 3-crop/2-year (early-season soybean, followed by winter wheat, and then followed by late-season soybean). There were four field replications for each cropping system. The experiment was established in 1970's and 36 samples were taken in 2002 in three depths, including 0-10, 10-20, and

20-30 cm. In addition to basic soil chemical properties, soil microbial biomass and activities of six C-transforming enzymes, including α- and β-glucosidase, α- and β-galacosidase, cellulase, and invertase, were also determined. Of the three cropping systems tested, pH values were significantly higher in the top 10 cm surface soils under MS and SW systems. Following over 30 years of crop rotations, contents of soil organic C, total N, and microbial biomass C and N were not significantly different through the soil profiles under different cropping systems. However, activities of C-transforming enzymes were significantly different in top 10 cm soils, but not in the 10-30 cm subsurface soils. In general, activities of C-transforming enzyme were lower in soils under the MSW system and higher under the MS and SW rotations. Enzyme activities decreased with increasing soil depths and were interrelated with each other, with r values ranging from 0.72*** to 0.97***. Activities of C-transforming enzymes were also significantly correlated with soil organic C content ($r > 0.84$***) and microbial biomass C and N ($r > 0.33$*). Of the parameters evaluated, enzyme activities were most sensitive to changes induced by crop rotations, and thus may be used to detect early changes in the soil ecosystem. Cellulase activity, organic C, total N, and microbial biomass C and N were drivers in ecosystem processes and functions under SW, while activities of C-transforming enzymes that release small molecular weight sugars governed soil processes and functions under MSW. Regardless of cropping systems evaluated, the ability of the microbial community to degrade complex carbohydrates (Cellulase/C_{mic}), the capacity of soil to support microbial life (C_{mic}/organic C), and microbial community structure

and composition (C_{mic}/N_{mic}) predominantly drive soil processes and functions. The relative activities of simple sugar releasing enzymes to overall detected microbial biomass C and soil organic C to total N ratios were also important factors that regulate soil processes and functions.

1. Introduction

Extensive studies demonstrated that continuous mono-crop systems contribute to accelerated soil erosion, reduction of soil organic matter, and the decline in soil quality. Crop rotation and residue management have been evaluated and practiced to minimize such negative impacts to the soil environment (Chander et al., 1997). Studies have shown that crop rotation not only minimized soil erosion, conserved soil organic matter, but also increased nutrients and water availability, and facilitated in weed and pathogen control (Bagayoko et al., 1992; Buman et al., 2004; Wood et al., 1991).

Soil organic matter is considered the most important indicator of soil quality due to its multiple functions on soil structural stability, water holding capacity, and nutrient retention and cycling (Biederbeck et al., 1994; Burke et al., 1995; Reeves, 1997). Considerable research has been conducted to evaluate changes of soil organic matter content as an indicator of changes in soil quality (Bowman et al., 1999; Bremer et al., 2008; Pulleman et al., 2000). However, changes in soil organic C is slow, which may not sensitively reflect changes in soil quality induced by management practices (Bandick and Dick, 1999; Staddon et al., 1998). Therefore, more recent studies were directed to evaluate microbial

properties, such as microbial biomass and enzyme activities, as indicators of soil quality (Jordan et al., 1995; Kaiser et al., 1995; Trasar-Cepeda et al., 2000).

Microbial biomass has been shown to be affected by agricultural management practices (Ajwa et al., 1999; Balota et al., 2003; Brookes, 1995). Crop rotations were suggested to stimulate soil microbiological activities due to deposition of diverse quantity and quality of crop residues at variable soil depth (Balota et al., 2004). The effects of crop rotations on soil microbial biomass, however, are not consistent in different studies. Moore et al. (2000) showed a significant increase in the contents of microbial biomass C and N under long-term crop rotation. On the contrary, Collins et al. (1992) reported that crop rotation led to a decrease in microbial biomass when compared with monoculture cropping systems. It is possible that the inconsistent results were due to limited sensitivity using microbial biomass to reflect changes induced by management practices.

Soil enzyme activities have been evaluated by numerous researchers as an indicator reflecting changes in the soil ecosystems, and were shown to be quick and sensitive to respond to the changes of soils. For example, β-glucosidase activity, an enzyme involved in the degradation of cellulose, changed significantly under a two-year cropping system, while there were no detectable changes in organic C content (Dick, 1994). Following two years of crop rotation practices, cellulase activity was significantly higher under maize–wheat rotation than under soybean-wheat or cotton-wheat rotations (Balota et al., 2004).

Moreover, it is generally accepted that no single parameter can be used universally when studying the responses of ecosystem processes to different

crop rotations (Brookes, 1995; Dick, 1992; Dick, 1994; Reeves, 1997). Although extensive studies were conducted to evaluate impact of crop rotation on soil properties, most of these studies evaluated responses of soil ecosystems using a single soil parameter (Acosta-Martinez and Tabatabai, 2001a; Acosta-Martinez and Tabatabai, 2001b; Balota et al., 2003; Ekenler and Tabatabai, 2003).

Therefore, the main objective of this study was to evaluate impacts of long-term crop rotation on soil chemical and microbiological parameters, including soil organic C, microbial biomass, and activities of C-transforming enzymes; to evaluate sensitivity of soil microbial parameters to potential changes in soil quality under different cropping systems, and to reveal the complex relationships and drivers of ecosystem processes and functions through multivariate analysis. Carbon-transforming enzymes, including α- and β-glucosidase, α- and β-galacosidase, cellulase and invertase, are important in nutrient cycling.

2. Material and methods

2.1. Soils

The long-term cropping system experiment was established in the 70's in central Oklahoma on a Wynona silt loam with 0 to 1% slope (Crabtree and Makonnen, 1981). In this study, three cropping systems were evaluated, including monocropped soybean (MS) (*Glycine max (L.) Merr.*), soybean-wheat (SW) (*Triticum aestivum L.*), and modified soybean-wheat (MSW). MSW involved 3-crop/2-year and consisted of early-season soybean planted in April and

harvested in September of year 1, followed by winter wheat planted in September and harvested in June of year 2, then followed by another planting of soybean in year 2 which was harvested in October or November (Farno et al., 2002; Keim et al., 2003). The plots were 36.6 m × 19.8 m. There were four replicated plots for each cropping system.

In this region of United States, planting soybean after harvesting winter wheat is an important cropping system. In the SW system, after harvesting wheat grain, no-tilling was applied prior to planting soybeans. The field was double disked only once a year before planting soybean. In the MSW system, the soil was disked three times per two year, including moldboard plowing in February, disking in late March, and double disking to plant wheat after harvesting soybean. No-till system was applied only to planting soybean after harvesting wheat in second year. In the MS system, moldboard plowing was applied in March; and the soil was disked in late April and again in June every year. No-till was not incorporated in the management practice (Farno et al., 2002).

Composite soil samples were taken in 2002 from each treatment plot at three different depths, including 0-10 cm, 10-20 cm, and 20-30 cm. Therefore, a total of 36 soil samples were obtained. Soils were sieved to pass a 2 mm sieve immediately following sampling. The field-moist soils were stored at 4^{o}C and used for microbiological and biochemical analyses. A portion of each sample was air-dried for chemical analysis; and a potion of the air dried sample was ground to pass an 80-mesh (180 m) sieve for the determination of soil organic matter and total N content.

2.2. Chemical parameters

Soil pH values were determined with a combination glass electrode (soil: water = 1:2.5). Soil moisture content was determined after drying at 105℃ for 48 h. Soil organic carbon content and total N was determined by dry combustion.

2.3. Microbiological parameters

Microbial biomass C (C_{mic}) and N (N_{mic}) were both determined by the chloroform-fumigation-incubation method (Jenkinson and Ladd, 1981). The content of C_{mic} was determined by titrating CO_2 released from microbial activity during a 10-day incubation with HCl; and N_{mic} was measured by extracting and quantifying inorganic N released through microbial N mineralization during a 10-day incubation. Contents of C_{mic} and N_{mic} were calculated using a k_c factor of 0.45 for C_{mic} (Jenkinson and Ladd, 1981) and k_N factor of 0.54 for N_{mic} (Jenkinson, 1988).

Assaying methods of C-transforming enzymes analyzed are listed in Tables 1. Briefly, the activities of α-glucosidase, β-glucosidase, α-galacosidase and β-galacosidase were determined based on colorimetric quantification of the *p*-nitrophenol released when soil was incubated at 37℃ for 1 h in buffered ρ-nitrophenyl glycoside solution (Eivazi and Tabatabai, 1988). The activities of cellulase and invertase were determined by quantifying the blue color developed from molybdenum blue reaction in the presence of reducing sugars and molybdenum reagents (Somogyi-Nelson method) following incubation of soils in

buffered substrate for 24 h (Deng and Tabatabai, 1994; Frankenberger and Johanson, 1983).

2.4. Statistical methods

Significant differences among treatments were determined by one-way analysis of variance. Comparison of treatment was done by lease significant difference test (LSD, $P \leq 0.05$) using the general linear model procedure of the SAS system. Correlations between soil chemical and microbial properties were calculated using the Pearson correlation coefficient. Principal component analysis (PCA) was applied to reduce the dimensionality of ten soil chemical and microbiological variables. PCA were conducted using correlation rather than covariance matrix because the tested soil variables were expressed in different units (Johnson, 1998). The first two principle analysis scores (PC1 and PC2) were used to test the significance among treatments. The obtained PC scores were plotted by Sigmaplot software (SigmaPlot, 2004).

Table 1. Methods used for assay of C-transforming enzymes in soils (modified from Deng and Tabatabai 1996).

Class/CE number	Enzyme name	Hydrolysis Reaction	Substrate	References
3.2.1.20	α-Glucosidase	α-D-glucose residues → α-D-glucose	*p*-Nitropheny-*α*-D-glucopyranoside	Eicazi and Tabatabai(1988)
3.2.1.21	β-Glucosidase	β-D-glucose residues → β-D-glucose	*p*-Nitropheny-β-D-glucopyranoside	Eicazi and Tabatabai(1988)
3.2.1.22	α-Galacosidase	α-D-galactose residues→ α-D-galactose	*p*-Nitropheny-α-D-galactopyranoside	Eicazi and Tabatabai(1988)
3.2.1.23	β-Galacosidase	β-D-galactose residues→ β-D-galactose	*p*-Nitropheny-β-D-galactopyranoside	Eicazi and Tabatabai(1988)
3.2.1.4	Cellulase	Cellulose→ glucose, cellobiose or higher molecular weight oligosaccharide	Cellulose	Deng and Tabatabai(1994)
3.2.1.26	Invertase	Sucrose → glucose and fructose	Sucrose	Frankenberger and Johanson(1983)

3. Results

3.1. Soil chemical properties

Soil pH values ranged from 5.75 to 6.22 over 0-30 cm soil profiles (Table 2). In the top 10 cm soils, crop rotation had significant effects on soil pH values which were in the order of SW > MS > MSW. The pH values in the 10-30 cm subsurface soils were not significantly different among the cropping systems.

Of the cropping systems evaluated, the highest amount of organic C and total N in 0-10 cm soil layers was found in the SW soils. However, the differences in organic C and total N contents between cropping systems within the same soil depth were not statistically significant. The contents of soil organic C and total N in 0-10 cm soil within a cropping system were significantly greater than those of subsurface soils taken from 10-20 and 20-30 cm. At 10-30 cm soil depth, the contents of organic C and total N were not significantly different among cropping systems or between soil depths.

3.2. Soil microbial biomass and enzyme activities

Even though the differences in the contents of C_{mic} and N_{mic} within and between soil depths in a cropping system were not significant (Table 2), the contents in 0-10 cm soil depth were over one-fold higher in the SW soils than in the MSW and MS soils, and were generally higher than in the 20-30 cm subsurface soils. The degree of impact by cropping systems was different in

Table 2. Effect of long-term crop rotation on soil chemical and microbial properties and ratios between the properties.

Crop rotation	pH	Organic C (g C/kg soil)	Total N (g N/kg soil)	Microbial biomass C (mg C kg^{-1} soil)	Microbial biomass N (mg N kg^{-1} soil)	Organic C:total N	C_{mic}:N_{mic}	C_{mic}: organic C (%)
				0-10 cm				
SW	6.2 a A	7.3 a A	0.9 a A	191 a A	32 a A	8.6 a A	7.3 a A	2.6 a A
MSW	5.8 b A	5.5 a A	0.8 a A	133 a A	26 a A	7.3 b A	6.9 a A	2.5 a A
MS	6.1 a A	6.6 a A	0.8 a A	162 a A	21 a A	8.2 ab A	9.4 a A	2.5 a A
				10-20 cm				
SW	6.1 a A	4.6 a B	0.6 a B	128 a A	16 a A	7.5 a B	13.5 a A	2.8 a A
MSW	5.9 a A	4.3 a B	0.6 a B	169 a A	14 a A	7.0 a A	13.0 a A	3.9 a A
MS	6.0 a A	4.5 a B	0.7 a B	166 a A	13 a A	6.9 a B	17.2 a A	3.7 a A
				20-30 cm				
SW	5.9 a A	3.9 a B	0.6 a B	149 a A	17 a A	7.2 a B	13.7 a A	3.7 a B
MSW	5.9 a A	3.9 a B	0.5 a B	102 a A	11 a A	7.3 a A	12.5 a A	2.6 a A
MS	6.0 a A	3.8 a B	0.6 a B	128 a A	11 a A	6.7 a B	11.2 a A	3.6 a A

SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean.

Smaller letters indicate significantly different means within the same depth according to least significant difference test; Uppercase letters indicate significantly different means across 0-30 cm soil depth (n=4, $p < 0.05$).

different soil depths. For example, the C_{mic} content for the SW system was considerable lower in the 10-20 cm soils than the 20-30 cm soil, while N_{mic} levels in these two subsurface layers were similar.

In the top 10 cm soils, cropping systems had significant effects on the activities of β-glucosidase, α- and β-galacosidase, invertase and cellulase, but not on the activity of α-glucosidase (Figure 1). However, activities of C-transforming enzymes were consistently lower in the MSW soils across cropping systems tested. Enzyme activities in 10-30 cm soil depths among cropping systems were not significantly different (Figure 2). Differences among cropping systems for each tested enzyme activity were smaller in the 10-20 cm soils than those in the 0-10 cm or 20-30 cm soils. Generally speaking, enzyme activities decreased with soil depths. Again, the degree of impact by cropping systems was different not only with respect to different soil depths but also for different enzymes.

3.3. Relationship between soil chemical and microbial properties

Significant correlations were observed among the activities of the six C-transforming enzymes tested, with r values ranging from 0.72*** to 0.97*** (Table 3). With the exception of pH values, these enzyme activities were positively correlated with most soil chemical and microbial properties. Correlation coefficients between enzyme activities and organic C contents ranged from 0.84*** to 0.91*** (Figure 3); and between enzyme activities and total N contents ranged from 0.80*** to 0.89*** (Table 3). It is interesting that the relationship

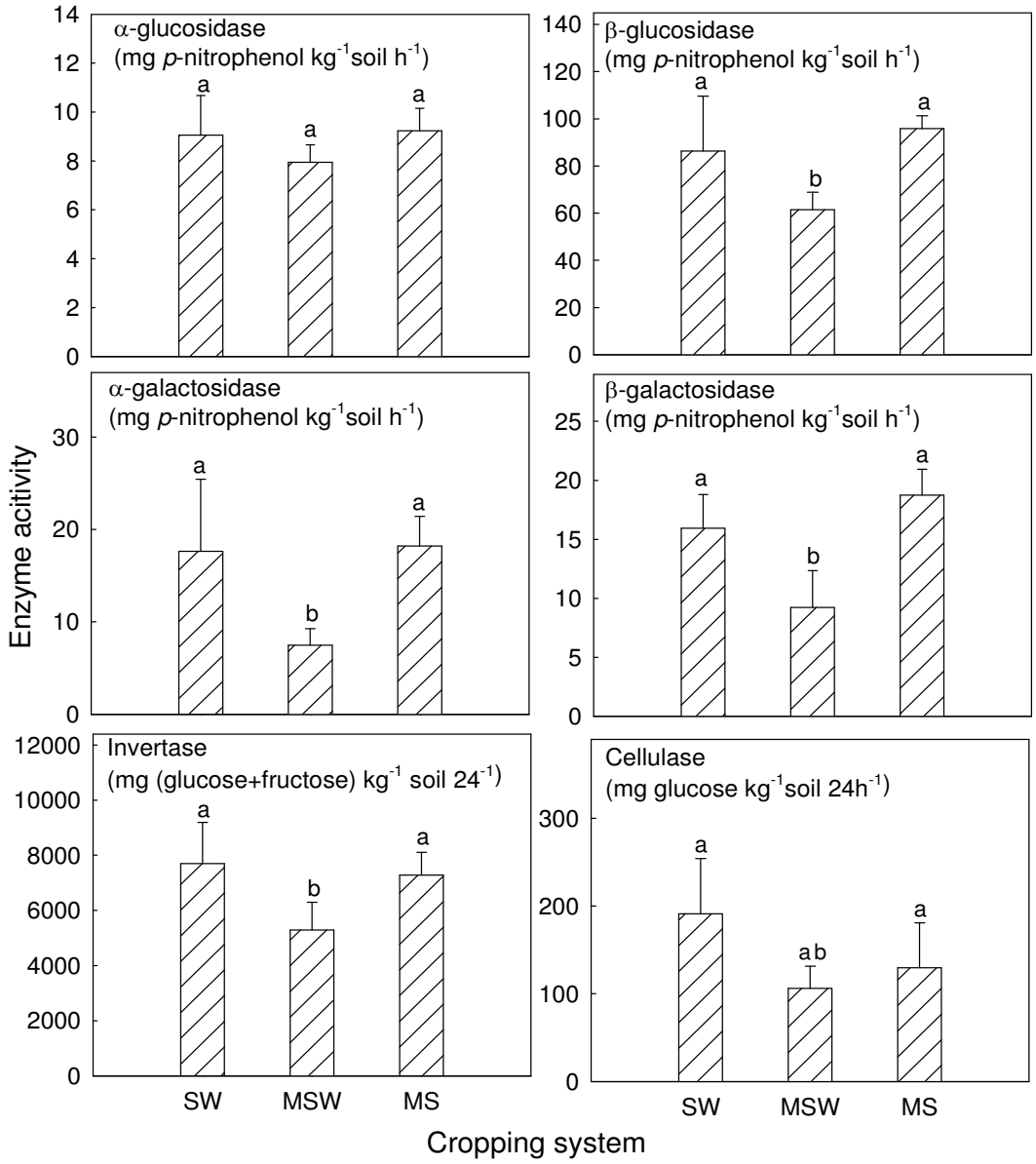

Figure 1. Effect of crop rotation on the activities of C-transforming enzymes in 0-10 cm soils. SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean. Bars indicate SE.

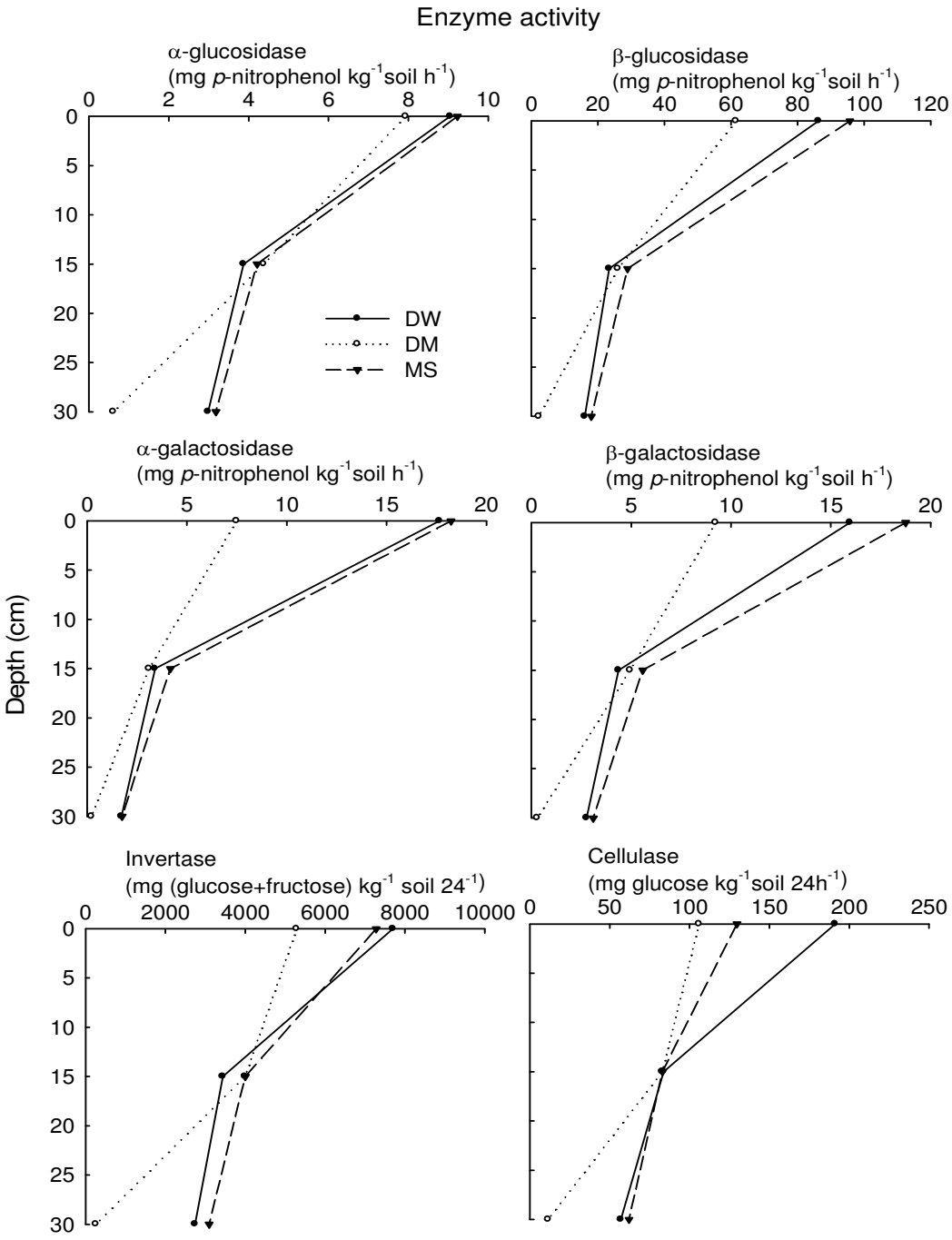

Figure 2. Distribution of C-transforming enzymes with 0-30 cm soil depths. SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean. Bars indicate SE.

Table 3. Correlation matrix (r) of soil chemical and microbial properties across the treatments and the depths.

	α-gluco -sidase	β-gluco -sidase	α-galacoto -sidase	β-galacoto -sidase	Invertase	Cellulase	pH	Total N	C_{mic}
β-glu	0.96***								
α -gala	0.90***	0.94***							
β -gala	0.93***	0.97***	0.94***						
Inver.	0.90***	0.94***	0.90***	0.92***					
Cell.	0.76***	0.75***	0.84***	0.72***	0.75***				
pH	0.04	0.14	0.22	0.14	0.17	0.1			
total N	0.89***	0.87***	0.87***	0.84***	0.83***	0.80***	0.09		
C_{mic}	0.27	0.27	0.40*	0.33*	0.33*	0.38*	0.31	0.39*	
N_{mic}	0.50**	0.47**	0.49***	0.46**	0.50**	0.59***	0.07	0.59***	0.13

Significance levels of correlations: * $p< 0.05$; ** $p< 0.01$; *** $p < 0.001$;

C_{mic} and N_{mic} mean microbial biomass C and N, respectively.

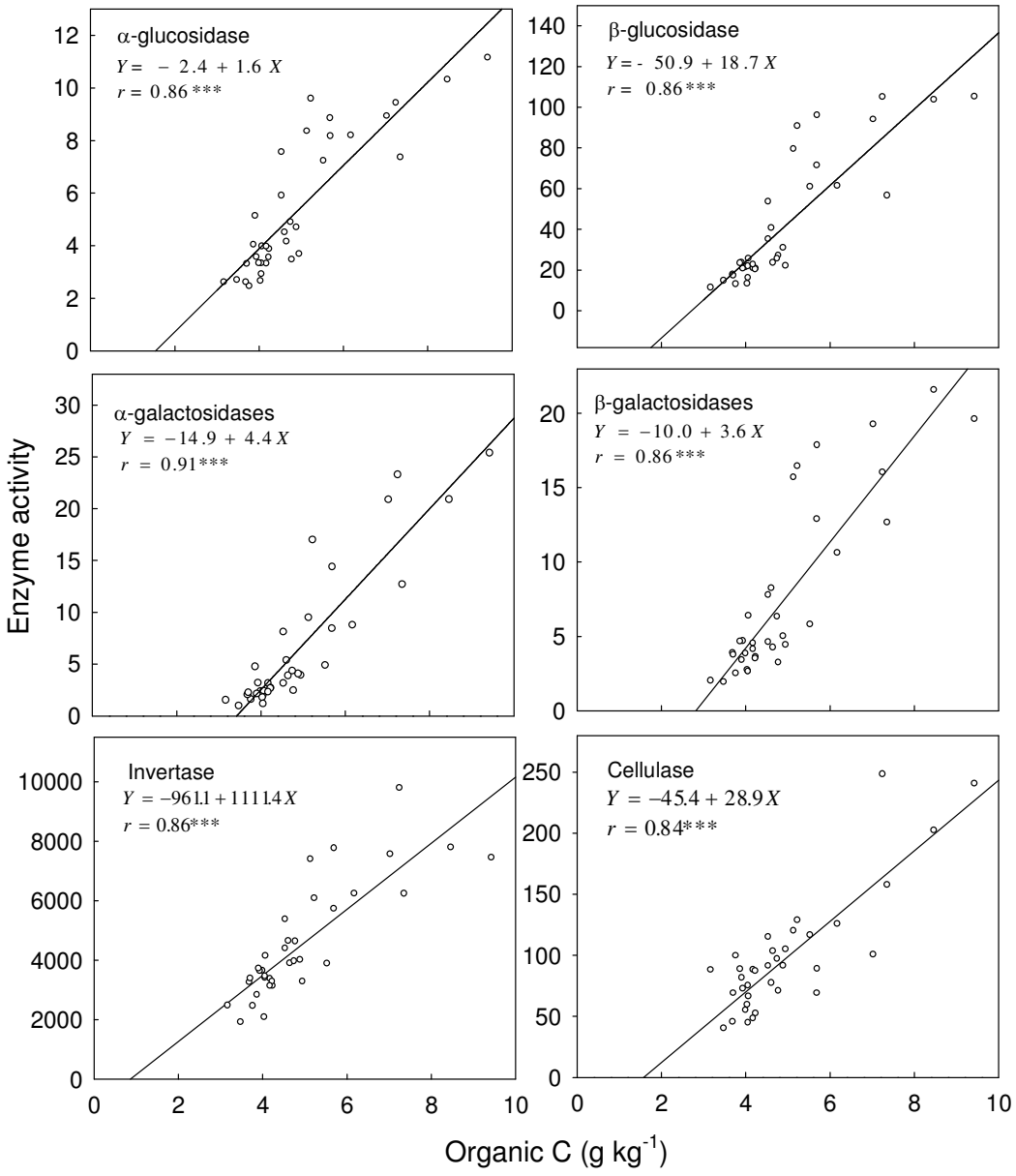

Figure 3. Correlation between activities of C-transforming enzyme and organic C content in 0-30 cm soil depths. Activities of α- and β-glucosidase, α- and β-galactosidase are expressed as mg *p*-nitrophenol kg^{-1} soil h^{-1}, of invertase as mg (glucose+fructose) kg^{-1} soil $24h^{-1}$, and of cellulase as mg glucose kg^{-1} soil $24h^{-1}$.

between enzyme activities and microbial biomass, C_{mic} and N_{mic}, were not as strong, with r values ranging from 0.27 to 0.59***. Microbial biomass contents were significantly correlated with Organic C content, with r values 0.49** and 0.59*** for C_{mic} and N_{mic} respectively (data not shown).

The ratios of organic C to total N in the top 10 cm soils were significantly different among cropping systems, but not in the 10-30 cm subsurface soils (Table 2). When the organic C to total N ratios within a cropping system between different soil layers were compared, the ratios were significantly higher in the top 10 cm soils for the SW and MS systems. These ratios for MSW were not significantly different in the three soil depths tested. Contrasting to the ratios of organic C and total N, ratios of C_{mic} to N_{mic} varied widely, ranging from 6.9 to 17.2 (Table 2). By average, the top 10 cm soils had the lowest ratios and the middle 10-20 cm soils had the highest ratios. Ratios of C_{mic} and N_{mic} were not significantly different within the same soil depth among crop rotations or between different depths within a cropping system. In the top 10 cm soils, ratios of C_{mic} to organic C and N_{mic} to total N were not significantly affected by cropping systems (data not shown). In general, ratios of enzyme activities to C_{mic} were not affected by cropping systems significantly (data not shown). This ratio for α- and β-galactosidase in the MS system was higher than in SW and significantly higher than in MSW. However, this trend was not shown for other tested enzymes.

The interrelationships among soil chemical and microbiological properties were further evaluated by PCA. The obtained results were expressed by PC1 and PC2, which explained 77 % of the total variances (Table. 4). PC1, accounts

to 63 % of the total variance, was positively correlated with most of the variables tested and contributed mostly by activities of α- and β-galactosidase, α- and β-glucosidase and invertase. PC2 explained 14 % of the total variance and was contributed mostly by organic C, total N, microbial biomass C and N and cellulase activity. When the principal scores of soil variables tested were plotted against different crop rotations, SW showed somewhat close relationship with PC2, while MSW and MS were not clearly related to the principal axes (Figure 4).

The interrelationships between the different ratios of soil chemical and microbial properties were evaluated using factor analysis. Three factor loadings explained 86 % of the total variation (Table 5). Factor I, which accounts for 51 % of the total variance, was defined by the ratios of enzyme activities to C_{mic} and C_{mic} to organic C. Factor II, which accounts for 18 % of the total variance, was defined by the ratios of C_{mic} to N_{mic} and N_{mic} to N. The ratios of α-galactosidase to C_{mic} and organic C to total N were contributed to factor III which explained 17% of total variance. When the principal scores of the different ratios of soil chemical and microbial properties were plotted against different crop rotations, three cropping systems were not clearly related to any of the three factors (data not shown).

Table 4. Principle component loadings of soil parameters tested.

	PC1	PC2
β -glucosidase	**0.97**	0.16
β -galactosidase	**0.89**	0.23
α -galactosidase	**0.84**	0.50
α -glucosidase	**0.74**	0.43
Invertase	**0.74**	0.25
Cellulase	0.42	**0.66**
Organic C	0.42	**0.91**
Total N	0.30	**0.94**
Microbial biomass C	0.35	**0.59**
Microbial biomass N	0.03	**0.42**
Eigenvalue	6.31	1.43
Explained Variance (%)	63%	14%

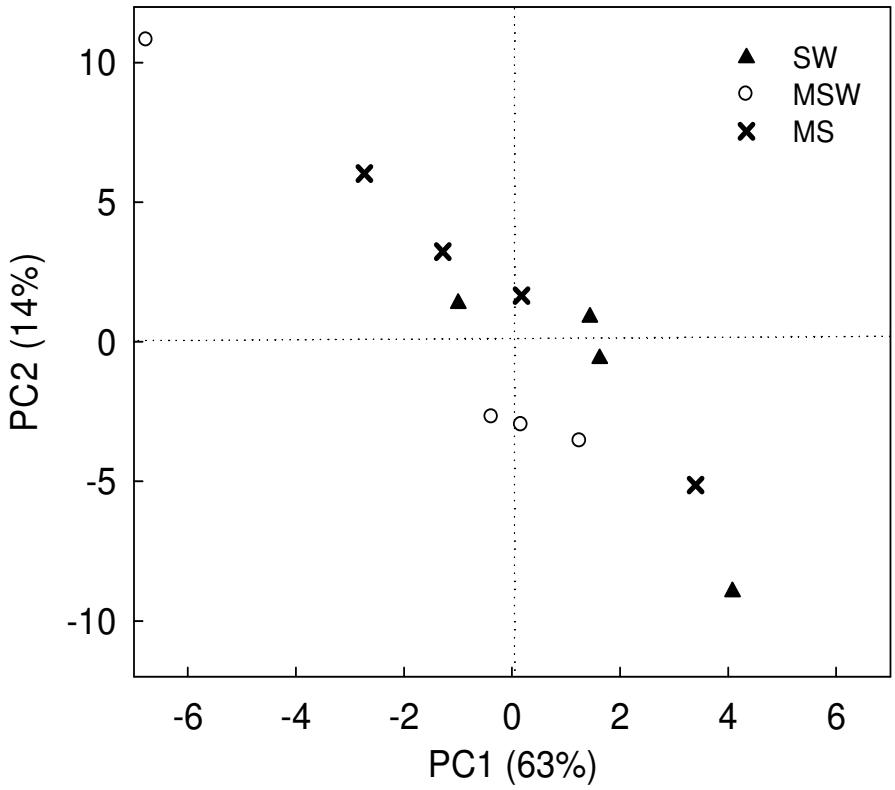

Figure 4. Factor scores of soil chemical and microbial properties and C-transforming enzyme activities against cropping systems. SW: Soybean/Wheat, MSW: Modified Soybean/ Wheat, MS: Monocrop Soybean.

Table 5. Factor loadings of the different ratios of soil chemical and microbial properties after varimax rotation.

	Factor I	Factor II	Factor III
α-glucosidase/C_{mic}	-0.67	**0.46**	-0.52
β-glucosidase/C_{mic}	-0.56	**0.77**	-0.26
α -galactosidase/C_{mic}	-0.13	**0.73**	0.48
β-galactosidase/C_{mic}	-0.19	**0.91**	0.03
invertase/C_{mic}	-0.48	**0.72**	-0.21
Cellulase/C_{mic}	**-0.71**	0.21	0.11
C_{mic}/ organic C	**0.93**	-0.36	0.09
C_{mic}/N_{mic}	**0.73**	-0.10	0.02
Organic C/ total N	0.01	0.02	**0.94**
Eigen value	5.06	1.69	1.15
Explained Variance (%)	56 %	19 %	13 %

4. Discussion

4.1. Soil chemical properties

The significantly lower pH values in the top 10 cm soils of MSW system when compared with MS and SW systems suggested that either early season soybean in the cropping systems could have lead to soil acidification or late season soybean reduced the rate of soil acidification. In this study, all soybeans planted in the MS and SW systems were late season soybeans, while those in the MSW were early and late season soybeans in alternative years. Studies by Godsey et al. (2007) showed that continuous soybean and wheat-soybean rotations reduced the rate of soil acidification compared with continuous wheat. In their study, late season soybeans were planted.

Both soybean and wheat plants were suggested to be exhaustive crops that deplete soil organic matter and nutrients (Swamp et al., 2000). Following over 20 years of rice-rice, maize-wheat, and soybean-wheat-maize rotations, the lowest soil organic C was found in soils under soybean-wheat rotation. Of these two crops, wheat produced more residues addition to soil than soybean. In a 10-year study, Wood and Edward (1992) found that the average annual soybean residue addition to soil was 2.4 Mg ha^{-1}, while that of wheat was 9.6 Mg ha^{-1}. The quality of crop residues also affects soil organic C content. Wheat residues have higher C/N ratios than the soybean residuals (Edwards et al., 1992). High C/N residues are more resistant to decomposition leading to accumulating soil organic matter in soil. Differences in organic C and N contents resulting from

different cropping systems may be also related to the frequency of tillage. Tillage was applied once per year in SW, comparing with three times per two years in MSW and three times per year in MS. Incorporate residues into soil by disking and plowing often lead to hastening decomposition. Therefore, it was not surprising that no-till led to increase in the content of organic C from 10 g kg^{-1} to 15 g kg^{-1} following 10 years practices (Edwards et al., 1992). When compared with tilled soils in a 30-year study, organic C contents were 2.3 g kg^{-1} higher in no-till soils (Godsey et al., 2007). Deng and Tabatabai (1996a) also reported a greater organic C content under no-till than moldboard plow. However, impacts of crop rotations on soil organic C and other nutrients were mainly on the top 10 cm soils, which were also observed by Wood and Edwards (1992).

4.2. Microbial biomass

The observed limited impacts of crop rotations on microbial biomass have been reported in other studies. In a 20-year study of soybean-wheat, maize-wheat, and cotton-wheat rotations, Balota et al. (2003) found that crop rotation did not have significant effects on the contents of C_{mic} and N_{mic} in 0-5 cm soils. Franchini et al. (2007) did not find significant difference in microbial biomass following five years of crop rotations involving soybean, maize, wheat, lupine, and oat. On the contrary, Moore et al. (2000) found that crop rotations involving corn, soybean, oats and alfalfa had significant influence on microbial biomass. The inconsistent results reported may be attributed to crop type as well as the sampling time. Microbial biomass content showed marked seasonal fluctuation in soils under continuous wheat, continuous wheat-soybean, or wheat-soybean-

sorghum rotation and was highest during flowering time of wheat growing season (Franzluebbers et al., 1994). In a winter wheat cultivated field, Lynch and Panting (1980) found that microbial biomass increased from May to July and decreased from August to April. Soil samples in this study were taken in April; those of Balota et al. (2003) were taken in August; and those by Moore et al. (2000) were taken in May and July. Sampling time could be another important factor contributing to the inconsistent result from these studies.

Of the cropping systems evaluated, SW provided great amount and diversity of residue to soil, but had least tillage. As a result, soils under SW had higher C_{mic} and N_{mic} contents. The close relationship between C_{mic} value and the quantity of crop residue input has been reported in previous studies (Moore et al., 2000). Crop residues stimulate microbial growth and promote the C_{mic} fraction of soil organic matter. Corn and wheat contribute more crop residues to soil than soybean (Wood and Edwards, 1992), and thus their involvements in cropping systems often enhance C_{mic} and N_{mic} contents (Moore et al., 2000). Regardless of the quality and quantity of crop residues addition, tillage promotes organic matter decomposition and accelerates the decline of organic C contents. Following evaluations of three crop rotations that were under till or no-till for 20 years, C_{mic} and N_{mic} contents in the 0-5 cm soils of the no-till systems were by average 2.0 and 1.5-fold of those in the tilled ones, respectively (Balota et al., 2003). Alvarez et al. (1995) also reported that microbial biomass C contents in 0-5 cm soils of the no-till systems were about twice of those under tillage.

4.3. Enzyme activities

The observed significant impacts of cropping systems on enzyme activities in surface soils are consistent with those found in other studies (Dodor and Tabatabai, 2005; Klose and Tabatabai, 2000). The lower activities of C-transforming enzymes in MSW may be due to relatively limited residue input and exasperate frequency of tillage. Soil disturbance and incorporation of crop residues affected the activities of glycosidase by affecting organic matter degradation and microbial growth and activities (Deng and Tabatabai, 1996b). Enzyme activities decrease with soil depths (this study, Deng and Tabatabai (1996b), because of decrease in organic matter content and microbial biomass in subsurface soils (Taylor et al., 2002).

Of soil properties evaluated, only enzyme activities in the surface soils were significantly affected by cropping systems, suggesting that enzymes are generally more sensitive in reflecting changes in soil processes. Linear regression between organic C content and enzyme activities showed that each unit change of organic C would lead to about 30 units change in celluase activities and over 1100 units changes in invertase activities (Figure 3). Similar results were reported by numerous other studies (Badiane et al., 2001; Dick, 1994; Dick, 1997; Dick et al., 1996; Dodor and Tabatabai, 2003).

4.4. Relationships among chemical and microbial properties

Although enzyme activities changed more quickly due to changes in the soil environment than organic C contents, these two soil parameters have

consistently shown to be highly correlated with each other, as supported by our results and other studies (Deng and Tabatabai, 1996b; Eivazi and Tabatabai, 1990). It is not surprising that activities of these enzymes were interrelated with each other because they all involved in C-transformations. However, it is interesting that there were little correlations between enzyme activities and pH values or microbial biomass. The little correlation between activities of some enzymes and pH were also reported by Balota et al. (2004) and Dick et al. (1988). It is well established that enzyme activities in biological systems are strongly related to pH values. Results obtained in this study suggested that pH played little role or was not the most limiting factor in governing synthesis and persistence of some enzymes in the soil environment. On the other hand, some other enzymes, such as activities of acid and alkaline phosphatase (Dick et al., 1988) and glucosidas and galactosidase, were shown to be significantly correlated with soil pH value (Acosta-Martínez and Tabatabai, 2000). The relatively weak relationships between enzyme activities and microbial biomass suggested that activities of most C-transforming enzymes in these soils might have been predominantly originated from accumulated enzymes that were free of microbial cells (Deng et al., 2006).

Not only cropping systems had significant impacts to soil enzyme activities, but also to microbial community composition and structure. The ratios of C_{mic} to N_{mic} were higher in the subsurface than surface soils, suggesting that microbial community composition shifted and there was increased dominance of fungi in 10-30 cm soils. Neville et al. (2002) also reported that fungal colonization

was higher in subsurface soils than surface soils. In this study, predominance of fungi coincides with lower soil C:N ratios in subsurface soils, supporting previous findings that fungi are predominant decomposers of recalcitrant organics (Deacon et al., 2006).

In an environmental study involving multiple variables, the complex relationships and drivers of ecosystem processes and functions can be revealed through multivariate analysis. Of the 10 variables evaluated, PC1 accounted for 63% of the total variance and was attributed mostly by C-transforming enzymes that release small molecular weight sugars. This suggested that activities of these enzymes were drivers in these soil environments. PC2 was loaded mostly by cellulase activity, organic C, total N, and microbial biomass C and N, indicating the importance of cellulase in breaking down complex carbohydrates and the dependence of microbes on organic matter and total N in soil. The close relationship between SW and PC2 indicated that cellulase activity, organic C, total N, and microbial biomass C and N were drivers in ecosystem processes and functions under this crop rotation (Figure 4). On the other hand, soil processes and functions in the MSW system were governed by activities of C-transforming enzymes that release small molecular weight sugars.

At the population/process level, the ability of the microbial community to degrade complex carbohydrates (Cellulase/C_{mic}), the capacity of soil to support microbial life (C_{mic}/organic C), and microbial community structure and composition (C_{mic}/N_{mic}) were drivers of soil functions (Table 5). Soil processes are also governed, to lesser degrees, by relative activities of simple sugar

releasing enzymes to overall detected C_{mic} and by soil organic C to total N ratios. The revealed relationships appeared to be intrinsic interactions that were not cropping systems specific (data not shown).

4.5. Environmental- Economic consideration among alternative crop rotations

Crop rotations are widely recommended to strengthen sustainability of agro-ecosystems (Buman et al., 2004). However, economic considerations may hinder application of such practices. Following a 6-year economical evaluation in systems evaluated in this study, Farno et al. (2002) showed that MSW produced the greatest net income, while SW had lowest net return. To maximum economic income, MSW rotation would be the choice of all systems evaluated in this study. However, results in our study showed that MSW led to degenerated soil quality and resulted in lower organic C content, microbial biomass, and enzymes activities than SW and MS did. This suggested that higher enzyme activities and greater nutrient cycling capacity in SW and MS soils did not translate into greater economic benefit.

5. Conclusions

Of the soil variables evaluated, cellulase activity, organic C, total N, and microbial biomass C and N were drivers in ecosystem processes and functions under SW system, while activities of C-transforming enzymes that release small molecular weight sugars governed soil processes and functions in MSW system. Regardless of cropping systems evaluated, the ability of the microbial community to degrade complex carbohydrates, the capacity of soil to support microbial life,

and microbial community structure and composition predominantly drive soil processes and functions. The relative activities of simple sugar releasing enzymes to overall detected microbial biomass C and soil organic C to total N ratios were also important factors that regulate soil processes and functions. However, higher enzyme activities and greater nutrient cycling capacity in soils did not translate into greater economic benefit.

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CHAPTER IV

EFFECT OF CROP ROTATION ON ACTIVITIES OF NITROGEN-TRANSFORMING ENZYMES

Abstract

Transformation of nitrogen (N), an important plant nutrient, is governed by activities of enzymes. However, our understanding on the effects of long-term cropping rotation on activity of N-transforming enzymes in soils is still limited. This study was initiated to evaluate the effects of crop rotation on the activities of N-transforming enzymes and thus to assess whether these enzyme activities are sensitive in reflecting changes in the soil ecosystem that were induced by management practices; to reveal the complex relationships and drivers of ecosystem processes and functions through multivariate analysis; and to determine relative importance of soil basic properties with respect to activities of enzymes involved in both C and N transformations Three crop rotations, including mono-crop soybean (MS), soybean-wheat (SW), and modified soybean-wheat (MSW) involved 3-crop/2-year, were evaluated. There were four field replications for each cropping system. Thirty-six soils were sampled in 2002 in three depths, including 0-10, 10-20, and 20-30 cm. Basic soil chemical and urease, L-glutaminase, L-asparaginase, β–glucosaminidase and nitrate

reductase, were determined. Although long-term cropping system did not significantly impact the contents of total N, and microbial biomass C and N, activities of N-transforming enzyme in top 10 cm soils were significantly different among different cropping systems. The observed differences were not shown in the 10-30 cm subsurface soils. In general, activities of N-transforming enzyme were lower in soils under the MSW system and higher under the MS and SW systems. Enzyme activities decreased with increasing soil depths, with the exception of urease, L-asparaginase and L-glutaminase in MSW. Activities of most N-transforming enzymes were significantly interrelated with each other, with r values ranging from 0.34* to 0.78***. Activities of these enzymes were also significantly correlated with soil organic C content ($r > 0.55^{**}$) and microbial biomass C and N ($r > 0.35^{*}$). Activities of urease, L-asparaginase and L-glutaminase were sensitive in revealing changes induced by crop rotations and can be used to detect impacts of cropping system. Of the N-transforming enzymes tested, β-glucosaminidase played a more dominant role contributing to nutrient cycling and dynamics of different cropping systems. SW management practice altered the soil environment leading to changes in microbial community composition to be less efficient in degrading complex amino-sugars. When considered all tested variables and both C- and N-transforming enzymes, nutrient availability and microbial community drive ecosystem functions and regulate soil processes.

1. Introduction

As populations increased and land source became scarcer, agriculture evolved to continuous monocropping (Reeves, 1997), leading to accelerated soil erosion, reduction of soil organic matter, and the decline in soil quality (Lal, 1998). Maintaining and improving soil quality is crucial for sustaining agricultural productivity and maintaining environmental quality for future generations of humans (Reeves, 1997). Crop rotations were shown to have a positive impact on soil quality and crop productivity (Bagayoko et al., 1992; Bullock, 1992), and thus are generating increasing interests.

Changes in soil ecosystem health and function are slow, making it challenging to evaluate effectiveness of different cropping systems in conserving soil quality and health. Commonly used soil quality indicators include chemical, biological, and biochemical parameters (Dick, 1992; Dick, 1994; Dick et al., 1988; Trasar-Cepeda et al., 2000). Of these parameters, soil enzyme activities are shown to be quick and sensitive in responding to changes in soil ecosystems (Dick, 1994; Dick, 1997). Activities of enzymes are involved in specific biochemical transformations, and thus selective activities may be indicators of specific processes or functions of interest. However, it is challenging to decide which enzyme to use in the evaluation. For C and N cycles alone, there are at least 500 enzymes involved (Schloter et al., 2003). Therefore, more studies are needed to evaluate impacts of cropping systems on activities of different enzymes involved in nutrient cycling and multiple soil processes and functions.

In the last chapter, studies were focused on C-cycling enzymes as affected by crop rotations. It has long been recognized that C- and N-cycle often coupled with each other, play predominant role in regulating nutrient transformations that governs soil productivity and function. Schloter et al. (2003) suggested N-cycling enzymes could be used indicators of soil quality.

Most widely studied N-transforming enzymes in soil are amidohydrolases, including L-asparaginase, L-glutaminase, urease, β-glucosaminidase, and nitrate reductase (Ekenler and Tabatabai, 2004). Activities of these enzymes directly or indirectly lead to the release of inorganic N for plant and microbial uptake and nutrition.

Therefore, the objective of this study was to evaluate the effects of crop rotation on the activities of five N-transforming enzymes, including L-asparaginase, L-glutaminase, urease, β-glucosaminidase, and nitrate reductase. In addition, effort was made to determine whether N-transforming enzymes are sensitive in revealing changes in soil that were induced by different cropping systems. We hypothesized that most N-transforming enzymes would more sensitive than organic C content to changes in soil ecosystems, and the degree of impact by different cropping systems would be enzyme and process specific.

2. Material and methods

2.1 Soils

The site and soils used in the research were described in Chapter III. Briefly, three cropping systems, including mono-crop soybean (MS), soybean-

wheat (SW), and modified soybean-wheat (MSW) involving 3-crop/2-year, were evaluated. Tillage was applied once per year in SW, comparing with three times per two years in MSW and three times per year in MS. No-tilling was only applied after harvesting wheat among cropping systems. Thirty-six soils were sampled in April 2002 from each treatment plot at three different depths, including 0-10 cm, 10-20 cm, and 20-30 cm. Soils were sieved to pass a 2 mm sieve immediately and stored at 4^{o}C for microbiological analyses, and some were air-dried and used for chemical analysis.

The methods of soil chemical and microbial properties such soil pH, organic C, total N, soil microbial biomass C (C_{mic}) and N (N_{mic}) were described in chapter III. Briefly, Soil pH values rang from 5.75 to 6.22 among the depths. Contents of organic C and total N were not significantly different through the soil profiles under different cropping systems, but lower in MSW than SW and MS.

2.2 Enzyme activity

Assaying methods of N-transforming enzymes analyzed are listed in Tables 1. Generally, activities of L-asparaginase, L-glutaminase, and urease were determined based on determination of NH_4^+-N released by enzymatic reactions during incubation of soil in THAM buffer with respective substrates at 37℃ for 2 h. The NH_4^+-N released was extracted by KCl-Ag_2SO_4 solution and quantified by steam distillation. Nitrate reductase activity was assayed by the analysis of NO_2-N using a colorimetric determination following incubating the soil with 2,4-dinitrophenol and KNO_3 at 25℃ for 24 h. β–Glucosaminidase activity was determined by analyzing *p*-nitrophenol released based on colorimetric

determination when soil was incubated with acetate buffer and *p*-nitrophenyl-N-acetyl-β-D-glucosaminide at 37℃ for 1 h. The *p*-nitrophenol released was extracted by filtration after addition of $CaCl_2$ and NaOH solutions.

2.3 Statistical methods

Detail statistical analyses were described in Charpter III. Briefly, significant differences among treatments were determined by analysis of variance, and mean comparison of treatment was done by LSD ($P \leq 0.05$). Correlations between soil properties were calculated by pairwise correlation. Principal component analysis (PCA) was applied to reduce the dimensionality of tested variables, and then the obtained PC scores were plotted by Sigmaplot software (SigmaPlot, 2004).

Table 1. Methods used for the assays of N-transforming enzyme activities (modified from Deng and Tabatabai 1996).

Class/CE number	Enzyme name	Reaction	Substrate	References
1.7.99.4	Nitrate reductase	$NO_3^- \rightarrow NO_2^-$	5mM KNO_3	Abdelmagid and Tabatabai(1987)
3.2.1.30	β-glucosaminidase	*N*-acetyl-β-D-glucosamine residues→ glucosamine residues	*p*-Nitropheny-*N*-acetyl-β-D-glucosamine	Parham and Deng (2000)
3.5.1.1	L-asparaginase	L-Asparagine→L-asparate +NH_3	L-Asparagine	Frankenberger and Tabatabai(1991a)
3.5.1.2	L-Glutaminase	L-Glutamine→L-glutamate+NH_3	L-Glutamine	Frankenberger and Tabatabai(1991b)
3.5.1.5	Urease	Urea→CO_2 + 2NH_3	Urea	Tabatabai and Bremner (1972)

3. Results

3.1. Enzyme activities

In the top 10 cm soils, cropping systems had significant effects on amidohydrolases activities, but not on the activity of β–glucosaminidase, and nitrate reductase (Figure 1). Of the three amidohydrolases tested, L-glutaminase was the most predominant, exhibiting greater potential to release NH_4^+-N in soil. On average, the amount of NH_4^+-N released by L-glutaminase activities in each unit of time is about 6 fold of L-asparaginase and twice as much as of urease. Of the cropping systems evaluated, activities of N-transforming enzymes were consistently lower in the MSW soils.

In 10-30 cm subsurface soils, enzyme activities among cropping systems were not significantly different (Figure 2). Generally speaking, enzyme activities decreased with soil depths in SW and MS, with the exception of urease, L-glutaminase and L-asparaginase activities in MSW. Similar to C-transforming enzymes, the degree of impact by cropping systems was also different in different depths and different enzymes. The activities of nitrate reductase in 0-10 cm soil were low, ranging from 0.04 to 18 mg NO_2-N kg^{-1} soil $24h^{-1}$. Activities of this enzyme in the subsurface soils were practically undetectable.

3.2. Relationship among soil properties

With exception of the relationship between urease and β-glucosaminidase, significant correlations were observed among the activities of N-transforming

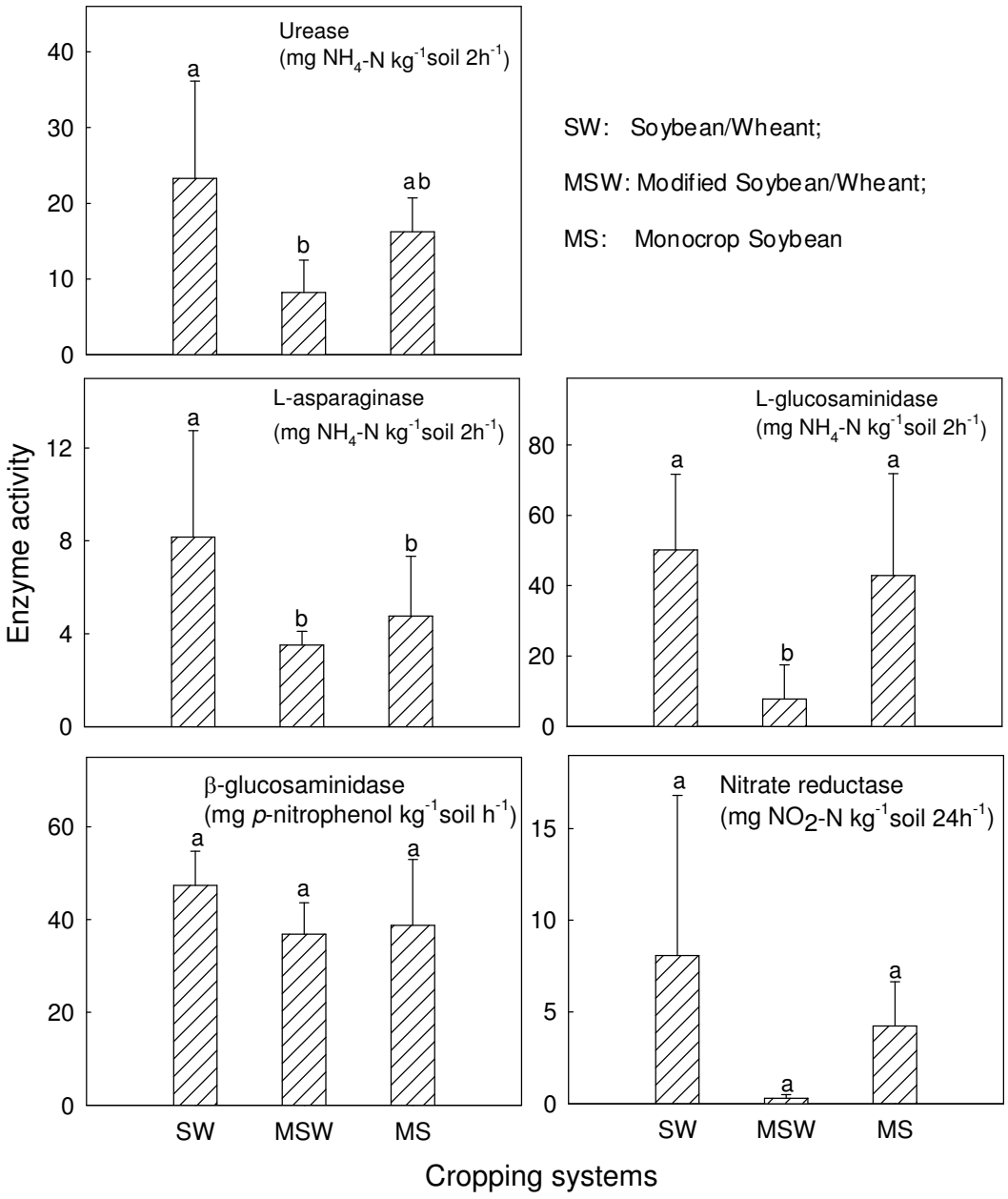

Figure 1. Effect of cropping systems on the activities of N-transforming enzymes in 0-10 cm surface soils. Bars indicate SE.

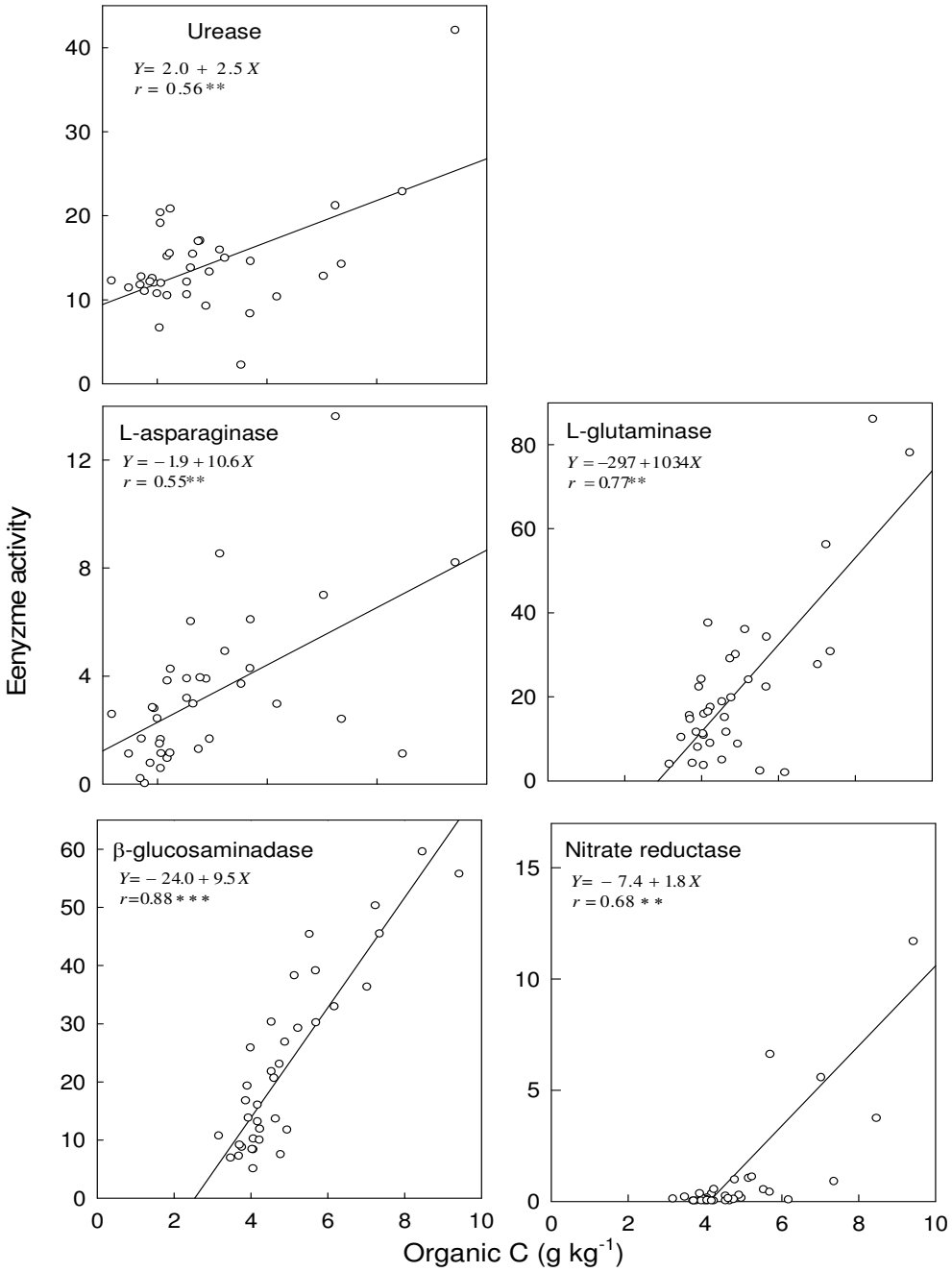

Figure 2. Distribution of N-transforming enzyme activities with 0-30 cm soil depths among cropping systems.

enzymes, with r values ranging from 0.34** to 0.78*** (Table 2). These enzyme activities were strongly correlated with organic C ($r > 0.55^{***}$) (Figure 3) and total N ($r > 0.46^{**}$) (Table 2), but showed little correlation or weak correlations with soil pH. Similar to C-transforming enzymes, activities of N-transforming enzymes were not strong correlated with microbial biomass C and N contents, with r values ranging from 0.22 to 0.55***.

When enzyme activities in the top 10 cm soils were expressed as per unit of C_{mic}, the specific activities of L-glutaminase and urease were significant different among different cropping systems (Table 3). However, such impacts of crop rotations on specific enzyme activities were not shown for activities of β-glucosaminidase, L-asparaginase and nitrate reductase. In general, specific enzyme activities were lower in MSW than MS and SW.

The interrelationships among soil chemical and microbial properties were also evaluated using factor analysis. Factor I, factor II, and factor III together accounted for 89 % of the total variances (Table. 4). Factor I, explained 61% of the total variance, was positively correlated with the activities of C_{mic}, organic C and total N. Factor II explained 16% of the total variance and was loaded by β-glucosaminidase activity, total N and N_{mic}. Factor III, accounts to 12% of the total variance, was loaded by the activity of amidohydrolases. When the factor scores were plotted against different crop rotations, SW was closely related to factor II and all four replicate SW systems were positively related to factor III. (Figure 4) MS was more closely related to factor I, while there was no clear trends for MSW (Figure 4).

Table 2. Correlation coefficient matrix among soil properties.

	Urease	L-asparaginase	L-glutaminase	β-glucosaminidase	Nitrate reductase
L-asparaginase	0.34*				
L-glutaminase	0.67***	0.46**			
β-glucosaminidase	0.31	0.59***	0.70***		
Nitrate reductase	0.57***	0.78***	0.66***	0.59***	
Total N	0.46**	0.56***	0.69***	0.90***	0.61***
Microbial biomass C	0.41*	0.24	0.41*	0.29	0.37*
Microbial biomass N	0.22	0.31	0.35*	0.55***	0.43**
pH	0.40*	0.16	0.23	0.002	0.30

Significance levels of correlations: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

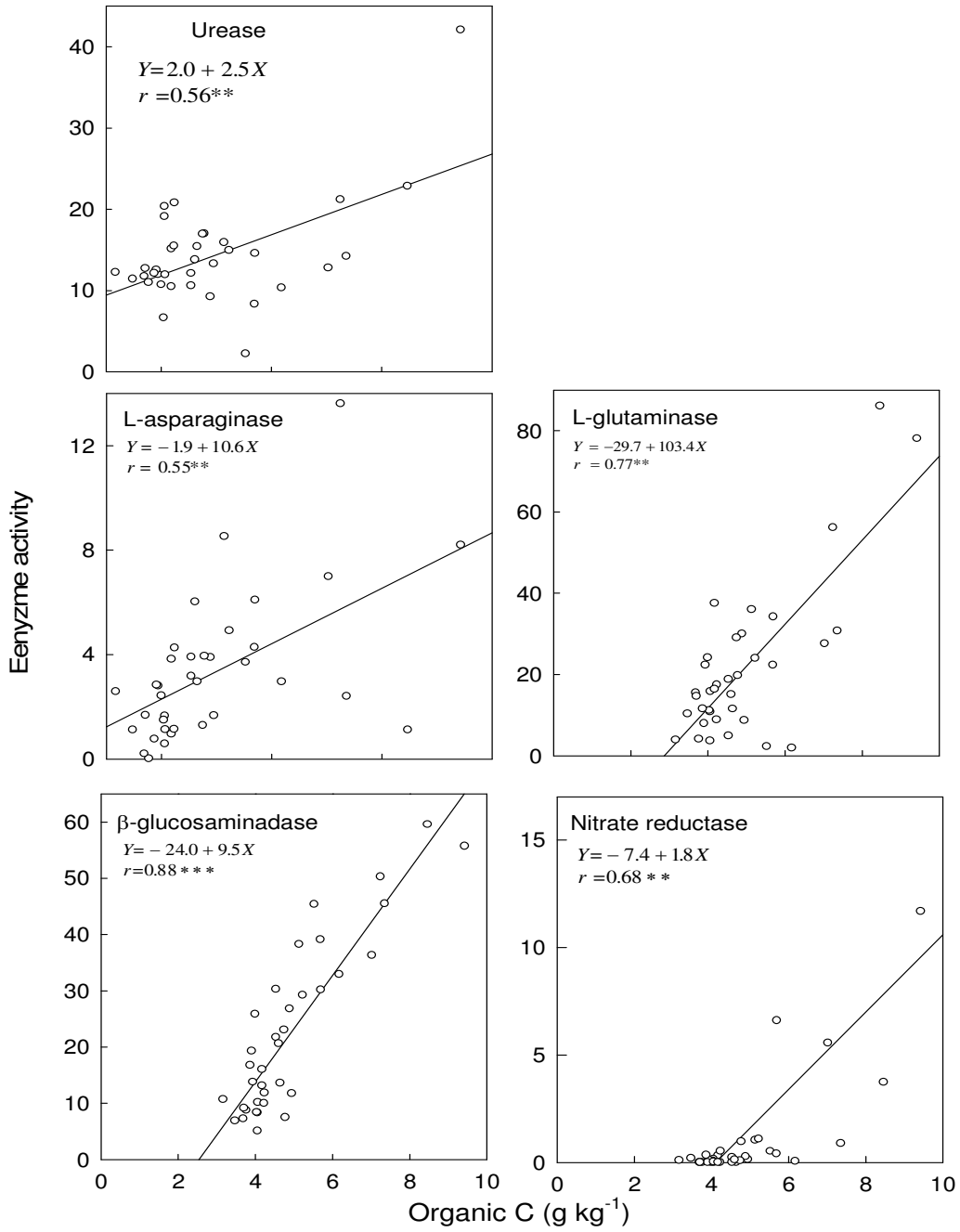

Figure 3. Correlation between the activities of N-transforming enzymes and organic C content in 0-30 cm soil depths. Activities of L-asparaginase, L-glutami-nase, and urease are expressed as mg NH_4-N kg^{-1} soil $2h^{-1}$, activity of β-glucosaminidase as mg *p*-nitrophenol kg^{-1} soil h^{-1}, and of nitrate reductase as mg NO_2^-N kg^{-1} soil $24h^{-1}$.

Table 3. The ratios of enzyme activities to microbial biomass C (C_{mic}) in 0-10 cm soils tested.

Treatments	Urease :C_{mic}	L-asp :C_{mic}	L-glu :C_{mic}	β-Gase:C_{mic} (mg *p*-nitro-phenol mg^{-1} C_{mic} h^{-1})	NR:C_{mic} (mg NO_2-N mg^{-1} C_{mic} $24h^{-1}$)
	(mg NH_4-N mg^{-1} C_{mic} $2h^{-1}$)				
SW	0.117	0.047	0.261	0.256	0.040
MSW	0.058	0.031	0.065	0.329	0.003
MS	0.105	0.031	0.270	0.243	0.027
LSD(P<0.05)	0.048	0.037	0.182	0.200	0.047

SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean. L-asp: L-asparaginase; L-glu: L-glutaminase; β-Gase: β-glucosa-minidase; NR: Nitrate reductase;

Table 4. Factor loadings of soil organic C, total N, microbial biomass, and N-transforming enzymes using factor analysis.

	Factor I	Factor II	Factor III
Organic C	**0.79**	0.57	0.22
Total N	**0.78**	**0.64**	0.05
Microbial biomass C	**0.84**	0.06	0.24
Microbial biomass N	0.04	**0.63**	-0.02
Urease	0.68	0.14	**0.74**
L-glutaminase	0.44	0.54	**0.60**
L-asparaginase	0.05	0.01	**0.46**
β-glucosaminidase	0.36	**0.85**	0.26
Cumulative variance (%)	61%	77%	89%

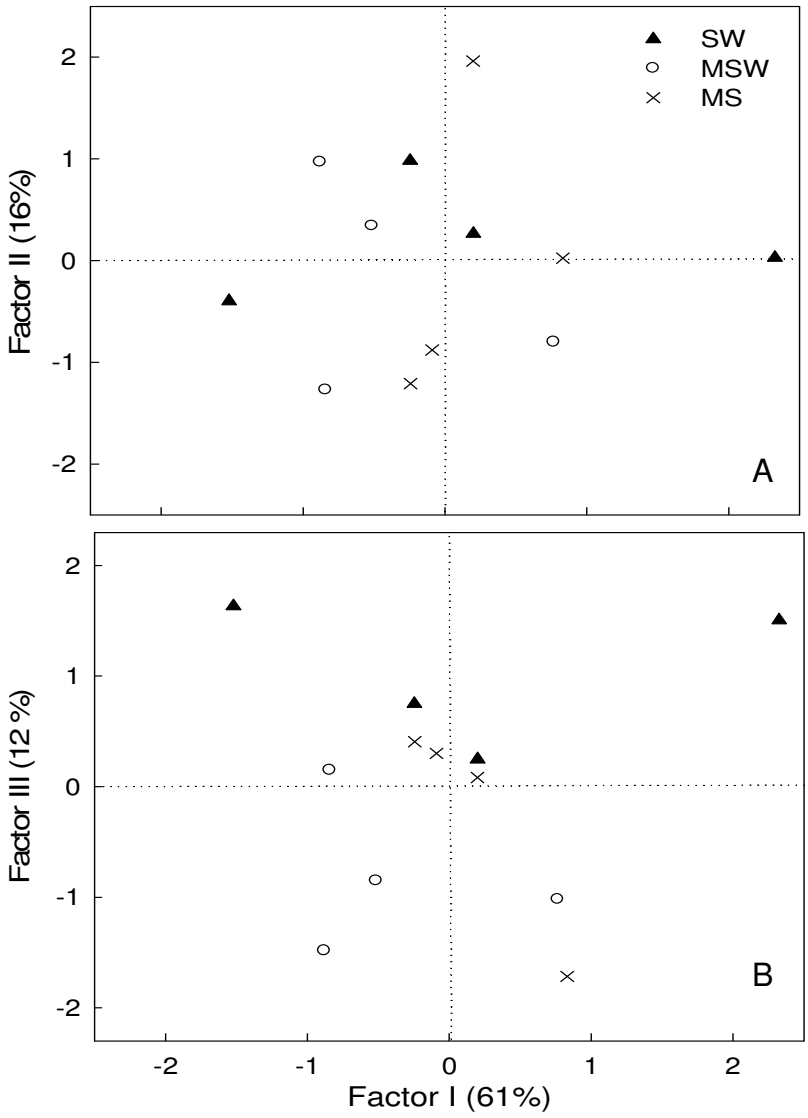

Figure 4. Factor scores of soil basic properties and N-transforming enzyme activities against cropping systems. SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean.

III (Figure 4B).

The interrelationships between the different ratios of soil chemical and microbial properties were evaluated using PCA factor analysis. Three factors explained 84 % of the total variation (Table 5). Factors I and II were almost equal importance, accounting for 70% of the total variance. Factor I was loaded by ratios of C_{mic} to organic C, C_{mic} to N_{mic}, and activities of β-glucosaminidase to C_{mic}, while factor II was loaded by ratios of organic C to total N, urease to C_{mic}, and L-glutaminase to C_{mic}. Specific activities of L-aparaginase and nitrate reductase contributed to factor III. SW system showed relatively close relationship to factor I and II, MS showed relatively close relationship to factor II and III, and MSW showed close relationship to factor III (Figure 5).

PCA was conducted using the data set including all of the basic properties and C- and N-transforming enzyme activities. Results showed that factor I was loaded with C_{mic} and activities of enzymes that release simple sugars or ammonium, with the exception of L-asparaginase (Table 6). Factor II was loaded by organic C, total N, N_{mic}, and activities of enzymes that release simple polysaccharides and amino-sugar, while factor III was loaded by pH and activities of invertase, L-asparaginase and nitrate reductase. Of the three cropping systems evaluated, SW showed somewhat close relationship to factor I, while MSW and MS were not clearly related to any of the three factors (Figure 6).

Table 5. Factor loadings of the ratios of soil chemical and microbial properties to microbial biomass C (C_{mic}).

	Factor I	Factor II	Factor III
Organic C:total N	0.12	**0.72**	-0.05
C_{mic}:organic C	**1.00**	-0.11	-0.13
C_{mic}:N_{mic}	**0.74**	-0.05	-0.03
Urease:C_{mic}	0.11	**0.81**	0.21
L-glutaminase:C_{mic}	-0.26	**0.94**	0.07
L-aparaginase:C_{mic}	-0.24	0.03	**0.98**
β-glucosaminidase:C_{mic}	**-0.85**	-0.24	0.13
Nitrate reductase:C_{mic}	0.02	0.53	**0.54**
Eigenvalue	2.95	2.67	1.13
Explained variance (%)	37 %	33 %	14 %

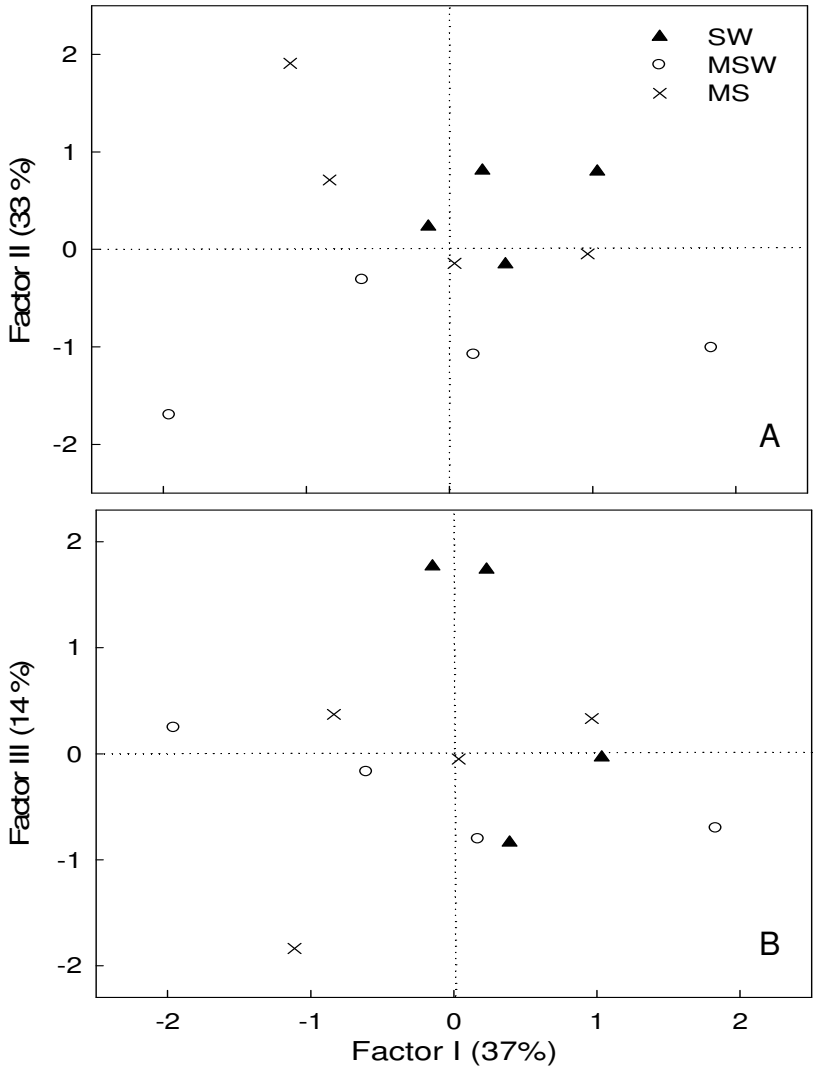

Figure 5. Factor scores of the ratios of soil basic properties and specific activities against cropping systems. SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean.

Table 6. Factor loadings of soil basic properties and C- and N-transforming enzyme activities.

	Factor I	Factor II	Factor III
Soil pH	0.34	0.18	**0.51**
Organic C	0.62	**0.76**	0.08
Total N	0.54	**0.81**	-0.03
Microbil biomass C	**0.52**	0.36	0.13
Microbil biomass N	-0.06	**0.56**	0.16
α-glucosidase	**0.81**	0.29	0.22
β-glucosidase	**0.78**	0.08	0.51
α- galactosidase	**0.80**	0.31	0.47
β-galactosidase	**0.90**	0.05	0.27
Invertase	**0.51**	0.17	**0.69**
Cellulase	0.37	**0.70**	0.40
Urease	**0.73**	0.40	0.27
L-asparaginase	0.10	-0.01	**0.93**
L-glutaminase	**0.73**	0.50	0.27
Nitrate reductase	0.32	0.37	**0.82**
β-glucosaminadase	0.30	**0.86**	0.10
Eigenvalue	9.53	2.09	1.39
Explained variance (%)	60 %	13 %	9 %

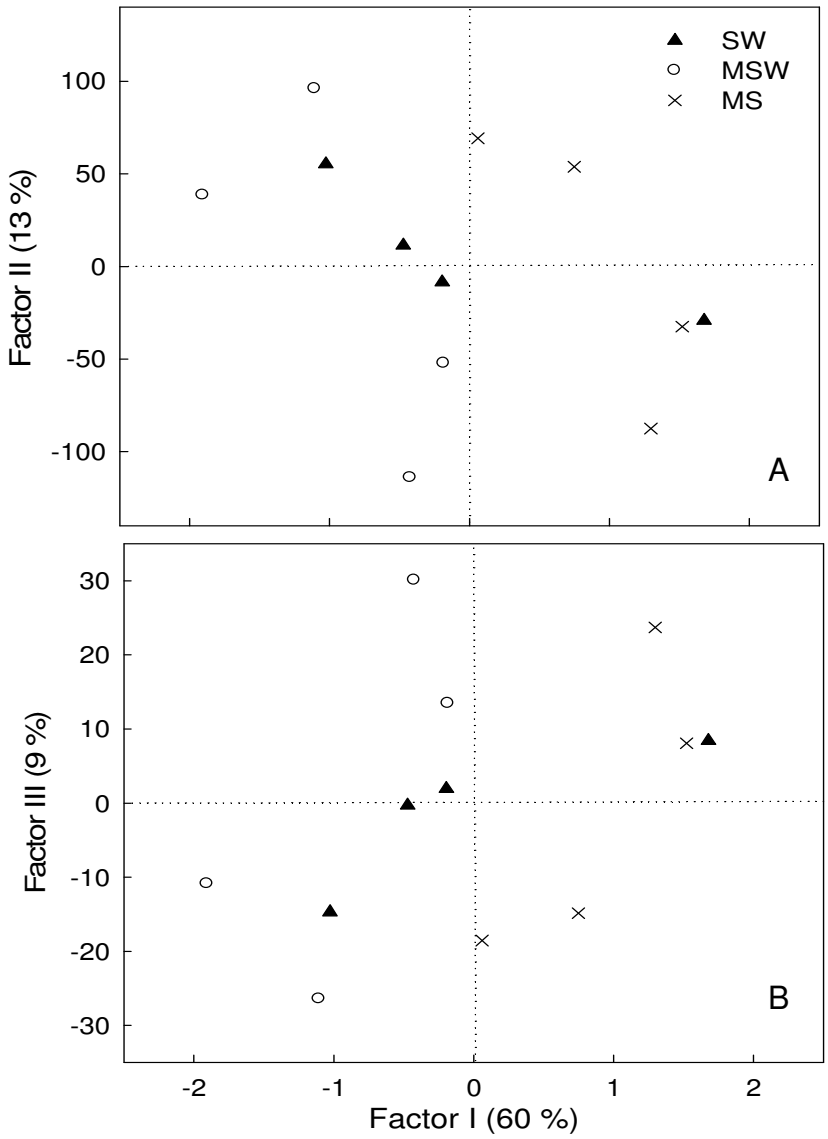

Figure 6. Factor scores of all soil variables tested, including soil organic C, total N, pH, microbial biomass and C- and N-transforming enzyme activities, against cropping systems. SW: Soybean/Wheat, MSW: Modified Soybean/Wheat, MS: Monocrop Soybean.

4. Discussion

4.1. Enzyme activities

The impacts of cropping systems on N-transforming enzymes were similar to those on C-transforming enzymes (Chapter III). The observed significant impacts of cropping system on amidohydrolase activities are consistent with those reported in other studies (Klose and Tabatabai, 2000; Miller and Dick, 1995). These effects were significant only in 0-10 cm surface soils. As discussed in Chapter III, the lower enzyme activities in MSW and MS may be due to little residue cover during fallow period and frequent tillage for the two systems, and limited diversity of crop residue for MS. During fallow period, soils were more susceptible to wind and water erosion, which often lead to reduction in soil organic matter content (Acosta-Martinez et al., 2003). Comparing to MS system, crop rotations provide the opportunity to return different amount and quality of residues, which could influence microbial activity and dynamics (Dodor and Tabatabai, 2003). The activity of β-glucosaminidase was higher under crop rotation than under continuous cotton (Acosta-Martinez et al., 2003). Compared to tilled soils, Dick (1984) reported that no-till soils significantly affected urease activity in the 0-7.5 cm soil. Studies showed that soil management practice affected enzyme activities by affecting the quantity and quality of organic matter, and its distribution over soil depth (Ekenler and Tabatabai, 2004).

Of the N-transforming enzyme activities tested, the significantly higher L-glutaminase activity indicated that this activity was most predominant. Similar

findings were reported by Ekenler and Tabatabai (2004), who examined six N-transforming enzymes and showed that $NH4^+$ released was mostly derived from hydrolysis of amides such as glutamine and asparagine. They concluded that glutaminase and asparaginase have greater catalytic efficiency than amidase, urease, arylamidase, and L-aspartase. However, L-asparaginase activities in this study by average were about half of those reported by Ekenler and Tabatabai (2004).

Nitrate reductase catalyzes the reduction of NO_3^- to NO_2^- and plays a crucial role in denitrification. Therefore activities of nitrate reductase have been proposed to be used an indicator to estimate the denitrification potential in soil (Singh and Kumar, 2008). Nitrate reductase activity was low in all the soils tested, and was practically undetectable in subsurface soils. The low nitrate reductase activity was in part related to low organic C and total N contents (Abdelmagid and Tabatabai, 1987). Another important factor is tillage because soil redox state affects synthesis of nitrate reductase. Arshad et al. (1999) and Singh and Kumar (2008) found that nitrate reductase activity was significantly higher in undisturbed or no-tilled soils than in cultivated soils.

Although different cropping systems did not lead to significantly different soil organic C and N contents, they did result in significantly different aminidohydrolase activities. This, again, suggested that enzyme activities are more sensitive in reflecting changes in soil processes than organic C and N contents. Of the N-transforming enzymes tested, L-glutaminase was the most

sensitive, showing 10.3 units changes for each unit change of organic C content (Figure 3).

4.2. Relationships among soil chemical, microbial biomass and enzyme activities

Unlike C-transforming enzymes and most other N-transforming enzymes tested, activities of β-glucosaminidase and urease were not strongly correlated with each other. This is surprising because both of these enzyme activities were significantly correlated with soil organic C contents. Many researchers proposed that intercorrelations between soil enzymes were primarily due to their close relationships with organic matter content (Deng and Tabatabai, 1996a; Eivazi and Tabatabai, 1990). Garcia et al. (1994) suggested that the management practices affected all of the enzyme activities equally. The limited relationship between β-glucosaminidase and urease could be due to the fact that these two enzymes originated from distinctly different groups of microbes. This is supported by the strong correlation between β-glucosaminidase activities and N_{mic}, but not with C_{mic}, suggesting bacteria as dominant contributors of this enzyme. On the contrary, urease activity was significantly correlated to C_{mic}, but not with N_{mic}, suggesting predominant contribution of this enzyme by fungal species.

Similar to C-transforming enzymes discussed in Chapter III, activities of most tested N-transforming enzymes were not strongly correlated with pH values. This suggested that pH did not play a dominant role governing synthesis and persistence of most enzymes in the soil environment. Urease activity on the other hand was more sensitive to soil pH, which is consistent with several other studies (Acosta-Martínez and Tabatabai, 2000; Deng and Tabatabai, 1996b).

Not only cropping systems had significant effects on enzyme activities, but also on specific activity of enzymes (ratios of enzyme activities to C_{mic}). Specific activity combines two different measurements in a single criterion, which indicate changes in microbiological activity (Landi et al., 2000). In this study, crop rotation significantly affected specific activities of urease and L-glutaminase, indicating that synthesis of these two enzymes was more sensitive to environmental conditions than others tested.

Among basic soil properties and N-transforming enzymes evaluated, microbial biomass and C and N contents, especially the available nutrients, were drivers of ecosystem processes and functions (Table 4). Of the N-transforming enzymes tested, β-glucosaminidase played a more dominant role contributing to nutrient cycling and dynamics of different cropping systems. The relatively close relationships between factor I and MS suggested that this cropping system led to C and N nutritional stress leading to impacting microbial growth and function. SW was more closely related to factor II, indicating limitations of complex N content, amino-sugar, and/or microbial biomass of low C:N ratios such as bacteria. Microbial biomass could be drivers of nutrient cycling, but could also serve as the mobile source of N nutrition in the system. The relatively close relationship between MS and factor III suggested that MS drove limitation of aminohydrolases in the system. Based on interactions of all variables, the capacity of soil to support microbial life (C_{mic}/organic C), microbial community structure and composition (C_{mic}/N_{mic}), and the ability of the microbial community to degrade complex carbohydrates (β-glucosaminidase /C_{mic} ratios) were drivers of soil

functions (Table 5). This result is similar to those obtained in the evaluation of C-transforming enzymes. Moreover, soil organic matter quality (organic C/total N) and specific activities of urease and L-glutamianase were almost equally important in governing soil process. SW management practice altered the soil environment, leading to changes in microbial community composition to be less efficient in degrading complex amino-sugars. This is evidenced by its close relationship with factor I (Table 5 and Figure 5).

PCA for all tested variables in this study was conducted to determine relative importance of soil basic properties with respect to activities of enzymes involved in both C and N transformations. In particular, we were interested in revealing whether C- or N-transforming enzymes were drivers of soil ecosystem processes and how cropping systems alter the complex relationships and the relative importance of the tested variables. When all tested variables and both C- and N-transforming enzymes were included in the analysis, C_{mic} and activities of enzymes that release simple sugars or inorganic N were the drivers of the soil ecosystems. This suggested that nutrient availability and microbial community drive ecosystem functions and regulate soil processes. The next important parameters were soil organic matter and activities of enzymes that release soluble polysaccharides because these parameters serve as the reservoir of the available nutrient pools. Although soils under SW systems had relatively high microbial biomass and significantly higher activities of C- and N-transforming enzymes of the three cropping systems evaluated (Figure 1), SW appeared to be limited by mobile C pool and supply of readily available nutrients (Table 6). This

is evidenced by the relative close relationship between SW and factor I (Figure 6). However, none of the tested variables were predominant drivers in MSW and MS cropping systems.

Although organic C content does not change quickly in response to changes in the soil environment, it is a well accepted indicator of soil quality and property. Recently, de la Paz Jimenez et al. (2002) proposed that a mathematical model can be constructed based on measurable soil parameters to provide a quantitative measure of important soil parameters that influence soil organic C contents. Of the variable tested in this study, the same five variables were selected in both stepwise and forward procedure in SAS program, which gave the following model:

Organic C= -1.77 + 11.04 total N - 0.26 α-glucosidase + 0.06 β-galacosidase +

0.01 urease + 0.03 cellulase

The obtained model suggested that total N content was most closed related to soil organic C content. Of enzyme activities evaluated, α-glucosidase was the most influential to soil organic C content, followed by β-galacosidase, and then urease and cellulase.

5. Conclusions

Activities of most N-transforming enzymes were significantly affected by long-term cropping systems and were shown to be more sensitive indicators in detecting early changes in soils that were induced by management practices. The highest activities were under soybean-wheat rotation, and lowest under modified soybean-wheat rotation. Of the N-transforming enzymes tested, β-

glucosaminidase played a more dominant role contributing to nutrient cycling and dynamics of different cropping systems. Multivariate analysis of all tested soil variables and enzyme activities suggested that nutrient availability and microbial community drive ecosystem function and regulate soil processes. SW management practice altered soil environment, leading to limited supply of mobile C and nutrients and changing microbial community composition to be less efficient in degrading complex organic substances, such as amino-sugars. However, none of the tested variables were predominant drivers in MSW and MS cropping systems. Based on constructed mathematical model, soil organic C content was most closed related to total N content, while activity of α-glucosidase was most influential of all tested enzymes on soil organic C content.

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CHAPTER V

SUMMARY AND CONCLUSIONS

Results from this studies indicated that contents of soil organic C, total N, and microbial biomass were not significantly different among three cropping systems following 30 years management practices. However, activities of most C- and N-transforming enzymes were significantly different among cropping systems in the top 10 cm soils, suggesting that activities of these enzymes were sensitive in responding to changes induced by crop rotations. Enzyme activities were generally higher under soybean-wheat rotation, lower under modified soybean-wheat rotation, and decreased with increasing soil depths. Activities of most tested enzymes were significantly correlated with organic carbon content and interrelated with each other.

Of the C-transforming enzymes tested, enzymes that release small molecular weight sugars were drivers in these soil environments. Of the N-transforming enzymes tested, β-glucosaminidase played a more predominant role contributing to nutrient cycling and dynamics of different cropping systems. Multivariate analysis of soil and microbial variables involved in C- or N-cycling suggested that the ability of the microbial community to degrade complex carbohydrates and amino-sugars (Cellulase/C_{mic}, β-glucosaminidase/C_{mic}), the capacity of soil to support microbial life (C_{mic}/organic C), and microbial community

structure and composition (C_{mic}/N_{mic}) were drivers of soil ecosystem processes and functions. The revealed relationships appeared to be intrinsic interactions that were not cropping system specific. Multivariate analysis of all soil variables and enzyme activities tested suggested that nutrient availability and microbial community drive ecosystem function and regulate soil processes. Soybean-Wheat management practice altered soil environment, leading to limited supply of mobile C and nutrients and changing microbial community composition to be less efficient in degrading complex organic substances. However, none of the tested variables were predominant drivers in the MSW or MS cropping systems. Based on a constructed mathematical model, soil organic C content was most closely related to total N content. Activity of α-glucosidase was most influential of all tested enzymes on soil organic C content.

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Master of Science

Thesis: ORGANIC MATTER CONTENT, MICROBIAL BIOMASS, AND ENZYME ACTIVITIES: INTERACTION AND VARIABILITY IN SOILS UNDER LONG-TERM CROP ROTATION

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Scope and Method of Study: The objectives of the study were (1) to evaluate the effects of long-term crop rotations on chemical, microbial and biochemical properties, (2) to evaluate sensitivity of soil parameters to potential changes in soils, and (3) to reveal the complex relationships of soil parameters and drivers of ecosystem processes and functions. Cropping systems tested included mono-crop soybean, soybean-wheat rotation, and modified soybean-wheat system involving 3-crop/2-year. Thirty-six soils were taken from three different depths (0-10, 10-20, 20-30 cm) with four replications of each cropping system. Soil chemical properties, microbial biomass C and N contents, and enzyme activities involved C- and N-cycling were tested.

Findings and Conclusions: The Contents of soil organic C, total N, and microbial biomass C (C_{mic}) and N (N_{mic}) were not significantly different among the three cropping systems. However, activities of most C- and N-transforming enzymes were significantly different in 0-10 cm soils, suggesting that activities of these enzymes were sensitive in responding to changes induced by cropping systems. Enzyme activities were generally higher under soybean-wheat rotation and lower under modified soybean-wheat rotation, and decreased with increasing soil depths. Activities of most tested enzymes were significantly correlated with organic carbon content and interrelated with each other. Results also suggested that the ability of the microbial community to degrade complex carbohydrates and amino-sugars, the capacity of soil to support microbial life (C_{mic}/organic C), and microbial community structure and composition (C_{mic}/N_{mic}) regulated soil ecosystem processes and function. Multivariate analysis of all tested soil variables suggested that nutrient availability and microbial community drive ecosystem functions and regulate soil processes. Soybean-Wheat rotation altered soil environment, leading to limited supply of mobile C and nutrients and changing microbial community composition to be less efficient in degrading complex organic substances.

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