

**SOIL QUALITY AS AFFECTED BY AGROFORESTRY  
AND GRASS BUFFERS IN GRAZED PASTURE AND  
ROW CROP SYSTEMS**

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by

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IN GRAZED PASTURE AND ROW CROP SYSTEMS**

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and hereby certify that, in their opinion, it is worthy of acceptance.

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# **To My Parents**

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## **ABSTRACT**

Establishment of agroforestry and grass buffers within agroecosystems is believed to improve soil quality. Soil enzyme activities and water stable aggregates have been identified as sensitive soil quality indicators to evaluate early responses to soil management. However, only a few studies compared these parameters among buffers, grazing pastures, and row crop systems. The objective of this study was to compare the activities of selected enzymes ( $\beta$ -glucosidase and  $\beta$ -glucosaminidase, fluorescein diacetate (FDA) hydrolase, dehydrogenase), water stable aggregates (WSA), soil organic carbon (SOC), total nitrogen (TN), and bulk density as soil quality parameters among four management treatments: grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row crop (RC). Two soil depths (0-10 and 10-20 cm) were analyzed in all treatments for two consecutive years, 2009 and 2010. The WSA was determined by wet sieving method while enzyme activities were colorimetrically quantified using a spectrophotometer in laboratory assays. Soil organic carbon, TN, and bulk density were also determined by standard procedures. Most of the soil quality indicators were significantly greater in perennial vegetation treatments compared to row crop. The

dehydrogenase activity in the GP treatment was  $323.8 \mu\text{g TPF g}^{-1}$  dry soil while it was  $174 \mu\text{g TPF g}^{-1}$  dry soil in RC treatment. Similarly, the GB treatment showed an activity of  $811.4 \mu\text{g fluorescein g}^{-1}$  dry soil in 2010 for FDA enzyme. Although there were numerical variations, the trends in response of quality parameters were consistent between years. The  $\beta$ -glucosaminidase activity increased slightly from 155.6 to 177.0  $\mu\text{g PNP g}^{-1}$  dry soil while  $\beta$ -glucosidase activity decreased slightly from 248.0 to 236.6  $\mu\text{g PNP g}^{-1}$  dry soil in GB treatment during two years. Water stable aggregates increased from 17.8 to 31.4% in row crop while all other treatments had similar values during the two-year study. Surface soil revealed greater enzyme activities and WSA than the sub-surface soil. The treatment by depth interaction was significant ( $P < 0.05$ ) for  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzymes in 2009 while the interaction was significant ( $P < 0.05$ ) for dehydrogenase and  $\beta$ -glucosaminidase in 2010. Soil enzyme activities were significantly correlated with soil organic carbon content ( $r = 0.78$  to  $0.94$ ;  $P < 0.0001$ ). The nature of enzyme activities observed in this study support the hypothesis that perennial vegetation provides favorable conditions for greater enzyme activities and microbial diversity compared with soils under row crop management. Hence the RC treatment can be assigned a soil quality index of 0.43, while the perennial vegetation treatments could attain an index of 0.63-0.67 with respect to a reference soil according to the arithmetic method. Assessing changes in selected enzyme activities appears to be a useful tool to determine soil degradation when reference values for similar systems are available. Implications can be made that perennial vegetation enhances organic matter accumulation in the soil, has minimum disturbance to the soil and will improve soil quality indicators.

# CHAPTER 1

## INTRODUCTION

Agroforestry is a collective term for land use practices that optimize the environmental as well as economic benefits when trees and/or shrubs are combined with crops and/or livestock in spatial or temporal arrangements (Gold and Garrett, 2009). Although these systems are popular and widely practiced in tropical climates, these are new to temperate climatic regions and are now receiving more attention due to their environmental as well as economic benefits (Udawatta et al., 2002). For example, agroforestry and grass buffer practices help reduce nonpoint source pollution losses from row crop and grazed pasture areas by improving soil hydraulic properties and reducing surface runoff (Udawatta et al., 2002; Abu-Zreig et al., 2003; Lovell and Sullivan, 2006). Research has also shown improvements in soil quality parameters and physical properties as influenced by agroforestry practices (Mungai et al., 2005; Kumar et al., 2008; Udawatta et al., 2009).

Soil quality is the capacity of a soil to perform a specific 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 (Karlen et al., 1997; Karlen et al., 2001). Soil quality is considered a key element of sustainable agriculture (Warkentin, 1996) because it is essential to support and sustain crop, range and woodland production and helps maintain other natural resources such as water and air. Soil quality assessment is a process by which soil resources are evaluated on the basis of soil functions. With the continuous degradation of agricultural soils, the concept

of soil quality has been developed to record the conditions of soil and to quantify the responses of soils to management changes. Since there is not a single parameter that can quantify soil quality, it is necessary that a data set be defined comprising measures of various soil attributes for quantifying soil quality (Larson and Pierce, 1991).

Soil quality indices developed to standardize measured soil quality parameters produce numeric values, which can be used to assess changes in soil over a period of time (Wienhold et al., 2004). Different soil assessment methods have been proposed to examine effects of management practices on overall soil quality (Zobeck et al., 2008). Those include Minimum Data Set (MDS), Soil Conditioning Index (SCI), Soil Management Assessment Framework (SMAF), and Agroecosystem Performance Assessment Tool (AEPAT). The minimum data sets help to recognize related soil indicators and to assess the link between chosen indicators and important soil and plant properties (Arsad and Martin, 2002). The SCI has been implemented by the USDA-NRCS (Natural Resources Conservation Service) to evaluate the effects of crop management on soil organic matter (USDA-NRCS, 2002). The SMAF is a comparatively recent method which relies on the consequences of management systems on changing soil properties and general soil functions (Andrews et al., 2004; Karlen et al., 2006). The AEPAT is a research oriented index methodology that quantifies performance of management practices for selected functions (Liebig et al., 2004). Hence, assessment of soil quality should be achieved most efficiently using a modeling framework based on collecting and synthesizing an array of soil quality indicators (Harris et al., 1996).

Good pasture management enhances nutrient cycling efficiency for livestock growth, soil health, and water quality (Bellows, 2001). Rotational grazing is an established method of increasing the efficiency of pasture systems (Rinehart, 2008). This grazing system has been shown to increase livestock productivity (Warren et al., 1986) and improve soil properties. It has been stated that grazing not only enhances the activity of soil microbial communities but also induces changes in the size and composition of microbial communities (Patra et al., 2005). According to the literature, poor management has been shown to deteriorate soil physical quality and cause soil deformation through soil compaction (Drewry et al., 2008; Kumar et al., 2008). There is a public concern as beef cattle production throughout the United States requires better forage management systems to reduce input costs and protect environmental quality (Sigua, 2003). Therefore, planning for sustainable pastoral development in the temperate region is very important. A better management of pastures would help to protect soil, enhance productivity, and improve environmental quality.

To evaluate the impact of management practices on the quality of soil, and thus to predict their consequences in the environment, studies have attempted to determine soil quality by using microbial parameters as indicators (Schloter et al., 2003). An assessment of soil enzyme activities in ecosystems will help to quantify and evaluate specific biological processes in the soil. It is important to have a better understanding of soil enzyme activities as these are easy to measure and they provide rapid responses to changes in management practices (Dick, 1997; Bandick and Dick, 1999). Soil microbes and enzymes play a crucial role in several soil biological activities and quantification of their activities would be a key to understand how they respond to changes in



management, agro-chemicals, and climate change. According to Six et al. (2006), soil microbes improve soil aggregation and thus water stable aggregates (WSA) can be used as an indirect measure of enzyme activity. Water stable aggregates provide internal and external surfaces for carbon storage and enhance microbial processes.

Although there have been extensive studies on soil enzymes (Wirth and Wolf, 1992; Lizarazo et al., 2005; Mungai et al., 2005) and environmental benefits of agroforestry practices (Udawatta et al., 2002; Seobi et al., 2005; Lovell and Sullivan, 2006), little has been reported on the role of soil enzyme activities in grazed pasture systems. Similarly, the percentage of WSA has not been evaluated in grazed pasture systems. Furthermore, quantifiable information is not available that could be used to evaluate management effects on microbial communities, WSA and other soil quality parameters on grazing systems. Basic understanding is needed on how tree buffers, grass buffers and grazed pastures affect soil and ecosystem processes with an ultimate goal to develop sustainable management systems.

The purpose of this study, which is composed of three main sections, is to examine the impact of permanent vegetation management on the overall soil quality. Chapter two provides a literature review pertaining to the study of selected soil quality parameters on grazed pasture and row crops systems. Chapter three evaluates the management effects on selected soil quality parameters. Chapter four compares the temporal variation in the treatment effects on soil quality indicators with two years of data. Chapter five describes the development of a soil quality index using soil quality parameters using a scale of 0 to 1 with an assigned value for each management soil type. This study provides information on the level of enzyme activities, water stable

aggregates, soil organic carbon, soil nitrogen, bulk density and general soil properties at the study sites. This project ultimately shows the status of soil under grazed pastures, buffers, and row crops and the importance of management in maintaining environmental quality. The findings of this research will help suggest the necessary steps to improve and conserve soil quality, and maintain the productivity of pastureland. In the long-run, the study will help to determine management plans to improve soil quality.

### **Objectives**

The main objective of this study was to evaluate the influence of agroforestry, and grass buffers on grazed pastures as compared to row crop management on selected soil quality parameters and to develop soil quality indices for each management. Specific objectives are:

- To assess selected soil enzymatic activities under grazed pasture, agroforestry buffer, grass buffer and row crop management systems.
- To assess water stable aggregates (WSA) and bulk density (Db) under grazed pasture, agroforestry buffer, grass buffer and row crop systems.
- To evaluate soil organic carbon and soil nitrogen as influenced by the treatments.
- To evaluate the depth effect, landscape effect and temporal variation of selected soil quality parameters with comparisons of two years of data.
- To develop a soil quality index based on measured soil quality parameters.

Results of the studies were written independently in the format of journal manuscripts for publication purposes. The first study is accepted for a publication in *Applied Soil Ecology*, and the second study is in review in *Soil and Tillage Research*.

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## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **Agroforestry: a Management Practice**

Agroforestry is defined as a practice that deliberately integrates shrub/grasses with agricultural crops and/or pastures on the same land unit for synergistic benefits due to biophysical interactions between components (Lundgren, 1982; Nair, 1993; Young, 1997; Gold and Garrett, 2009). Agroforestry is a management practice that optimizes limited resources through combination of complementary components within a landscape unit. The interactions in agroforestry systems provide multiple benefits, including additional income sources, increased and diversified production, improved water quality, and enhanced habitat for humans and animal life. The maintenance and improvement of soil quality through organic matter inputs to the soil; nitrogen fixation and nutrient recycling; carbon sequestration; and biodiversity conservation are other environmental benefits of agroforestry practices (Young, 1997).

The cultivation of trees with agricultural crops began concomitantly with the beginning of plant and animal domestication (Smith, 1929; King, 1987; Williams et al., 1997). Although land management practices that integrate trees and agricultural crops on the same land area have been reported since the early 20th century (Cook, 1901), real agroforestry research did not begin until the early 1980s (Oelbermann et al., 2004). As a consequence of increased environmental concerns, the International Development Research Centre (IDRC) in Canada concluded that priority should be given to systems combining trees and crops to optimize sustainable land-use in areas with high population

pressures (King, 1987). As a result, the publication entitled *Trees, Food and People-Land Management in the Tropics* (Bene et al., 1977) was developed, where the term ‘agroforestry’ was first used. Until now, research in temperate and tropical agroforestry systems has paid more attention on the efficiency of these systems in soil and water conservation, crop productivity, nutrient cycling, and changes in soil physical and chemical properties (Oelbermann et al., 2004).

Agroforestry practices such as alley cropping and silvopasture have the greatest potential for conserving and sequestering carbon because of the close interaction between crops, pasture, trees and soil (Nair, 1998). Therefore, agroforestry systems have great potential to store carbon in above-, below-ground biomass, and soils, and have the potential to counteract greenhouse gas release associated with shifting cultivation and deforestation (Dixon, 1995; Nair and Nair, 2002).

Sanchez (2000) mentioned that agroforestry systems are better than other land-use practices at the global and local scale because of their role in food production and ecological conservation. As a result of rigorous land management practices in temperate (Rosenzweig and Hillel, 2000) as well as in tropical (Schroeder, 1994) regions, a large area of degraded land has emerged that is suitable for initiating agroforestry land management practices. There is a great potential to improve these lands by establishment of agroforestry. Although there has been extensive research in agroforestry, a need still exists for information regarding the ecological services of agroforestry. In particular, the assessment of impacts of various types of agroforestry practices on soil quality, microbial ecology and non-point source pollution is still a field needing further exploration.

## **Pasture Management**

Good pasture management enhances nutrient cycling efficiency for livestock growth, soil health, and water quality (Bellows, 2001, Udawatta et al., 2008, 2009). Grazing management can be defined as the manipulation of livestock grazing to accomplish desired results. Grazing management is an important tool for efficient utilization of the pasture resource which strongly influences pasture and animal performance. Sustainable grazing management depends on manipulation of livestock and knowledge of significant threshold (Vallentine, 1990; Holechek et al., 1999).

Rotational grazing is an established method of increasing the effectiveness of pasture management (Rinehart, 2008). Rotational grazing is characterized by the intermittent movement of livestock to fresh paddocks to facilitate the regrowth of pastures. Some popular rotational grazing systems include management-intensive grazing, multiple-pasture rotation, and short-duration grazing (Gerrish, 2004). This grazing system requires skillful management which includes electric fencing and novel water-delivery devices.

The rotational grazing system has been shown to increase livestock productivity (Warren et al., 1986) and improve soil properties (Kumar et al., 2008). Jones (2000) stated that livestock grazing can affect vegetation, soils and animal communities. Livestock plays a role in altering vegetation by redistributing plants and seeds. They may cause soil trampling and can disrupt microbiotic crusts (Miller et al., 1994; West, 1996; Belnap and Lange, 2001). It has been stated that grazing not only enhances the activity of soil microbial communities but also induces changes in the size and composition of microbial communities (Patra et al., 2005). According to the literature, poor management



has been shown to deteriorate soil physical quality and cause soil deformation through soil compaction (Drewry et al., 2008; Kumar et al., 2008).

There is a public concern as beef cattle production throughout the United States requires better forage management systems to reduce input costs and protect environmental quality (Sigua, 2003). Therefore, planning for sustainable pastoral development in temperate regions is very important. A better management of pastures would help to protect soil, enhance productivity, and improve environmental quality. To evaluate the impact of management practices on the quality of soil, and to predict their consequences on the environment, studies have attempted to determine soil quality by using microbial parameters as indicators (Schloter et al., 2003).

### **Soil Quality**

The notion of soil quality was first reported in the literature in the early 1990s (Doran and Safely, 1997; Wienhold et al., 2004), and the first official application of the term was approved by the Soil Science Society of America Ad Hoc Committee on Soil Quality (S-581) and reviewed by Karlen et al. (1997). Soil quality has been defined as “the capacity of a reference 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.” Soil quality has also been defined as "fitness for use" (Larson and Pierce, 1991) and "the capacity of a soil to function" (Karlen et al., 1997).

The terms soil quality and soil health have been extensively used to explain soil productivity and environmental quality. Soil health may be defined as the capacity of a

soil to function as a living and dynamic system to support biological productivity during a given period of time (Harris et al., 1996). As soil health depends upon various processes, specific indicators may be used to quantify or assess soil health (Doran, 2002). On the other hand, soil quality is a term used to indicate a specific purpose of the soil. Hence, soil quality can vary depending upon land use. Also, it focuses on the capacity to meet defined needs such as the growth of a particular crop (Doran and Parkin, 1994). In short, the term soil health is used under sustainable conditions of plant productivity, whereas soil quality considers optimizing soil conditions for one particular preferred objective. Although the two terms differ conceptually, the terms are often used interchangeably as they are related (Karlen et al., 2001).

Retention of soil quality under rigorous land use and rapid economic development is a major challenge for sustainable land use (Doran et al., 1996). Deterioration of soil quality occurs due to nutrient imbalance in soil, excessive fertilization, soil pollution and soil loss (Zhang et al., 1996; Hedlund et al., 2003). A basic evaluation of soil quality is very important to assess the degradation trends with various land uses (Lal and Stewart, 1995). The degradation of soil quality is governed by many physical, chemical and biological components of soil and their interactions (Papendick and Parr, 1992).

The soil quality concept is consistently being improved as the knowledge base expands on soils and soil quality attributes (Karlen and Stott, 1994). There is not a direct measure of soil quality. There are certain soil attributes which are sensitive to changes in management practices which can be used as indicators of soil quality (Andrews et al., 2004). Hence, the soil quality approach has emerged as an evaluation process which

consists of a sequence of activities. The following are important considerations for soil quality assessment:

- Selection of soil quality indicators
- Determination of a minimum data set
- Development of an interpretation scheme of indices
- On-farm assessment and validation

### **Soil Quality Indicators and Parameters**

Soil quality is a dynamic index and can affect the sustainability and productivity of land use. There exist interconnections among physical, chemical and biological properties of soil. Assessing soil quality requires evaluation of a number of factors related to soil quality. Biological indicators scrutinize the biodynamic nature of ecosystems and are most vulnerable to degradation by land management practices (Brady and Weil, 2002). Several authors (Doran and Parkin, 1994; Abawi and Widmer, 2000) have reported that the identification of biological indicators of soil quality is critically important because of the influence of microbial processes on soil quality. The biological indicators of soil quality that are commonly measured include soil organic matter, respiration, soil pH, enzyme activities, microbial biomass and mineralizable nitrogen. To be useful as biological indicators, Dalal (1998) suggested the following criteria: measurement of more than one function, sensitivity to management change, the presence of threshold values and accepted interpretation.

Soil organic matter is a very important parameter which contributes significantly to soil function and soil quality control, water holding capacity, and susceptibility of soil

to degradation (Feller et al., 2001). Highly productive agroforestry systems, including silvopastoral systems, play an important role in carbon sequestration in soils and in above- and below-ground biomass (Veldkamp, 1994). An increase in the soil carbon content is related to higher microbial biomass and elevated respiration (Sparling et al., 2003).

Soil respiration is defined as oxygen uptake or carbon dioxide evolution by the soil microbial faunal community and includes the gas exchange of aerobic and anaerobic metabolism (Anderson, 1982). Soil respiration results from the degradation of organic matter, with the formation of carbon dioxide. When soil is disturbed, a change in soil respiration can be observed due to more rapid growth and greater mineralization of microorganisms (Singh and Gupta, 1977). Carbon dioxide evolution from a soil is thus a measure of the total soil biological activity, including microbial activity (Alef and Nannipieri, 1995). Bacterial biomass can be useful to determine active organisms and relative proportion of fungal to bacterial biomass. Soil microbial biomass is the principal reserve of nutrients such as nitrogen in the soil (Havlin et al., 2005). Literature suggests that the microbial biomass, microbial respiration rate and their relation to soil organic matter levels remain important components of soil quality (Wardle, 2002).

### **Microbial Biomass, Diversity and Activity**

Microbial indicators that have been accepted to evaluate soil quality are microbial biomass, microbial diversity and microbial activity. Microbial biomass and activity can be used to understand the effects of crop rotation and cultivation practices on soil quality (Limon-Ortega et al., 2006). Literature shows a strong correlation between soil microbial

biomass, fertility and health (Vanlauwe et al., 1999; Ladd et al., 2004). Microorganisms are the main source of enzymes in soils (Tabatabai, 1994), and thus the soil microbial communities affects the enzyme mediated processes in soil (Kandeler et al., 1996).

Microbial diversity and genetic, taxonomic, and physiological structure can be studied in relation to soil quality and health because these indicate functional diversity in soil. Soil microbial biomass is used to quantify populations and also can be used to assess nutrient dynamics in soil. Soil microbial community changes more rapidly with the changes in management and environment. Hence these can be used as good indicators of soil quality.

### **Soil Enzymes**

The term soil enzyme activity is linked to microbial activity and reflects the physiological work of all living organisms in soil, together with plant roots (Ladd, 1978). Soil enzymes are pertinent for assessing soil health because they are essential for organic matter decomposition and the metabolic activity of soil microorganisms (Nannipieri et al., 2002). Several techniques have been used to determine soil microbial activity such as the evolution of carbon dioxide, nitrification activity, DNA synthesis in bacteria, fluorescein diacetate (FDA), and activity of dehydrogenase (Nannipieri et al., 1990). Some specific metabolic pathways of interest can also be evaluated with enzyme assays of  $\beta$ -glucosidase and  $\beta$ -glucosaminidase (Anderson et al., 2004; Pavel et al., 2004).

Enzyme activities in soil are mainly the expression of fungi, bacteria, and plant roots, and play an important role in the biogeochemical cycling of carbon, nitrogen and other essential elements. Enzyme activities have been used to examine soil microbial

activity (Anderson et al., 2004), approximate soil resilience to wastes (Benitez et al., 2004), and assess soil biological properties after fumigation with methyl bromide (Klose and Ajwa, 2004).

Measuring enzymatic activities and understanding the factors that affect enzyme expression and substrate turnover rate are significant. Enzymatic activities illustrate soil metabolic capacity, quality, fertility as well as resilience of the soil when subjected to various natural and anthropogenic factors (Bloem et al., 2006). Soil enzyme assays are process level indicators and are presented as a means of determining the potential of a soil to degrade or transform substrates (Dick, 1994). Because enzymes are difficult to extract from soils and usually lose their integrity, enzymes in soils are characterized by measuring their activity under a strict set of conditions (e.g., temperature, pH, buffer and substrate concentration). Soil enzyme activities are closely related to important soil quality parameters such as organic matter, soil physical properties and microbial activity or biomass (Dick, 1994).

Knowledge of several soil enzyme activities can provide information on the soil degradation potential (Trasar-Cepeda et al., 2000). The assessment of soil enzyme activities requires low costs compared to other biochemical analysis (Ndiaye et al., 2000), and the results can be correlated to other soil properties (Moore et al., 2000; Ndiaye et al., 2000; Trasar-Cepeda et al., 2000). Further, it has been reported that any change in soil management and land use is reflected in the soil enzyme activities, and that they can anticipate changes in soil quality before they are detected by other soil analyses (Ndiaye et al., 2000, Udawatta et al., 2009). Previous studies with soils from various regions have shown that enzyme activities are sensitive to soil changes due to tillage (Kandeler et al.,

1999; Acosta-Martinez and Tabatabai, 2001), cropping systems (Ndiaye et al., 2000; Ekenler and Tabatabai, 2002), and land use (Staben et al., 1997; Gewin et al., 1999; Acosta-Martinez et al., 2003). The measurements of soil enzyme activities are useful to detect biological activity as soil enzymes catalyze all biochemical transformations. Enzyme activities in soil reflect potential estimation of in situ activity. The contrasting conditions of the assay relative to the field site and the various enzyme sources affect the measured activity (Nannipieri et al., 2002). Soil enzyme activity measurements have been used as indices of soil quality (Bandick and Dick, 1999; Badiane et al., 2001). The enzyme activities have also been used as tools to understand how human activity is altering biogeochemical cycles in ecosystems (Wick et al., 2000; Saviozzi et al., 2001).

### **Soil Enzyme Procedures**

As presented by Dick (1997), soil enzymes are a precursor of numerous developments in cells and mediate transformation of organic matter, discharge of nutrients, biological nitrogen fixation, nitrification, de-nitrification and detoxification. Enzymes vary significantly with minor variations in space and time (Webster and Oliver, 1990), and their activity is related to the distribution of other soil properties such as moisture, temperature, organic matter content, substrate (Jordan et al., 1995; Bergstrom et al., 1998). Enzymatic activity is greatly influenced by management practices (Bandick and Dick, 1999; Ekenler and Tabatabai, 2003), drainage, and root distribution (Amador et al., 1997).

Soil enzymes include oxidoreductases, transferases, hydrolases and lyases. The enzymes most active in soil include  $\beta$ -glucosidase, protease, dehydrogenase, urease,

phosphatase, cellulase,  $\beta$ -glucosaminidase, saccharase, amylase, and pectinase (Alef and Nannipieri, 1995).

### **Fluorescein Diacetate (FDA) Hydrolase Enzyme in Soil**

There are several enzymatic methods for measuring total microbial activity. One method is fluorescein diacetate (FDA) hydrolysis (Schnurer and Rosswall, 1982). Green et al. (2006) have optimized the method to assay FDA in soils by using a static incubation, using a reducing solvent to terminate the hydrolysis, and covering a large range of activities. As a result, FDA can be used as a biochemical and biological indicator of soil quality. The product of this enzyme conversion is fluorescein, which can be identified by fluorescence microscopy or quantified by spectrophotometry (Schnurer and Rosswall, 1982).

Fluorescein diacetate is a colorless compound hydrolyzed by membrane-bound as well as free enzymes resulting in the release of fluorescein. This can be absorbed in the wavelength and can be measured by spectrophotometry. Several enzymes, such as non-specific esterases, proteases, and lipases, are responsible for FDA hydrolysis and are plentiful in the soil environment (Schnurer and Rosswall, 1982). This assay provides a broad-spectrum indicator of soil biological activity.

The FDA method is recommended for its sensitivity, simplicity and precision to be studied for soil microbial activity. The FDA hydrolase has been correlated with most accurate measures of microbial biomass and adenosine triphosphate (ATP) content (Federle and Ventullo, 1990). The activity of FDA is abundant in a variety of decomposers and it has been correlated with soil organic matter and carbon contents



(Dick et al., 1996; Gasper et al., 2001). The activity of these decomposers provide a precise assessment of total microbial activity as most of the energy flow in the soil system passes through microbial decomposers (Adam and Duncan, 2001). Also, soil FDA hydrolase activities are involved directly in the transformation of organic materials in soil (Sicardi et al., 2004).

### **Dehydrogenase Enzyme in Soil**

Another enzymatic assay to measure total microbial activity is through dehydrogenase activity. Dehydrogenase activity reveals the total oxidative capacity of soil microorganisms, which is important in oxidation of soil organic matter (Alef and Nannipieri, 1995). Dehydrogenase activity can be used as a measure of the intensity of microbial metabolism in soil (Skujins, 1978; Trasar-Cepeda et al., 2000). Dehydrogenase activity has also been used as an indicator of microbial activity in response to consecutive addition of toxic wastes (Benitez et al., 2004). One of the most frequently used methods to estimate dehydrogenase activity is using triphenyltetrazolium chloride (TTC) as an artificial electron acceptor (Lenhard, 1956; Tabatabai, 1994). The TTC is reduced to triphenylformazan (TPF) (Smith and Pugh, 1979; Tabatabai, 1994). Nearly all microorganisms reduce TTC to TPF, which can be measured spectrophotometrically.

Dehydrogenase is considered as an intracellular enzyme which may also be extracellularly located in soil due to cell lysis and may be associated with organic matter or soil colloidal surfaces (Nannipieri et al., 2002). Active dehydrogenases exist as integral parts of intact cells in soils. Dehydrogenase activity in soils provides information on the microbial activity in the soil.

## **$\beta$ -glucosidase and $\beta$ -glucosaminidase Enzymes in Soil**

$\beta$ -glucosidase is used to measure soil carbon decomposition. This enzyme assay is found to be sensitive in determining soil management effects in several soil types (Bandick and Dick, 1999). The  $\beta$ -glucosidase, the most predominant glycosidase in soil, is very important because it is the substrate involved in the last limiting step of cellulose degradation. The importance of this enzyme in biological systems has long been recognized.

The  $\beta$ -glucosidase enzyme is useful as a soil quality indicator, and may reflect past biological activity and the capacity of a soil to stabilize soil organic matter, as well as an indicator of management effects on soils (Bandick and Dick, 1999; Ndiaye et al., 2000). Generally,  $\beta$ -glucosidase activities can provide evidence of changes in organic carbon before it can be accurately measured by other methods (Dick, 1994; Dick et al., 1996; Wick et al., 1998). Recently, scientists have begun to explore the role of this enzyme in microorganisms, plants and invertebrates. This has greatly facilitated its adoption for soil quality testing (Bandick and Dick, 1999; Stott et al., 2010).

Study of  $\beta$ -glucosaminidase activity, another glycosidase, is important because very little information is available about chitin degradation in soils from tropical as well as temperate environments.  $\beta$ -glucosaminidase is a key extracellular enzyme involved in N mineralization that hydrolyzes C-N bonds of organic sugars to free  $\text{NH}_4^+$  that can be taken up by plants (Dick, 1997; Ekenler and Tabatabai, 2002).  $\beta$ -glucosaminidase is a key enzyme involved in the hydrolysis of N-acetyl- $\beta$ -D-glucosamine (NAG) residues of chito-oligosaccharides (Parham and Deng, 2000). This hydrolysis is considered to be

important in carbon and nitrogen cycling in soils. This is involved in the processes where chitin is converted to amino sugars, a major source of mineralizable carbon and nitrogen in soils (Stevenson, 1994; Ekenler and Tabatabai, 2002).  $\beta$ -glucosaminidase activity has been correlated with the nitrogen mineralized in soils (Ekenler and Tabatabai, 2002), microbial biomass carbon, and fungal populations (Parham and Deng, 2000; Acosta-Martinez et al., 2004). The substrates for this enzyme are chitobiose and glycoproteins. Chitin consists of NAG residues in  $\beta$ -1,4 linkages and it is the second most abundant biopolymer on earth (Stryer, 1994).

### **Soil Organic Carbon and Nitrogen**

Agroforestry systems have a great potential to sequester above- and below-ground biomass, conserve soil and help in mitigating greenhouse effects (Albrecht and Kandji, 2003). The conversion of intensively cropped agricultural fields to extensive land uses such as afforested ecosystems helps to sequester carbon in soil. Forest plantations may sequester SOC especially with establishment on cultivated lands where SOC has been depleted (Johanson, 1992). The restoration of degraded soils and the adoption of recommended management practices on agricultural soils can reverse degradative trends and lead to SOC sequestration (Lal, 2003).

Soil texture, drainage conditions, and slope vary with land uses and control SOC accumulation (Tan et al., 2004; Awasthi et al., 2005), because it is related largely to vegetation and topographical features (Franzmeier et al. 1985). Conservation and restoration degraded lands may greatly contribute in enhancing soil quality (Lal, 2000; Awasthi et al., 2005). Predicting the distribution of soil organic carbon (SOC) and

nutrient pools under different land use are important to understand carbon budgets at the watershed level.

The environmental standpoint includes improvement of soil quality, increase in biodiversity and removal of carbon dioxide (CO<sub>2</sub>) from the atmosphere (Batjes and Sombroek, 1997). Soil carbon sequestration implies removal of atmospheric CO<sub>2</sub> by plants and storage of this as soil organic carbon. Mycorrhizal fungi also contribute to SOC sequestration and increase aggregation (Rillig et al., 2002). Land use and soil management practices can significantly influence SOC dynamics and C flux from the soil (Post and Kwon, 2000; McGuire et al., 2001). However, the mechanisms and processes of soil carbon sequestration are not fully understood (Bajracharya et al., 1998). Spatial distribution of SOC pools and flux are important for understanding the role of soils in the global carbon cycle and for assessing potential biosphere responses to climatic change (Schimel et al., 2007).

### **Physical Properties, Water Stable Aggregates (WSA) and Bulk Density**

Changes in topsoil thickness, subsoil exposure, compaction, infiltration, tillage, soil structure, and loss of vegetative cover are some of the important physical attributes which affect soil quality. Soil bulk density (Db) is used as an indicator of soil resistance to root elongation. Seobi et al. (2005) found soil under perennial grass and tree buffers had lower bulk density and higher porosity than soil under row-crop management. In the same way, Rachman et al. (2004) demonstrated that areas under perennial grass for more than ten years had lower bulk density and higher porosity and saturated hydraulic conductivity than areas under row crop cultivation for the same soil. Bulk density is

inversely related to total porosity (Carter and Ball, 1993), which gives an estimate of space left in the soil for air and water movement. Bulk density is related to natural soil characteristics such as texture, organic matter, soil structure (Cassel, 1982; Chen et al., 1998) and varies over the year due to action of several processes: freezing and thawing (Blevins et al., 1983; Unger, 1991), settling by desiccation, and kinetic energy of rainfall (Cassel, 1982), and loosening by root action and animal activity.

The percentage of water stable aggregates (WSA) measures the resistance of the soil to breakdown by water and mechanical stress. Six et al. (2006) stated that soil microbes improve soil aggregation, and thus WSA can reflect relative microbial activity. Water stable aggregates are formed by the aggregation of clay (smallest particles), followed by accumulation of macro-aggregates bound together with bacterial secretions, fungal hyphae, and fine roots. As described by Tisdall and Oades (1982), soils consist of dynamic aggregates of different sizes bound together by organic and inorganic compounds. According to the aggregate hierarchy model (Tisdall and Oades, 1982), soil organic matter is considered as the principal binding agent of aggregate formation which starts with the microaggregates. Macroaggregates are considered a secondary soil structure associated with formation of pores, microbial habitat and physical protection of organic matter (Carter, 2004). Studies report the proportion of WSA is associated with perennial vegetation and reduced disturbance of soil (Balesdent et al., 2000).

## **Soil Quality Index**

### **Minimum Data Set**

A general outline is necessary to evaluate soil quality. That outline can be used to check changes in the environment associated with agricultural management. A minimum data set of soil factors has been proposed by Larson and Pierce (1994). It is generally accepted that such factors should be easy to calculate and represent differences in management (Visser and Parkinson, 1992).

A minimum data set (MDS) was proposed to measure soil quality and its changes due to management practices through selection of key indicators such as organic matter, pH, nutrient status, bulk density, and rooting depth (Larson and Pierce, 1994). Collecting a minimum data set helps to identify the relevant soil indicators and correlate them with significant soil and plant properties (Arshad and Martin, 2002). It is a minimum set of indicators required to obtain a complete understanding of the soil indicators examined. Moreover, they provide a useful tool for evaluating the status, health, and quality of soil (Doran et al., 1996; Larson and Pierce, 1994; Doran and Parkin, 1994). Sufficiently detailed experiments need to be conducted to develop meaningful assessments of soil status, often expressed as an index of soil quality (Kang et al., 2005).

### **Scorecards and Soil Quality Kits**

The use of scorecards for on-farm soil quality assessment is useful where qualitative observations of soil health are scored to obtain an overall measure of soil quality and soil health (Romig et al., 1995). These cards may be developed to evaluate soil health through farmer observations of soil physical, chemical and biological properties (Romig et al., 1996). These soil characteristics are classified in terms of

descriptive indicators which are interpreted on a graded scale. The soil quality test kits are used to examine physical, chemical and biological characteristics of soil.

Assessment tools such as soil quality test kits (Liebig et al., 1996) rely on farmer-based evaluations regarding various soil management practices. This has been aimed to create an educational tool to increase public awareness of the importance of soil quality.

### **Soil Quality Indices**

Various soil quality indexing methods (Granatstein and Bezdicek, 1992; Andrews and Carroll, 2001) have been applied to develop a range of critical test values. The soil quality assessments can be defined within these values (Arshad and Martin, 2002).

A soil quality index is developed to standardize measured soil quality parameters and produce a numeric value which can be used to assess changes in soil over a period of time and to compare soils (Wienhold et al., 2004). Various soil assessment methods have been proposed to examine effects of management practices on overall soil quality (Zobeck et al., 2008). Those include Minimum Data Set (MDS), Soil Conditioning Index (SCI), Soil Management Assessment Framework (SMAF) and Agroecosystem Performance Assessment Tool (AEPAT). The SCI has been implemented by the USDA-NRCS (Natural Resources Conservation Service) to evaluate the effects of crop management on soil organic matter (USDA-NRCS, 2002). The SMAF is a comparatively recent method which relies on the consequences of management systems on changing soil properties and general soil function (Andrews et al., 2004; Karlen et al., 2006). The AEPAT is a research oriented index methodology that quantifies performance of management practices for selected functions (Liebig et al., 2004). Hence,

assessment of soil quality should be achieved most efficiently using a modeling framework based on collecting and synthesizing an array of soil quality indicators (Harris et al., 1996).

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## CHAPTER 3

### AGROFORESTRY AND GRASS BUFFER EFFECTS ON SOIL QUALITY PARAMETERS FOR GRAZED PASTURE AND ROW-CROP SYSTEMS

#### ABSTRACT

Establishment of buffers and incorporation of trees and shrubs are believed to improve soil quality and thereby improve water quality from grazed pasture systems. Although enzyme activities and water stable aggregates have been identified as measurable soil quality parameters for early responses to changes in soil management, the literature lacks information on those parameters for grazing systems with agroforestry buffers. The objective of this study was to examine the activities of fluorescein diacetate (FDA) hydrolase, dehydrogenase,  $\beta$ -glucosidase and  $\beta$ -glucosaminidase, the percentage of water stable aggregates (WSA) and soil organic carbon and nitrogen as soil quality parameters for grazed pasture and row-crop systems. The study consisted of four management treatments: grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row-crop (RC). The WSA was determined by wet sieving method while the enzyme activities were colorimetrically quantified using a spectrophotometer in laboratory assays. Soil organic carbon (SOC) and total nitrogen (TN) contents were also determined. Two soil depths (0-10 and 10-20 cm) were analyzed for all treatments. The row-crop treatment showed significantly lower activities compared to all other treatments for  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzymes along with lower WSA. The dehydrogenase activities were significantly higher in GP treatment compared to RC treatment. The FDA hydrolase activities were not significantly different among

treatments. Surface soil revealed higher enzyme activities and higher WSA than the sub-surface soil. The treatment by depth interaction was significant for  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzymes. The soil organic carbon and total nitrogen data strongly supported the results of enzyme activities and WSA. Implications can be made that perennial vegetation enhances organic matter accumulation in the soil, has minimum disturbance to the soil and will have positive impacts on the ecosystem.

*Keywords:* carbon, cottonwood, depth effect, soil enzyme activities, soil nitrogen, water-stable aggregates.

## **Introduction**

Beef cattle production throughout the United States requires better forage management systems to reduce input costs and protect environmental quality. Good pasture management enhances nutrient cycling efficiency for livestock growth, soil health, and water quality (Bellows, 2001). It has been stated that grazing not only enhances the activity of soil microbial communities but also concurrently induces changes in the size and composition of these communities (Patra et al., 2005). Despite improvements in certain soil biological parameters, grazing systems have also been scrutinized for degradation of water, soil and air quality (Abu-Zreig et al., 2003; Acosta-Martinez et al., 2003; Amador et al., 1997). One possible solution could be to establish perennial vegetative buffers with grass and tree species.

Agroforestry is a collective term for land use practices that optimize the environmental as well as economic benefits when trees and/or shrubs are combined with crops and/or pasture in spatial or temporal arrangements (Gold and Garrett, 2009).

Agroforestry practices have been shown to improve soil quality, carbon sequestration, and water quality in cropping systems (Lal, 2004, Nii-Annang et al., 2009). Also, these practices are believed to reduce nonpoint source pollution from row-crop areas by improving soil hydraulic properties and reducing surface runoff (Abu-Zreig et al., 2003; Gilliam, 1994; Lovell and Sullivan, 2006; Udawatta et al., 2002) as well as increase or maintain soil organic carbon (SOC) through litter fall, reduction in soil erosion and increased land productivity (Escobar, 2002).

Soil quality has been defined as the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Benedetti and Dilly, 2006; Doran and Parkin, 1994; Karlen et al., 1997). Soil quality assessment is a process by which soil resources are evaluated on the basis of soil function (Karlen et al., 1997; Weil and Magdoff, 2004). Periodic assessment of soil quality with known indicators, thresholds and other criteria for evaluation will make it easier to quantify these parameters. Hence, assessment of soil quality and health should be achieved most efficiently using a modeling framework based on collecting and synthesizing an array of soil quality indicators (Harris et al., 1996).

To evaluate the impact of management practices on the quality of soil, and thus to predict their consequences in the environment, studies have attempted to determine soil quality by using microbial parameters as indicators (Schloter et al., 2003). Among the microbial parameters enzyme activities have been identified as possible indicators of the quality of soil because of their rapid responses to changes in soil management (Bandick and Dick, 1999).

Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh et al., 1991). Soil enzyme activities have been related to soil physio-chemical characters (Amador et al., 1997), microbial community structure and vegetation (Sinsabaugh et al., 2002) and disturbance (Boerner et al., 2000). Studies show that enzyme activity and microbial diversity are greater in agroforestry alley cropping practices due to differences in litter quality and quantity, and root exudates (Mungai et al., 2005; Myers et al., 2001; Udawatta et al., 2009). Previous research suggests that relationships between organic matter, microbial activity, and microbial biomass are good indicators of soil quality (Anderson and Domsch, 1990).

The percentage of water stable aggregates (WSA) measures the resistance of the soil to breakdown by water and mechanical stress. Six et al. (2006) stated that soil microbes improve soil aggregation and thus WSA can reflect relative microbial activity. Water stable aggregates are formed by the aggregation of clay (smallest particles), followed by accumulation of macro-aggregates bound together with bacterial secretions, fungal hyphae, and fine roots. As described by Tisdall and Oades (1982), soils consist of dynamic aggregates of different sizes bound together by organic and inorganic compounds. According to the aggregate hierarchy model (Tisdall and Oades 1982), soil organic matter is considered as the principal binding agent of aggregate formation and which starts with the microaggregates. The lowest hierarchical order of this model, i.e. the microaggregates, consists of clay particles attached to organic molecules by polyvalent cations. Macroaggregates are considered a secondary soil structure associated with formation of pores, microbial habitat and physical protection of organic matter



(Carter, 2004). Studies report the proportion of WSA is associated with perennial vegetation and reduced disturbance of soil (Balesdent et al., 2000).

An assessment of soil enzyme activities in the ecosystem will help to quantify and evaluate specific biological processes in the soil. It is important to have a better understanding of soil enzyme activities as these are easy to measure and they provide rapid responses to changes in management practices (Dick, 1997; Bandick and Dick, 1999). Although there have been extensive studies on soil enzymes (Lizarazo et al., 2005; Mungai et al., 2005; Wirth and Wolf, 1992), little has been reported on their roles in grazed pasture management systems with agroforestry practices. Understanding and maintaining biodiversity has become an increasingly important field of research, as well as a resource management goal. More research is needed for a comprehensive understanding of buffer effects on overall soil quality (Lovell and Sullivan, 2006) and to develop environmentally friendly management plans. We hypothesized that there is an effect of grazed pasture with buffers and row-crop management on soil quality parameters. The objective of this research was to compare the effects of grazed pasture, agroforestry buffer, grass buffer, and row-crop management on activity of selected enzymes, WSA, and soil organic carbon and total soil nitrogen contents.

## **Materials and Methods**

### **Study Area**

The experimental site is located at the Horticulture and Agroforestry Research Center (HARC) of the University of Missouri in New Franklin, MO (92°74' W and 37°2' N; 195 m above sea level). Four small watersheds under grazed pasture (GP) were used

for the study, which include replicate watersheds with agroforestry buffers (AgB) (tree-grass buffers) and grass buffers (GB). Pastures were seeded with red clover (*Trifolium pratense* L.) and lespedeza (*Kummerowia stipulacea* L.) in 2003. The size of each watershed is about 0.64 ha (6420 m<sup>2</sup>) and this area was divided into six paddocks. The size of each buffer is about 0.16 ha (1605 m<sup>2</sup>). The cattle were introduced in 2005 and were rotationally grazed (Kumar et al., 2008). The previous land use for GP, AgB and GB was similar. The land was under tall fescue grass (*Festuca arundinacea* Schreb.) without grazing before the establishments of watersheds. The GB buffer areas were reseeded with tall fescue (*Festuca arundinacea*; Kentucky 31) in 2000. The AgB buffers consisted of eastern cottonwood trees (*Populus deltoides* Bortr. ex Marsh.) which were planted into fescue in 2001. Soils for the row-crop (RC) treatment were sampled from an adjacent corn field on the north side of the pasture areas. This area was under corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation and during the sampling year the crop was corn. The size of row-crop treatment was similar to the grazing watersheds. Soil at the pasture and row-crop sites was classified as Menfro silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalfs). Average slope for the site is 12.5%. The annual precipitation of the experimental site for the last 50 years is 925 mm; mean maximum temperature is 18.8°C and minimum temperature is 6.9°C. The mean annual temperature in 2009 was 12.6°C. (<http://agebb.missouri.edu/weather/history/index.asp>).

### Experimentation and Sampling

The management treatments were grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row-crop (RC). The AgB and GB treatments were in the buffer

areas of the small watersheds with respective buffer type and the GP treatment was in the pasture areas in the small watersheds with buffers. The experimental design was completely randomized with a split plot for soil depths (0-10 and 10-20 cm). There were two replicates for treatments and three sampling locations per treatment plot. An additional factor, landscape position, was evaluated for the GP and RC treatments, resulting in soil samples collected at three landscape positions.

Altogether, 96 total samples were taken from the four treatments during June 2009. The sampling procedure was different between buffer areas and the other two treatments. For GP and RC treatments, soil samples were collected at three landscape positions from each treatment: upper, middle and lower (three sub-samples at each position) and in three transects. Since buffer areas were narrow relative to GP and RC treatments, only three sub-samples were collected from the middle area within each buffer. The soil samples for the GB buffer treatment were taken from the center of the buffer. Samples for the AgB buffer treatment were sampled about 40 cm from the base of a tree trunk. Hence, each buffer treatment consisted of six sample locations (three sub-samples and two replications).

Soils were collected with a soil auger and were placed in labeled plastic bags. The sampling bags were sealed and transported to the laboratory in a cooler. All samples were maintained at field moist condition and were stored at 4°C until analyzed. Three separate sub-samples were taken from each sample. One sub-sample was used for enzyme assays after passing through a 2-mm sieve. The second sub-sample was air-dried for water stable aggregates and soil carbon and nitrogen analysis. The third sub-sample was used to determine soil moisture content.

## Laboratory Analyses

WSA and enzyme activities were analyzed in duplicate for each sample. Water stable aggregates (<250  $\mu\text{m}$  diameter) were determined from a 10-g air dried soil sample using the wet-sieving method (Angers and Mehuys, 1993). The aggregate content was corrected for soil moisture and expressed on an oven-dry weight basis.

Fluorescein diacetate (FDA) hydrolase was colorimetrically quantified at 490 nm to indicate the broad-spectrum of soil biological enzyme activities (Dick et al., 1996). A sieved 1-g moist soil sample was shaken for 15 min with 20 mL of sodium phosphate buffer and subsequently shaken with 4.8 mM of FDA for 105 min. The absorbance was measured on the filtrate following acetone hydrolysis. A standard calibration curve was used to measure the concentration and the concentration was expressed in  $\mu\text{g}$  fluorescein released  $\text{g}^{-1}$  dry soil.

Dehydrogenase enzyme activity was determined as described by Tabatabai (1994). Six grams of moist soil sample were used in this analysis. Soil was incubated with 2, 3, 5-triphenyltetrazolium chloride substrate at 37°C for 24 h. A previously developed standard curve was used to calculate the concentration of triphenyl formazan (TPF) product colorimetrically at 485 nm. The enzymatic activity was expressed in  $\mu\text{g}$  TPF released  $\text{g}^{-1}$  dry soil.

$\beta$ -Glucosidase enzyme activity was determined according to Dick et al. (1996). The method was based on colorimetric determination of p-nitrophenol (PNP) released by  $\beta$ -glucosidase with 1-g sieved moist soil samples incubated with buffered (pH 6.0) p-nitrophenol- $\beta$ -D-glucoside. The p-nitrophenol released was extracted by filtration and

determined colorimetrically. Soil was incubated with the p-nitrophenyl- $\beta$ -D-glucoside substrate for 1 h at pH 6.0 at 37°C. A pre-developed calibration relationship was used to determine the concentration of p-nitrophenol colorimetrically (410 nm) and the enzyme activity was expressed in  $\mu\text{g}$  p-nitrophenol released  $\text{g}^{-1}$  dry soil.  $\beta$ -Glucosaminidase enzyme activity was determined as described by Parham and Deng (2000). Moist soil samples (1 g) were used in this analysis. Soil was incubated with the p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide substrate for 1 h at 37°C. A regression equation developed with standards was used to determine the concentration of p-nitrophenol produced colorimetrically (405 nm) and the enzymatic activity was expressed in  $\mu\text{g}$  p-nitrophenol released  $\text{g}^{-1}$  dry soil.

Soil organic carbon (SOC) and total nitrogen (TN) contents were determined by dry combustion analysis at 950°C using LECO TruSpec CN analyzer based on methodology of Nelson and Sommers (1996).

### Statistical Analyses

The data were analyzed as a completely randomized design with a split plot for soil depth using Proc GLM in Statistical Software Package SAS version 9.2 (SAS, 2008). Soil depth was considered as the split plot. A separate analysis was conducted to assess whether landscape positions influenced enzyme activities, WSA, SOC and TN; this was conducted for only two treatments, GP and RC. An analysis was run for the landscape positions along with their interactions with GP and RC treatments. Since landscape positions and their interactions were not significantly different, an additional analysis was run comparing only treatments. To compare all treatments (GP, AgB, GB and RC), the

landscape positions were averaged for the GP and RC treatments since these landscape positions were not significantly different. The parameters measured were analyzed taking into account the four management treatments and two depths. The main effects consisted of treatment effects (management) and the subplot consisted of depth effects. The least significant difference tests (Duncan's LSD) were used for pair-wise comparisons of treatment means. Differences were declared significant at the five percent level of significance ( $p \leq 0.05$ ).

## **Results**

### Landscape Effects

The WSA and enzyme data were analyzed first to test for landscape effects. Landscape effects were not significant for the measured parameters within the grazed pasture (GP) and row-crop (RC) treatments (Table 3.1). Also, the treatment by landscape interactions were not significant. Data comparisons were only made with treatments that contained landscape positions, the GP and the RC treatments. For further analyses in this study, the enzyme activities and percentage of WSA were averaged across landscape positions for treatment comparisons.

### Water Stable Aggregates (WSA)

Water stable aggregate percentages ranged from 17.8% to 70.5% among the study treatments. The RC treatment (17.8%) had the lowest WSA level and it was significantly lower than all other treatments (Fig. 3.1). The GB treatment had the highest WSA percentage (70.5%). The differences among the AgB, GB, and GP treatments were not

significant. The AgB, GB, and GP areas had more than three times WSA compared with the RC treatment. Although the WSA percentage was not significantly different between the two buffers, it was numerically higher in the GB treatment than in the AgB treatment.

### Enzyme Activities

FDA hydrolase activity was highest under GP managed soils, but similar levels were observed in soils under AgB, GB and RC treatments (Table 3.2). There were no significant differences among treatments. Soils under perennial vegetation treatments had 1.1 to 1.3 times the FDA activity compared to soils from cultivated land (RC). The average FDA hydrolase activity for the perennial vegetation treatments was found to be 937  $\mu\text{g}$  fluorescein  $\text{g}^{-1}$  dry soil while the activity on the conventionally managed RC treatment was only 749  $\mu\text{g}$  fluorescein  $\text{g}^{-1}$  dry soil.

There were significant differences between GP and RC treatments for dehydrogenase activity. The GP treatment showed the highest activity (225.6  $\mu\text{g}$  TPF  $\text{g}^{-1}$  dry soil) and the RC treatment showed the lowest activity (62.4  $\mu\text{g}$  TPF  $\text{g}^{-1}$  dry soil; Table 3.2). Similar dehydrogenase activities were observed in soils of the treatments AgB and GB.

Analysis of  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activity revealed significant differences ( $p \leq 0.01$ ) between the RC treatment and all other treatments (Table 3.2). The GP (243 and 159  $\mu\text{g}$  PNP  $\text{g}^{-1}$  dry soil) treatment showed the highest activity and RC (123 and 74  $\mu\text{g}$  PNP  $\text{g}^{-1}$  dry soil) showed the lowest activity for  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities, respectively. We observed that  $\beta$ -glucosidase

and  $\beta$ -glucosaminidase enzyme activities were more than two times higher under perennial vegetation treatments than the RC treatment.

### Soil Carbon and Nitrogen

The soil organic carbon (SOC) and total nitrogen (TN) contents were significantly higher in perennial vegetation treatments compared to the RC treatment (Table 3.3). The SOC content for the RC treatment was 1.2% while those of other three treatments were greater than 1.7%. Similarly, the TN content for the RC treatment was 0.13%, while in other treatments it was greater than 0.19%. The GP treatment had the highest SOC content while the TN content was highest both in GP and AgB. However, the differences among perennial vegetation treatments were not significant. These results followed the same pattern as  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities as well as WSA.

### Depth Effects

The depth effect was significant for all parameters at  $p \leq 0.01$  (Table 3.4). This supports the hypothesis that enzyme activities and water stable aggregates are greater in the surface soil compared to sub-surface soil. Among the treatments, the AgB showed the greatest differences in WSA, and FDA hydrolase and  $\beta$ -glucosidase enzyme activities between the two depths. Similarly for dehydrogenase and  $\beta$ -glucosaminidase enzyme activities, the highest difference was observed in GP and GB treatments, respectively. The smallest differences were observed in the RC treatment for all parameters. The treatment by depth interaction was significant only for  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities (Figs. 3.2 and 3.3). This indicates that the slopes for



the enzyme activities were different between the perennial vegetation treatments and the RC treatment. In essence, greater differences existed between the two depths for measured parameters in perennial vegetation treatments compared to the RC treatment.

## **Discussion**

The results show that there were no significant variations of parameters with landscape position. The literature suggests that spatial variations in enzyme activities may occur when the litter quality and microclimate are significantly different (Mungai et al., 2005). The non-significance of landscape effects in these soil quality parameters may be due to lack of variation in litter quality and quantity, micro-climate and other influencing factors. In support of this argument, Decker et al. (1999) and Mungai et al. (2005) reported that spatial variation occurs in long-term studies (greater than 10 years duration). It appears that significant changes in soil physical properties may require more time at the current study site, which is less than 10 years old. Studies on soil organic carbon accumulation in temperate zone alley cropping systems show that these practices require longer time frames to detect changes in the SOC content due to colder climatic conditions and low C inputs (Oelbermann et al., 2006a; Oelbermann et al., 2006b; Peichl et al., 2006).

One outcome of our study showed that WSA in the RC treatment was significantly lower compared to the AgB, GB, and GP treatments. These results are similar to previous research in which WSA of uncultivated native prairie was 68% compared to 23% for crop management with conventional tillage (Kremer and Li, 2003), and WSA of 8% for crop areas and 15% for grass and agroforestry buffers in claypan soils (Udawatta et al., 2008). Biological activities of soil microorganisms were usually

greater under perennial vegetation than under row-crop management (Guggenberger et al., 1999). Differences among studies could be due to soil type, soil and crop management, vegetation, and duration of management, which influence stability of soil aggregates. Studies by Kremer and Li (2003) and Udawatta et al. (2008) were on Mexico silt loam and Armstrong soils, respectively, while the current study was conducted on Menfro soils. Although numeric values differ, the percentages of WSA in this study follow a similar pattern; the RC treatment had a significantly lower level compared to perennial vegetation treatments. The bulk density for the RC treatment was found to be  $1.42 \text{ g cm}^{-3}$  as compared to the average bulk density of  $1.31 \text{ g cm}^{-3}$  in buffers and grazing areas (Kumar et al., 2008), supporting our results showing the highest WSA in GB and the lowest in the RC treatment.

In this study, the perennial vegetation treatments revealed significantly higher  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities compared to RC treatment. Numerous studies including Bandick and Dick (1999); Kremer and Li (2003); Acosta-Martinez et al. (2003); Mungai et al. (2005); and Udawatta et al. (2009) also reported significantly higher activities of these enzymes in perennial vegetation areas compared to continuously cropped areas. The higher  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities in perennial vegetation treatments can be correlated with increased organic matter accumulation and higher root activity in these treatments compared to row-crop areas. Another factor that may have attributed to observed differences could be the growth rates and biomass accumulation of perennial vegetation in these sites. For example, in central Missouri, Pallardy et al. (2003) reported a biomass accumulation of 2.7 and 14  $\text{Mg ha}^{-1}$  for first and second year harvests of poplar clones (*Populus deltoids*

and *P. nigra* Bortr. ex Marsh) which translates to 1.3 and 6.5 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, assuming 50% C in the biomass. The greater accumulation of biomass and C in these sites may have increased WSA and enzyme activities under perennial vegetation treatments.

Decomposition of plant and animal residues, root exudates, soil biota and microorganisms, add soil organic molecules to the soil. A study by Liu et al. (2005) showed that cover crops increased soil aggregate stability. Dense roots and large root systems supply large quantities of organic materials to soils during their growing periods (Goodfriend et al., 2000). Other studies have indicated that root exudation and senescence as the major source of the organic matter (Goodfriend et al., 2000; Lu et al., 2002). Although the current study was conducted on a grazing management system, the results are consistent and the effect of root activity on the soil carbon can be described in a similar way. The exudates and other organic constituents result in the production of binding agents in soils. This can lead to greater binding within the surface soil by polysaccharides (Degens, 1997; Haynes et al., 1991). The greater organic carbon supplied by the roots of the perennial vegetation promote greater microbial activity and biomass accumulation. These activities help produce extracellular polysaccharides, which also have the capacity to stabilize soil aggregates (Lynch and Bragg, 1985; Roberson et al., 1995). Additionally, plant polysaccharides and fungal hyphae associated with the perennial vegetation help form more stable macroaggregates (Degens, 1997; Oades and Waters, 1991; Tisdall and Oades, 1982).

Readily available substrates also contribute to greater microbial and enzyme activity (Zablotowicz et al., 1998). Mungai et al. (2005) found that FDA hydrolase activity was significantly higher in tree rows compared to crop alleys for surface soil in a

temperate alley cropping practice and the differences were attributed to tree age and soil water content. The FDA hydrolase represents a broad spectrum of enzymes like esterases and lipases. The FDA hydrolase activity was not significantly different among treatments in the current study possibly due to high variability within samples. The significant variation of  $\beta$ -glucosidase,  $\beta$ -glucosaminidase and dehydrogenase enzyme activities suggests that there was a significant difference in functional microbial diversity as these enzymes are involved in carbon and nitrogen cycling and organic matter decomposition (Acosta-Martinez et al., 2003; Mungai et al., 2005).

In this study, enzyme activities strongly followed the distribution of soil carbon and nitrogen among treatments. The SOC content was highly correlated with dehydrogenase,  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities ( $r=0.81, 0.94,$  and  $0.93,$  respectively; Table 4.5). The greater correlations between enzyme activity and organic matter were consistent with previously published research (Kremer and Li, 2003; Mungai et al., 2005; Myers et al., 2001; Udawatta et al., 2008, 2009). It can be hypothesized that perennial vegetation provided environmental conditions suitable for greater accumulation of SOC and TN. In a recent study by Kremer and Kussman (2011), increased total soil organic carbon and total nitrogen in all vegetation sites were attributed to carbon additions through rhizodeposition from roots of perennial kura clover (*Trifolium ambiguum* M. Bieb.). Kumar et al. (2010) conducting a study on the same watersheds at HARC showed that root carbon was 3% greater in the buffers compared to rotationally grazed pastures. Similarly, root length density was 4.5 times higher in buffer treatments compared to grazed pasture. The available soil carbon is likely used for plant re-growth and maintenance at a high physiological rate due to stress imposed in a grazing

system (Baron et al., 2002). While in agroforestry and grass buffers, these areas are not being grazed and hence there has been higher root carbon storage in these treatments.

Soil OM, soil N and enzyme activities are greater in the surface soil as compared to sub-surface soil and these findings agree with published research (Shamir and Steinberger, 2007; Tangjang et al., 2009). They attributed these differences to higher organic matter accumulation, favorable moisture and temperature in the surface soil as compared to sub-surface soil. In this current study, SOC and TN contents were greater in the surface soil as compared to the sub-surface soil. Furthermore, perennial vegetation treatments had more SOC and TN compared with the row-crop treatment. Differences in enzymes activities and percentage of WSA were minimal in RC as there is little variation of organic matter and microbial activities in surface compared to sub-surface soil.

Literature also suggests that management practices, tillage operations and cropping systems affect microbial populations and enzyme activities (Knight and Dick, 2004; Mungai et al., 2005). Dehydrogenase has been shown to be sensitive to soil management effects (Martens et al., 1992) and indicates activity of viable microorganisms. Kremer and Li (2003) found significantly higher dehydrogenase activities in native prairie vegetation but similar activities in other agroecosystems. Similarly, a study conducted by Acosta-Martinez et al. (2003) reported crop rotation and conservation tillage management increased enzyme activities compared to continuous crop cultivation. However, higher enzyme activities are not limited to increased activity by microbial communities but may be associated with humic compounds within the soil matrix. Research suggests that humic extracts be responsible for as much as 50% of the  $\beta$ -glucosidase activity of the soil (Busto and Perez-Mateos, 1995); additional studies have

shown that  $\beta$ -glucosidase may be stabilized by humic or clay colloids thereby contributing to the retention of considerable enzyme activity (Busto and Perez-Mateos, 2000; Hayano and Katami, 1977). Based on microwave irradiation, Knight and Dick (2004) showed that long-term management effects on  $\beta$ -glucosidase activity depended on the changes in the abiotic forms of enzymes. Less disturbed soils or soils with greater carbon inputs may improve stabilization of enzymes within the soil matrix. Thus for our study, the treatments with higher SOC (GP, AgB, GB) exhibited higher  $\beta$ -glucosidase and  $\beta$ -glucosaminidase activities, likely due to greater potential for protecting enzymes exposed to the soil environment during microbial cell lysis over a period of years.

## **Conclusions**

The objective of this study was to evaluate the changes in water stable aggregates, enzyme activities, soil carbon and soil nitrogen as soil quality parameters in grazed pasture systems with buffers in comparison with row-crop management. In the study, two soil depths (0-10 and 10- 20 cm) were evaluated. In addition, landscape effects were evaluated for the row crop (RC) and grazed pasture (GP) treatments. All soil quality parameters measured were either significantly higher or numerically greater in perennial vegetation treatments compared to the row-crop treatment. The landscape effect and its interaction with treatments were not significant. Depth effect was significant for all parameters and there were significant treatment by depth interactions for  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activity. This supports the hypothesis that greater microbial activities and functional diversity exist in perennial vegetation areas compared to row-crop areas. Soils under row-crops are annually disturbed, which negatively

influences soil quality. Higher soil enzyme activities and microbial biomass are enhanced by conservation practices that may lead to increases in other soil quality parameters such as organic matter content, aggregation and soil water infiltration, soil sustainability and productivity, and consequently soil and ecosystem functions.

Results of the study show that establishment of agroforestry and grass buffers in grazing pasture systems improve organic matter content in soils. This adds new information to the knowledge base as the literature lacks information in grazed pasture management systems with agroforestry buffers. The study will help improve our understanding relative to soil microbial activity and functional diversity in soil and soil carbon sequestration. These improvements may in turn help enhance water and soil quality.

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Table 3.1. Mean water stable aggregates (WSA) along with FDA hydrolase, dehydrogenase,  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities averaged across soil depths for three landscape positions (includes two treatments; grazed pasture, GP and row-crop, RC).

Landscape position	WSA		FDA		Dehydrogenase		$\beta$ -glucosidase		$\beta$ -glucosaminidase	
	GP	RC	GP	RC	GP	RC	GP	RC	GP	RC
Lower	62.5a	16.7a	935.3a	709.9a	211.6a	70.7a	241.7a	153.1a	149.2a	98.2a
Middle	63.4a	19.0a	979.4a	756.6a	251.8a	74.6a	236.9a	111.6a	150.8a	63.3a
Upper	58.0a	17.6a	1077.4a	779.9a	210.6a	41.5a	249.8a	103.1a	176.0a	60.7a

Data followed by the same letter within a column were not significant at  $p \leq 0.05$ .

Table 3.2. Fluorescein Diacetate (FDA) hydrolase, dehydrogenase,  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities for grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row-crop (RC) treatments.

Treatment	FDA	Dehydrogenase	$\beta$ -glucosidase	$\beta$ -glucosaminidase
	----- $\mu\text{g g}^{-1}$ dry soil -----			
GP	997.4 a	225.6 a	242.8 a	158.7 a
AgB	986.0 a	163.2 ab	238.1 a	152.6 a
GB	827.8 a	88.8 ab	248.0 a	155.6 a
RC	748.8 a	62.4 b	122.6 b	74.1 b

Data followed by the same letter within a column are not significantly different at  $p \leq 0.05$

Table 3.3. Soil organic carbon and nitrogen for the grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row-crop (RC) management treatments.

<b>Treatment</b>	<b>Soil Organic carbon</b>	<b>Total soil nitrogen</b>
	-----% mass basis-----	
GP	1.8 a	0.20 a
AgB	1.7 a	0.20 a
GB	1.7 a	0.19 a
RC	1.2 b	0.13 b

Data followed by the same letter within a column are not significantly different at  $p \leq 0.05$ .



Table 3.4. Variation of water stable aggregates and enzymes activities with depth for agroforestry buffer (AgB), grass buffer (GB), grazed pasture (GP) and row-crop (RC) treatments.

Treatment	Depth -cm-	WSA --%--	FDA	Dehydrogenase ----- $\mu\text{g g}^{-1}$ soil -----	$\beta$ -glucosidase	$\beta$ -glucosaminidase
GP	0-10	69.7 a	1145.8 a	300.0 a	309.7 a	209.9 a
	10-20	52.9 b	849.0 b	151.2 b	176.0 b	107.4 b
AgB	0-10	78.3 a	1185.6 a	235.2 a	327.5 a	208.6 a
	10-20	58.9 b	786.2 b	91.2 b	148.8 b	96.6 b
GB	0-10	78.4 a	995.0 a	136.8 a	321.9 a	220.9 a
	10-20	64.1 b	660.6 b	40.8 b	174.1 b	90.3 b
RC	0-10	24.0 a	896.8 a	76.8 a	146.0 a	87.9 a
	10-20	11.9 b	601.0 b	48.0 b	99.3 b	60.2 b

Data followed by different letters within a column within a treatment are significantly different at  $p \leq 0.01$ .

Table 3.5. Correlation coefficients (r) between FDA hydrolase, Dehydrogenase,  $\beta$ -glucosidase, and  $\beta$ -glucosaminidase enzyme activities, and soil organic carbon and total nitrogen contents.

<b>Parameters</b>	<b>FDA</b>	<b>Dehydrogenase</b>	<b><math>\beta</math>-glucosidase</b>	<b><math>\beta</math>-glucosaminidase</b>
Carbon	0.78 (p=0.0003)	0.81 (p=0.0001)	0.94 (p<0.0001)	0.93 (p=0.0001)
Nitrogen	0.78 (p=0.0004)	0.83 (p<0.0001)	0.93 (p=0.0001)	0.92 (p=0.0001)

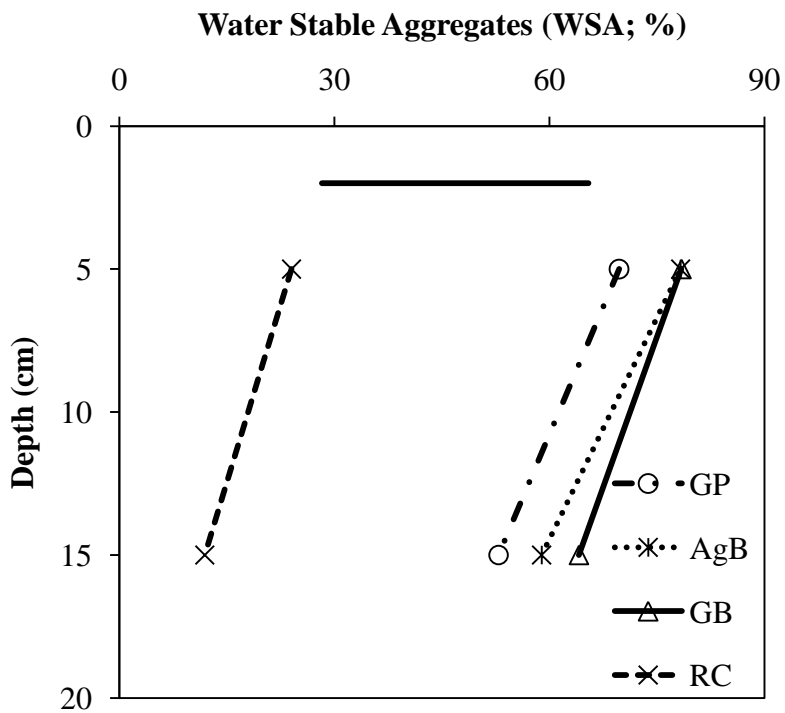


Figure 3.1. Mean water stable aggregate levels (WSA, %) for the grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row-crop (RC) management treatments. Samples were from the 0 to 20 cm soil depth. The bar indicates the LSD value (35.27).

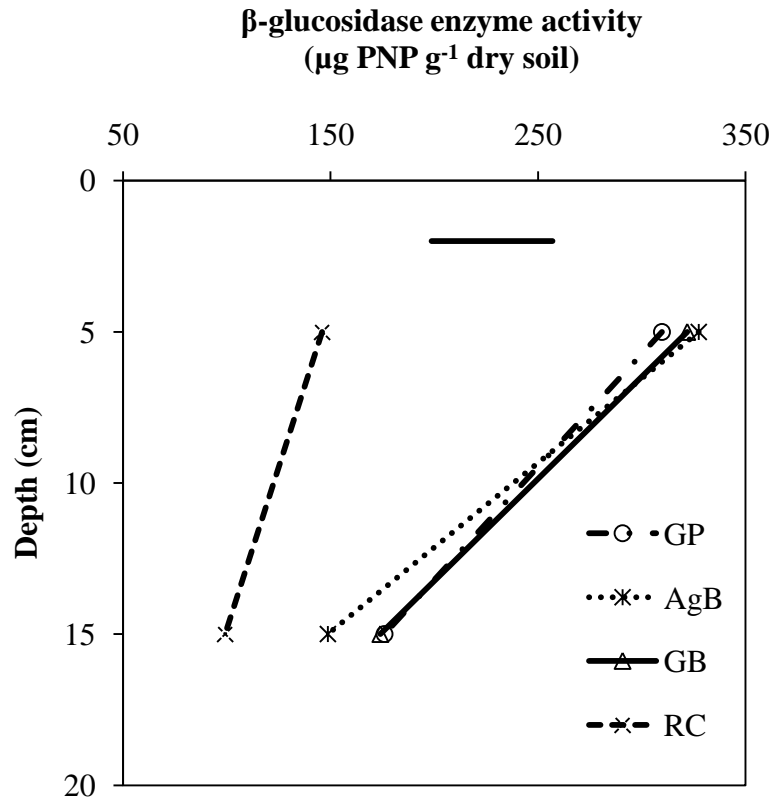


Figure 3.2.  $\beta$ -glucosidase enzyme activity as a function of depth for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row-crop (RC). Samples were from the 0 to 20 cm soil depth. The bar indicates the LSD value (58.3).

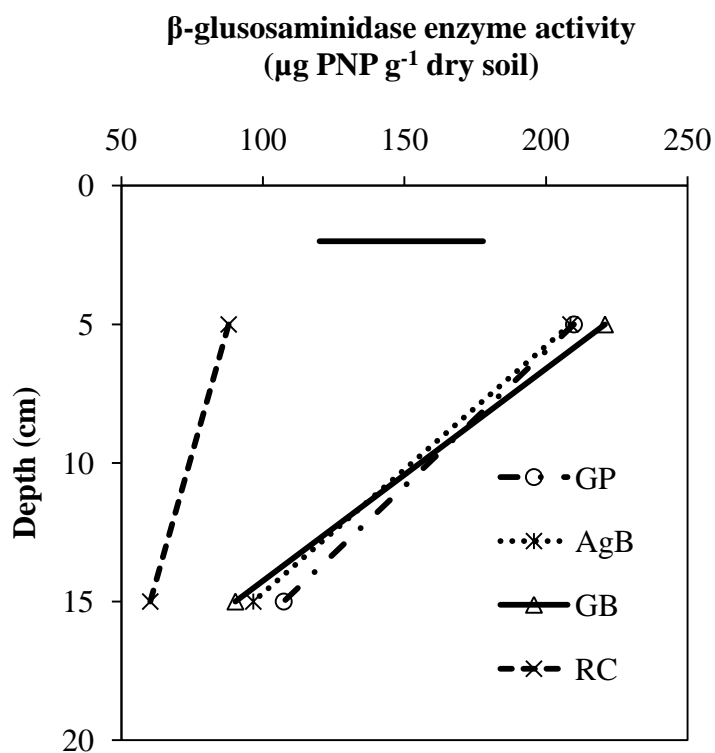


Figure 3.3.  $\beta$ -glucosaminidase enzyme activity as a function of depth for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row-crop (RC). Samples were from the 0 to 20 cm soil depth. The bar indicates the LSD value (57.7).

## CHAPTER 4

### VARIATION IN SOIL QUALITY INDICATORS IN GRAZED PASTURE WITH AGROFORESTRY BUFFERS AND ROW CROP SYSTEMS

#### ABSTRACT

Incorporation of agroforestry buffers within agroecosystems is believed to enhance soil quality. Soil enzyme activities and water stable aggregates have been identified as indicators to evaluate early responses to management. However, few studies exist that compare these parameters among buffers, grazing pastures and row-crop systems. The soil quality indicators examined were the selected enzymes ( $\beta$ -glucosidase,  $\beta$ -glucosaminidase, fluorescein diacetate (FDA) hydrolase, dehydrogenase), water stable aggregate (WSA), soil organic carbon and total nitrogen as soil quality parameters. The study consists of grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row crop (RC). Two soil depths (0-10 and 10-20 cm) were analyzed in 2009 and 2010. Most soil quality indicators were significantly greater in perennial vegetation treatments compared to row crop. The trend of response was consistent between years. The  $\beta$ -glucosaminidase activity increased from 155.6 to 177.0  $\mu\text{g PNP g}^{-1}$  dry soil while  $\beta$ -glucosidase activity decreased from 248.0 to 236.6  $\mu\text{g PNP g}^{-1}$  dry soil in GB treatment during two years. The treatment by depth interaction was significant for  $\beta$ -glucosaminidase in both years. Soil enzyme activities were significantly correlated with soil organic carbon. Conclusions can be made that interactions between soil management and quality indicators are of great significance in agroecosystems.

*Keywords:* agroecosystem interactions, microbial activity, perennial vegetation, soil enzymes, soil organic carbon.

## **Introduction**

The interactions between soil biological parameters and management practices and subsequent effects on environmental quality are of great agricultural and ecological significance (Watt et al., 2006). Despite the important roles of the soil microbiota in agroecosystem functions (Verhoef and Brussaard, 1990), very little is known of their activities, composition, and abundance under grazing pasture systems. The sustainability of management systems depends on the diversity of the soil microbial community and their biochemical processes (Pankhurst et al., 1996). The change in microbial community structure, biomass and activity rates occurs with the severity and duration of the disturbance (Schloter et al., 2003). Microbial and biochemical soil properties are suggested as early indicators of changes in soil quality. A better understanding on how to manipulate environmental conditions to fully utilize the microbial potential will help in developing more sustainable agroforestry systems.

Agroforestry is an intensive land-use management practice that optimizes the economic and environmental benefits from biophysical interactions when trees and/or shrubs are deliberately combined with crops and/or livestock in spatial or temporal arrangements (Gold and Garrett, 2009). Agroforestry buffers help in reducing nonpoint source pollution from row crop areas by improving soil hydraulic properties and reducing surface runoff (Udawatta et al., 2002; Lovell and Sullivan, 2006; Kumar et al., 2008). Agroforestry buffers have also been shown to increase the soil organic carbon (SOC)

through litter accumulation and root activity (Young, 1989), reduce soil erosion (Escobar et al., 2002; Schultz et al., 2004) and increase land productivity (Noble et al., 1998).

Silvopasture is a type of agroforestry management system that is believed to provide environmental, economical and social benefits. Tree or tree-grass buffers are used in these systems to protect water resources where animal access is restricted. In silvopasture systems, grazing and stocking rates affect animals, affect utilization of nutrients by soil plant systems, and enhance soil microbial activities and thereby soil ecology of pasture soils (Haynes and William, 1993; Sigua, 2003). The extent to which these properties can change within a season or pasture management system is of interest from several viewpoints, including their likely value as indicators of soil quality (Doran and Parkin, 1994). Thus, incorporation of agroforestry into pastures is believed to improve soil quality.

Soil quality is defined by Doran and Parkin (1994) as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health. Soil Quality Assessment is a process by which soil resources are evaluated on the basis of soil functions (Weil and Magdoff, 2004). Soil quality assessment involves measurement of multiple soil parameters representing chemical, physical, and biological characteristics (Doran and Parkin, 1994). Periodic assessments of soil quality with known indicators and thresholds help to assess the capacity of land for a particular function. Selection of soil quality indicators depend on soil characteristics, land use and management goals, and environmental protection (Stott et al., 2010).



Enzyme activities are recognized as possible indicators of the changes in soil management. The activities are believed to show generally the early responses to changes in management practices (Dick 1994; Bandick and Dick, 1999). Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Burns, 1983; Sinsabaugh et al., 1991). However, the natural variation within and among soils is the major constraint (Trasar-Cepeda et al., 2000). Possibly due to this reason, studies have often stated that results obtained for a particular soil cannot be generalized due to differences in their inherent soil properties (Gianfreda et al., 2005; Bielinska and Pranagal, 2007). A number of observations may be required to determine variability in space and time. It is possible to develop specific measures of functional diversity with the studies typically dealing with differences in soil enzyme activities.

Information on grazing systems with agroforestry and grass buffer interactions within the temperate agroforestry zone on soil quality and conservation is limited; therefore research designed to explore new species and management combinations are necessary for sustainability of these systems (Jose et al., 2004). To ensure that grazing pasture systems with agroforestry practices improve soil functioning and environmental quality, soil quality assessment can be used to provide information needed to evaluate the impact of implementing these management systems (Andrews et al., 2004). Furthermore, a better understanding of overall microbial activity and carbon dynamics in an agroforestry practice will contribute to estimates of environmental and economic benefits and assist policy and management decisions for these systems (Lee and Jose, 2003). The objectives of this study were to evaluate the effects of agroforestry and grass buffers on soil parameters in grazed pasture and row-crop systems and compare temporal variation

of parameters. We hypothesized that there is an effect of grazed pasture with buffers and row-crop management on soil quality parameters and that parameter values vary annually due to variation in soil characteristics.

## **Materials and Methods**

### **Study Area**

The study was carried out at the Horticulture and Agroforestry Research Center (HARC) of the University of Missouri in New Franklin, MO (92°74' W and 37°2' N; 195 m above sea level). Four small watersheds under grazed pasture (GP) were used for the study, which include replicate watersheds with agroforestry buffers (AgB) (tree-grass buffers) and grass buffers (GB). The size of each watershed with buffers is about 0.8 ha. The grazed pasture area was divided into six paddocks. The cattle were introduced in 2005 and were rotationally grazed (Kumar et al., 2008). The land was under tall fescue grass (*Festuca arundinacea* Schreb.) without grazing before the establishments of watersheds. The GB buffer areas were reseeded with tall fescue (*Festuca arundinacea*; Kentucky 31) in 2000. Pastures were seeded with red clover (*Trifolium pratense* L.) and lespedeza (*Kummerowia stipulacea* L.) in 2003. The AgB buffers consisted of eastern cottonwood trees (*Populus deltoides* Bortr. ex Marsh.). Soils for the row-crop (RC) treatment were sampled from an adjacent field on the north side of the pasture areas. The crop was corn in 2009 and it was soybean in 2010. Soils at the study site were classified as Menfro silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalfs).

### **Experimental Design and Sampling**

The management treatments were GP, AgB, GB, and RC. The AgB and GB treatments were in the buffer areas of the small watersheds with respective buffer type and the GP treatment was in the rotationally grazed area in the watersheds. The experimental design was completely randomized with a split plot for soil depths (0-10 and 10-20 cm). There were two replicates for treatments and three sampling locations per treatment plot.

Soil sampling was conducted during June of two consecutive years, 2009 and 2010. There were three sampling positions per treatment plot and two replications. For GP and RC treatments, samples were taken from middle landscape positions only. The soil samples for the GB buffer treatment were taken from the center of the buffer. Samples for the AgB buffer treatment were sampled about 40 cm from the base of a tree trunk. Hence, treatments consisted of six sample locations (three sub-samples and two replications). Soils were collected from two depths (0-10 and 10-20 cm). In 2010, 48 core samples were collected to determine bulk density from all treatments representing two depths (0-10 and 10-20 cm). Water stable aggregate and enzyme soil samples were collected with a soil auger and were placed in labeled plastic bags. The sampling bags were sealed and transported to the laboratory in a cooler. All samples were maintained at field moist condition and were stored at 4°C until analyzed.

#### Laboratory Analyses

Water stable aggregates were determined from a 10-g air-dried soil sample using the wet-sieving method on aggregates > 250 µm diameter (Angers and Mehuys, 1993). The aggregate content was adjusted for soil moisture and expressed on an oven-dry

weight basis. Soil bulk density was determined by the core method (Blake and Hartge, 1986). Soil organic carbon (SOC) and total nitrogen (TN) contents were determined following the methodology of Nelson and Sommers (1996). LECO TruSpec CN Analyzer was used and dry combustion analysis performed at 950°C.

All enzymes were colorimetrically quantified in laboratory assays.  $\beta$ -Glucosidase enzyme activity was determined based on the procedure of Dick et al. (1996). The method was based on colorimetric determination of p-nitrophenol (PNP) released by the substrate with 1-g sieved moist soil samples incubated with buffered (pH 6.0) p-nitrophenol- $\beta$ -D-glucoside. Soil was incubated with the p-nitrophenyl- $\beta$ -D-glucoside substrate for 1 h at pH 6.0 at 37°C. A pre-developed calibration equation was used to calculate the concentration of p-nitrophenol colorimetrically (410 nm) and the enzyme activity was expressed in  $\mu\text{g}$  p-nitrophenol released  $\text{g}^{-1}$  dry soil.  $\beta$ -glucosaminidase enzyme activity was determined according to Parham and Deng (2000). Soil was incubated with the p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide substrate for 1 h at 37°C. A regression equation developed with standards was used to determine the concentration of p-nitrophenol released colorimetrically (405 nm) and the enzymatic activity was expressed in  $\mu\text{g}$  p-nitrophenol released  $\text{g}^{-1}$  dry soil.

Fluorescein diacetate (FDA) hydrolase was colorimetrically quantified at 490 nm (Dick et al., 1996). A sieved 1-g moist soil sample was shaken for 15 min with 20 mL of sodium phosphate buffer and subsequently shaken with 100  $\mu\text{L}$  of 4.8 mM of FDA for 105 min. The absorbance was measured on the filtrate after acetone hydrolysis. A standard calibration curve was used to measure the concentration which was expressed in  $\mu\text{g}$  fluorescein released  $\text{g}^{-1}$  dry soil.

Dehydrogenase enzyme activity was determined as described by Tabatabai (1994) using six grams of moist soil sample. Soil was incubated with 2, 3, 5-triphenyltetrazolium chloride substrate at 37°C for 24 h. A standard curve was used to calculate the concentration of triphenyl formazan (TPF) product colorimetrically at 485 nm. The enzyme activity was calculated in  $\mu\text{g}$  TPF released  $\text{g}^{-1}$  dry soil.

The water stable aggregates (WSA) and enzyme activities were analyzed in duplicate for each sample.

### Statistical Analyses

The data were analyzed as a completely randomized design with a split plot for soil depth using Proc GLM in Statistical Software Package SAS version 9.2 (SAS, 2008). Soil depth was considered as the split plot. Data collected in each of two years were analyzed separately to determine the treatment effects and the interactions with depth. The parameters measured were analyzed taking into account the four management treatments and two depths. The main effects consisted of treatment effects (management) and the subplot consisted of depth effects. The least significant difference tests (Duncan's LSD) were used for pair-wise comparisons of treatment means. Differences were declared significant at the five percent level of significance ( $p \leq 0.05$ ).

## Results

### Water Stable Aggregates (WSA)

Water stable aggregate (WSA) percentages ranged from 17.8% to 70.5% in 2009 and 31.4 % to 65.5% in 2010 among the study treatments. The RC treatment (17.8% and

31.4%) had the lowest WSA level and it was significantly lower than all other treatments in both years (Tables 4.1 and 4.2). The GB treatment had the highest WSA percentage (70.5% and 65.5%) in both years. Variation in WSA levels within perennial vegetation treatments for two years was not significant. But the variation of WSA for the RC treatment in two years was high compared to the other treatments. The WSA was almost double in the second year compared to the first year within the RC treatment. The differences among the AgB, GB, and GP treatments were not significant in the first year. In the second year, the variation of WSA between AgB and GP was not significant and both the treatments showed significantly lower WSA than the GB treatment. There were significant depth effects in both years (Tables 4.3 and 4.44; Fig. 4.1).

#### Soil Bulk Density

Bulk density was estimated only in 2010. The differences in bulk density among treatments were not significant but the row crop treatment had the highest value (1.42 g cm<sup>-3</sup>) and AgB had the lowest value (1.31 g cm<sup>-3</sup>; Table 2). The bulk density values decreased in the order RC>GP>GB>AgB. Although there were no significant differences, values trended in expected ways; differences did not exist due to the low number of replications (two). There were significant depth effects (Fig. 4.2).

#### Soil Carbon and Nitrogen

Soil organic carbon (SOC) and total nitrogen (TN) contents varied slightly between the two years. In 2009, the SOC and TN concentrations were significantly higher in perennial vegetation treatments compared to RC treatment (Table 4.1), but these

were not significantly different among treatments in 2010 (Table 4.2.). There was a slight decrease in SOC content (1.8 to 1.6 %) and TN content (0.20 to 0.18 %) in GP treatment. But the buffer treatments showed slightly higher concentrations in 2010. In the AgB treatment, SOC content increased from 1.70 to 1.91 % and TN content increased from 0.20 to 0.22 %. In the GB treatment, SOC content changed from 1.70 to 1.88% while the TN content changed from 0.19 to 0.20 %. Soil organic carbon and TN contents in the RC treatment increased from 1.20 to 1.26 % and TN increased from 0.13 to 0.16 %, respectively. There were significant depth effects in SOC and TN (Fig. 4.3a and b). The perennial vegetation treatments showed a greater decrease in SOC and TN contents from surface to sub-surface compared to row crop agriculture.

## Enzyme Activities

### $\beta$ -glucosidase and $\beta$ -glucosaminidase Enzyme Activities

Analysis of  $\beta$ -glucosidase and  $\beta$ -glucosaminidase activity revealed significant differences between the RC treatment and all other treatments in both years (Tables 4.1 and 4.2). The  $\beta$ -glucosidase activities were very similar in 2009 and 2010 in the GP treatment (242.8 and 240.7  $\mu\text{g PNP g}^{-1}$  dry soil, respectively). For the AgB treatment,  $\beta$ -glucosidase activity slightly increased from 238.1 to 246.2  $\mu\text{g PNP g}^{-1}$  dry soil over the two years. Similarly for the GB treatment,  $\beta$ -glucosidase activity decreased slightly from 248.0 to 236.6  $\mu\text{g PNP g}^{-1}$  dry soil during two years. However, the year to year variation in  $\beta$ -glucosidase activity in the RC treatment was greater (122.6 vs. 165.3  $\mu\text{g PNP g}^{-1}$  dry soil, respectively).

There were comparatively higher activities of  $\beta$ -glucosaminidase enzyme in the second year than first year for all treatments. The GP treatment showed  $\beta$ -glucosaminidase enzyme activity of  $158.7 \mu\text{g PNP g}^{-1}$  dry soil in 2009, while in 2010, it was  $170.8 \mu\text{g PNP g}^{-1}$  dry soil. The  $\beta$ -glucosaminidase enzyme activity increased from 152.6 to  $166.5 \mu\text{g PNP g}^{-1}$  dry soil in the AgB treatment and whereas in the GB treatment, the activity increased from 155.6 to  $177 \mu\text{g PNP g}^{-1}$  dry soil. The RC treatment increased by  $18.1 \mu\text{g PNP g}^{-1}$  dry soil from 74.1 in 2009 to  $92.2 \mu\text{g PNP g}^{-1}$  dry soil in 2010. Among all treatments and years, the RC treatment had the lowest activities.

The treatment by depth interaction was significant for  $\beta$ -glucosaminidase enzyme in both years while the interaction for  $\beta$ -glucosidase enzyme activity was significant only in 2009 (Fig. 4.4; Fig.4.5a. and b.).

#### Fluorescein Diacetate(FDA) Hydrolase Activity

Higher variability in FDA activities was observed during the two-year study compared to other enzymes. The FDA activity decreased in all treatments except the GB treatment in 2010 compared to 2009. In the GP treatment, the activity decreased from  $997.4 \mu\text{g fluorescein g}^{-1}$  dry soil in 2009 to  $759.7 \mu\text{g fluorescein g}^{-1}$  dry soil in 2010. Similarly, the AgB treatment showed FDA activity of 986 and  $804.6 \mu\text{g fluorescein g}^{-1}$  dry soil in 2009 and 2010, respectively. The GB treatment showed similar FDA activity during the two years ( $806.2$  and  $811.4 \mu\text{g fluorescein g}^{-1}$  dry soil in 2009 and 2010, respectively). The RC treatment had an FDA activity of  $748.8 \mu\text{g fluorescein g}^{-1}$  dry soil in 2009 whereas it reduced to  $705.4 \mu\text{g fluorescein g}^{-1}$  dry soil in 2010. The FDA hydrolase activity was not significant among treatments in 2009 (Table 4.1). In contrast,



management treatment significantly affected activity in 2010. The RC treatment was not significantly different as compared to the GP and AgB treatments but was significantly lower compared to the GB treatment in 2010 (Table 4.2). The differences in activities among the perennial vegetation treatments were not significant.

#### Dehydrogenase Enzyme Activity

Dehydrogenase activities differed significantly in both years among treatments (Tables 4.1 and 4.2). In 2009, the GP treatment revealed significantly greater activity compared to the RC treatment but the variations among buffers and pasture treatments was not significant. In 2010, all perennial vegetation treatments showed significantly higher activity than the RC treatment (Table 4.2). Variations in dehydrogenase activities were greater among years for this enzyme compared to the other enzymes studied. In fact, the activities were higher in 2010 compared to 2009 for all treatments. The dehydrogenase activity in the GP treatment increased from 225.6  $\mu\text{g TPF g}^{-1}$  dry soil in 2009 to 323.8  $\mu\text{g TPF g}^{-1}$  dry soil. In the AgB treatment, the dehydrogenase activity was 160.8  $\mu\text{g TPF g}^{-1}$  dry soil in 2009 and 310.2  $\mu\text{g TPF g}^{-1}$  dry soil in 2010. The GB treatment showed the greatest difference between the two years. It increased from 84  $\mu\text{g TPF g}^{-1}$  dry soil in 2009 to 337.9  $\mu\text{g TPF g}^{-1}$  dry soil in 2010. The activity also increased from 62.4  $\mu\text{g TPF g}^{-1}$  dry soil to 174  $\mu\text{g TPF g}^{-1}$  dry soil in 2010 for the RC treatment.

The depth effect was significant for all enzyme activities in both years (Tables 4.3 and 4.4). There were no significant treatment by depth interactions in 2009; however, these interactions were significant in 2010 (Fig. 4.6). The difference in activities between the surface and sub-surface soil was significant for both years.

## **Discussion**

### Water Stable Aggregates (WSA)

The results showed that WSA percentages within soils under RC management were significantly lower as compared to the GP, AgB, and GB treatments which closely parallel previous findings. Studies demonstrate that water stable aggregates in natural grassland, agroforestry, prairies, and managed natural vegetation were found to be significantly higher compared to cultivated areas with row crop management (Kremer and Li, 2003; Mungai et al., 2005; Udawatta et al., 2008; 2009; Guo et al., 2010; Kremer and Kussman, 2011). The greater aggregate stability was attributed to the increased stabilization of carbon and nitrogen mediated by higher microbial activity and permanent root biomass associated with perennial vegetation while the lower WSA in the cultivated areas was attributed to carbon losses and disturbance in the soil structure associated with prolonged cultivation practices.

Soil organic matter and biological activity in soil highly affect water stable aggregates. Soil organisms are concentrated in litter, around roots, and surface of aggregates where organic matter is available (Ingham, 2000). Organic glues resulting from biological decomposition of organic matter bind soil particles to each other and stabilize WSA (Tisdall and Oades, 1982). Research indicates that complex polysaccharide molecules are more important in promoting aggregate stability than lighter, simpler molecules (Elliot and Lynch, 1984). In the RC treatment, physical disturbance and tillage operations accelerate organic matter decomposition, and destroy fungal hyphae and soil aggregates (Frey et al., 2003; Green et al., 2005). Long-term

cropping practices decrease the length and mass of fine roots and deplete soil organic matter resulting in a reduction of macro-aggregates (Tisdall and Oades, 1980; Cambardella and Elliot, 1992).

In contrast, perennial vegetation systems improve soil aggregation and organic matter accumulation (Franzluebbers et al., 2000). Grass can act as a cover crop, improve particulate organic matter content, and aggregation by providing continuous grass and root residues (Franzluebbers and Stuedemann, 2005; Handayani et al., 2008). The carbon inputs, root penetration and morphology, as well as mycorrhizal association affect aggregation (Denef et al., 2002). In addition, grassland soils are known for high levels of organic matter and greater structural stability (van Veen and Paul, 1981).

The reason behind lower aggregate stability in the GP treatment compared to GB could be due to disturbance on the soil surface by grazing animals, unfavorable effects on aggregate stability, and low organic matter input (Bird et al., 2007). Grazing causes soil to break apart, and exposes the organic matter to degradation. Although rotational grazing is more likely to increase aggregate stability (NRCS 2001), heavy grazing disrupts the formation of aggregates. Due to differences in management, species composition, and disturbance, WSA in the GP treatment was comparatively lower among perennial vegetation treatments but significantly higher than the RC treatment.

Also, the bulk density for the RC treatment was found to be  $1.42 \text{ g cm}^{-3}$  as compared to the average bulk density of  $1.33 \text{ g cm}^{-3}$  in buffers and grazing areas. This supports our results showing the highest WSA in GB and the lowest in the RC treatment. The bulk density values were not significantly among treatments probably due to low replication (only two replicates). Kremer and Li (2003) also found that soils under grass

vegetation held greater organic matter and had a higher proportion of WSA when compared with traditionally cropped areas.

### Soil Carbon and Nitrogen

The soil organic matter pools (C and N) were affected by management practices. The SOC and TN contents were significantly greater in perennial vegetation treatments compared to row crop systems in 2009. The higher root activity, microbial decomposition and continuous vegetative cover might have contributed greater carbon and nitrogen accumulation compared to row crop where tillage and cultivation practices caused losses of carbon and nitrogen. Greater WSA levels also lead to accumulation of soil organic matter within macroaggregates and protects soil carbon from faunal action and microbial consumption (Beare et al., 1994; Six et al., 2000). Variations in plant biomass and morphology can also cause the variation in nitrogen accumulation in soil (Clements and Williams, 1967). According to Lal (2002), conventional tillage can deplete soil organic matter as a result of accelerated mineralization, leaching and translocation. As organic matter increases, soil biological activity increases. This enhances the diversity of organisms and the ecosystem functions they perform.

The variation of SOC and TN between the two years might be due to crop rotation and biomass turnover. Rhizodeposition, root exudates, as well as biomass turnover also varied between two years. In RC treatment, these variations might be contributed by the crop rotation and time of sampling. The different significance levels in the two years might be due to these variations. The highest contents were observed in GP in 2009 while these were highest in GB in 2010. The lowest contents were observed in the RC

treatment in both years. More interestingly, there were significant variations among treatments in 2009 while these were not significantly different in 2010.

Undoubtedly, there were significant depth effects. There was a greater decline of SOC content in perennial vegetation from surface to sub-surface soil compared to the row crop treatment (Shamir and Steinberger, 2007; Tangjang et al., 2009). This supports the hypothesis that enzyme activities and water stable aggregates are greater in the surface soil compared to sub-surface soil.

### Enzyme Activities

Following the dynamics of WSA and organic matter, the study showed significant differences in selected enzyme activities. The  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzyme activities were most consistent between the two years. These activities were significantly higher in perennial vegetation treatments compared to row crop management in both years and these findings agree with results from related research (Acosta-Martinez et al., 2003; Dick et al., 1996; Kremer and Li, 2003; Mungai et al., 2005; Udawatta et al., 2008; 2009; Kremer and Kussman, 2011). In a study by Ekenler and Tabatabai (2003), significantly reduced  $\beta$ -glucosaminidase activity has been attributed to soil disturbance and conventional tillage. The higher activities of these enzymes can also be attributed to the increased organic matter and greater activities of roots compared to conventionally cultivated crop areas (Myers et al., 2001; Kremer and Li, 2003; Mungai et al., 2005) and the enzyme activities were highly correlated with the soil carbon and nitrogen (Tables 4.5 and 4.6). Moreover these enzymes have been

associated with functional microbial diversity as these are involved in carbon and nitrogen cycling in soil.

Nevertheless the variation of the other two enzymes (FDA and dehydrogenase) was diverse between the two years. The FDA activities were not significantly different in 2009 among treatments. However, GB treatment showed significantly higher FDA activities compared to the RC treatment in 2010. Dehydrogenase activities were significantly higher in the GP treatment compared to the RC treatment in 2009. Higher activity in GP may be due to slight increase in surface bulk density that seems to stimulate microbial activity (Pengthamkeerati et al., 2011). In 2010, all perennial vegetation treatments revealed significantly higher dehydrogenase activities compared to the row crop treatment. The varied nature of these enzymes could be due to their broad spectrum of activities which represent viable microorganism activities in the soil (Miller et al., 1998; Gasper et al. 2001; Kandeler, 2007). The higher variation of dehydrogenase activities in the two years in the RC treatments could be due to crop rotation and time of sampling. Studies show that soil management and cover type influence soil microorganism population, diversity, and soil microbial processes. These in turn cause the changes in the quantity and quality of plant residue, accumulation of biomass, and root carbon in the soil profile and by providing a vigorous environment (Bandick and Dick, 1999; Boerner et al., 2000; Doran, 1980; Kandeler et al., 1999). Additionally, varying tillage operations, crop rotation, perennial vegetation, residue decomposition and cropping systems influence microbial diversity and enzyme activity due to changes in substrate quantity, soil moisture, and temperature (Doran et al., 1998; Mungai et al., 2005). A similar agroforestry (Mungai et al., 2005) and aforested ecosystem study

(Myers et al., 2001) showed that microbial communities and enzyme activities were directly correlated with quality and quantity of vegetation cover. Differences were attributed to quantity and biochemical properties of the organic materials.

The nature of enzyme activities observed in this study support the hypothesis that perennial vegetation provides favorable conditions for greater enzyme activities and microbial diversity compared with soils under row crop management. Inferring results from this study and the studies of Kremer and Li (2003) and Udawatta et al. (2008; 2009) it appears that permanent vegetation leads to carbon accumulation and consequently increases in selected soil quality parameters compared to row crop areas. These differences can also be attributed to land management. Increased enzyme activities contribute to favorable soil carbon and nitrogen balance, which favors root growth and promotes microbial activity.

## **Conclusions**

The objective of this study was to evaluate the changes in water stable soil aggregates, soil organic carbon, soil nitrogen, and enzyme activities as influenced by permanent vegetative buffers in grazed pasture management compared with row crop management. In this study, two soil depths (0-10 and 10-20 cm) were sampled which contained the highest biological activity and were most likely to demonstrate changes in soil management and vegetation. Based on water stable aggregates and enzyme activities, it is obvious that regular disturbance has significantly reduced soil quality in row crop agriculture. The study showed that establishment of agroforestry and grass buffers in grazed pasture areas has a significant effect on measured soil quality indicators.

The buffers were established in 2001 and therefore, the changes reported here occurred in less than 10 years. The soil quality parameters were significantly greater in permanent vegetation areas compared to row crop agriculture and the parameters were consistent during two measurement years. These results showed that organic matter additions from vegetation as well as soil disturbance from cultivation practices undoubtedly hold a strong influence over the various enzyme activities taking place within the soil. Therefore, evaluating soil enzyme activities and soil properties for various management systems will help in assessing management effects on soil quality.

Measurement of soil organic matter for estimating soil quality reflects long term effects as accumulation of soil organic matter occurs over several years. Estimation of water stable aggregates and enzyme activity can be conducted in a short period of time which will help to understand the effects of management practices. Assessing changes in selected enzyme activities might be a useful tool to determine land degradation under certain management practices when reference values for similar systems are available. As other studies similar to study area of this project have reported that the establishment of buffers may help to reduce non-point source pollution from agricultural lands. Conclusions can be made that the establishment of agroforestry and grass buffers in grazed pasture will enhance soil quality and help maintain ecosystem sustainability. The findings of the current study will certainly add to the soil quality knowledge base and will help our understanding of these management systems.

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Table 4.1. Water stable aggregates (WSA), soil organic carbon (SOC), Total Nitrogen (TN), Total Nitrogen (TN),  $\beta$ -glucosaminidase (GS),  $\beta$ -glucosidase (GC), dehydrogenase (DH) and Fluorescein Diacetate (FDA) hydrolase enzyme activities for grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row crop (RC) treatments (year 2009, n=12).

Treatment	WSA ---%---	SOC -----%-----	TN -----%-----	GS ----- $\mu\text{g g}^{-1}$ dry soil-----	GC ----- $\mu\text{g g}^{-1}$ dry soil-----	DH ----- $\mu\text{g g}^{-1}$ dry soil-----	FDA ----- $\mu\text{g g}^{-1}$ dry soil-----
GP	61.3a	1.8a	0.20a	158.7a	242.8a	225.6a	997.4a
AgB	68.6a	1.7a	0.20a	152.6a	238.1a	163.2ab	986.0a
GB	70.5a	1.7a	0.19a	155.6a	248.0a	88.8ab	827.8a
RC	17.8b	1.2b	0.13b	74.1b	122.6b	62.4b	748.8a

Data followed by the same letter within a column were not significantly different at  $p \leq 0.05$

Table 4.2. Water stable aggregates (WSA), bulk density (Db), soil organic carbon (SOC), Total Nitrogen (TN),  $\beta$ -glucosaminidase (GS),  $\beta$ -glucosidase (GC), dehydrogenase (DH) and Fluorescein Diacetate (FDA) hydrolase enzyme activities for grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row crop (RC) treatments (year 2010, n=12).

Treatment	WSA ---%---	Db --g cm <sup>-3</sup> --	SOC -----%-----	TN -----%-----	GS ----- $\mu\text{g g}^{-1}$ dry soil-----	GC ----- $\mu\text{g g}^{-1}$ dry soil-----	DH ----- $\mu\text{g g}^{-1}$ dry soil-----	FDA ----- $\mu\text{g g}^{-1}$ dry soil-----
GP	55.6b	1.38a	1.60a	0.18a	170.8a	240.7a	323.8a	759.7ab
AgB	59.2b	1.31a	1.92a	0.22a	166.5a	246.2a	310.2a	804.6ab
GB	65.5a	1.32a	1.88a	0.20a	177.0a	236.6a	337.9a	811.4a
RC	31.4c	1.42a	1.26a	0.16a	92.2b	165.3b	174.6b	705.4b

Data followed by the same letter within a column were not significantly different at  $p \leq 0.05$

Table 4.3. Variation of water stable aggregates and enzymes activities with depth for agroforestry buffer (AgB), grass buffer (GB), grazed pasture (GP) and row crop (RC) treatments (year 2009).

Treatment	Depth	WSA	FDA	Dehydrogenase	$\beta$ -glucosidase	$\beta$ -glucosaminidase
-cm-	--%--	----- $\mu\text{g g}^{-1}$ soil-----				
GP	0-10	69.7a	1145.8a	300.0a	309.7a	209.9a
	10-20	52.9b	849.0b	151.2b	176.0b	107.4b
AgB	0-10	78.3a	1185.6a	235.2a	327.5a	208.6a
	10-20	58.9b	786.2b	91.2b	148.8b	96.6b
GB	0-10	78.4a	995.0a	136.8a	321.9a	220.9a
	10-20	64.1b	660.6b	40.8b	174.1b	90.3b
RC	0-10	24.0a	896.8a	76.8a	146.0a	87.9a
	10-20	11.9b	601.0b	48.0b	99.3b	60.2b

Data followed by different letters within a column within a treatment are significantly

different at  $p \leq 0.01$ .



Table 4.4. Variation of water stable aggregates and enzymes activities with depth for agroforestry buffer (AgB), grass buffer (GB), grazed pasture (GP) and row crop (RC) treatments (year 2010).

Treatment	Depth -cm-	WSA --%--	FDA	Dehydrogenase	$\beta$ -glucosidase ----- $\mu\text{g g}^{-1}$ soil-----	$\beta$ -glucosaminidase
GP	0-10	68.0a	935.1a	452.0a	324.3a	234.6a
	10-20	43.0b	584.4b	195.7b	157.2b	107.1b
AgB	0-10	71.4a	1006.1a	416.2a	342.1a	229.5a
	10-20	47.0b	603.0b	204.2b	150.2b	103.6b
GB	0-10	76.2a	1005.2a	471.8a	319.4a	247.2a
	10-20	55.0b	617.7b	204.1b	153.8b	106.8b
RC	0-10	40.0a	930.6a	213.0a	198.8a	106.7a
	10-20	23.0b	480.2b	136.3b	131.8b	77.6b

Data followed by different letters within a column within a treatment were significantly different at  $p \leq 0.05$

Table 4.5. Correlation coefficients ( $r$ ) of  $\beta$ -glucosidase,  $\beta$ -glucosaminidase, dehydrogenase and FDA enzyme activities, with soil organic carbon and total nitrogen content (year 2009).

Parameters	$\beta$ -glucosidase	$\beta$ -glucosaminidase	Dehydrogenase	FDA
Soil organic Carbon	0.94 ( $p < 0.0001$ )	0.93 ( $p = 0.0001$ )	0.81 ( $p = 0.0001$ )	0.78 ( $p = 0.0003$ )
Total Nitrogen	0.93 ( $p = 0.0001$ )	0.92 ( $p = 0.0001$ )	0.83 ( $p < 0.0001$ )	0.78 ( $p = 0.0004$ )

Table 4.6. Correlation coefficients ( $r$ ) of  $\beta$ -glucosidase,  $\beta$ -glucosaminidase, dehydrogenase and FDA enzyme activities, with soil organic carbon and total nitrogen content (year 2010).

Parameters	$\beta$ -glucosidase	$\beta$ -glucosaminidase	Dehydrogenase	FDA
Soil organic Carbon	0.86 ( $p < 0.0001$ )	0.88 ( $p < 0.0001$ )	0.89 ( $p < 0.0001$ )	0.84 ( $p < 0.0001$ )
Total Nitrogen	0.84 ( $p < 0.0001$ )	0.84 ( $p < 0.0001$ )	0.85 ( $p < 0.0001$ )	0.82 ( $p < 0.0001$ )

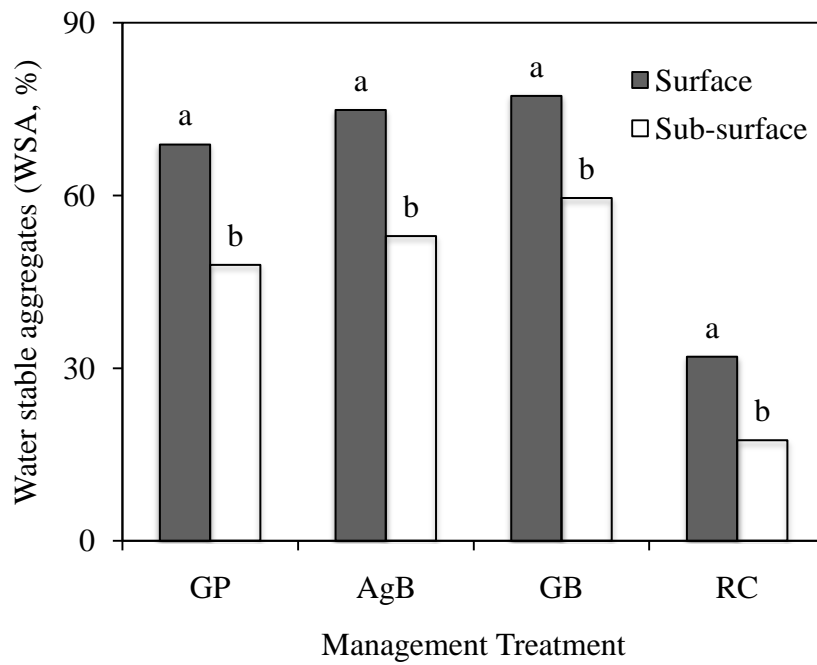


Figure 4.1. Water stable aggregate levels (WSA, %) for the grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row crop (RC) management treatments. Samples were from the 0 to 20 cm soil depth and data presented were the average of sampling years, 2009 and 2010.

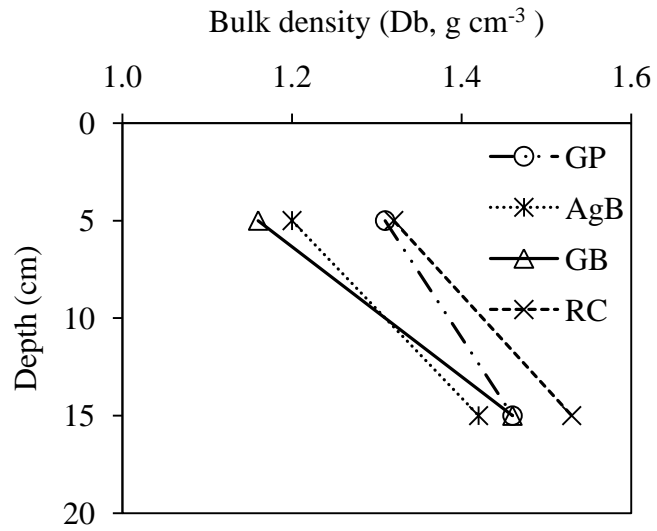
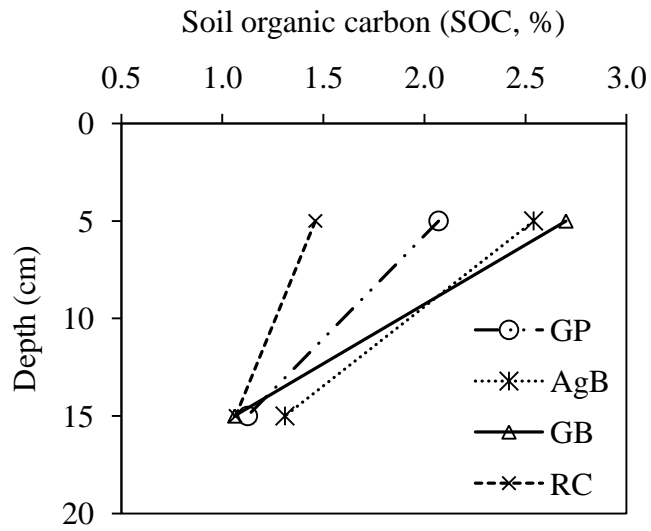
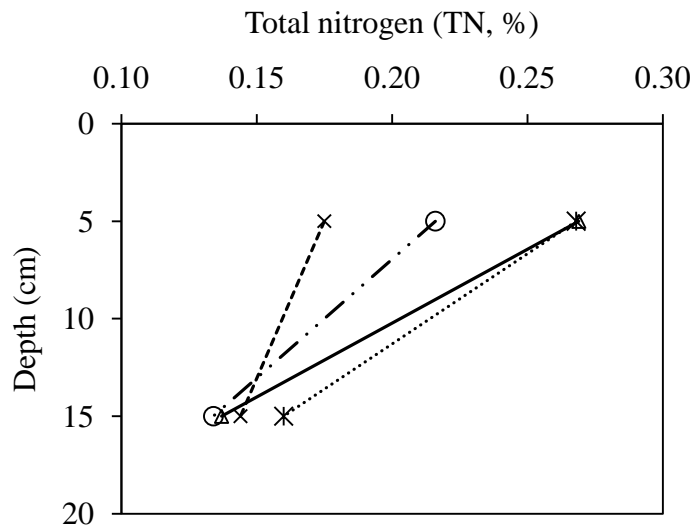


Figure 4.2. Soil bulk density as a function of depth for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row crop (RC). Samples were from the 0 to 10 and 10-20 cm soil depths and sampling was done in 2010.



a.



b.

Figure 4.3. Soil organic carbon (a.) and total nitrogen (b.) as a function of depth for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row crop (RC) for the year 2010. Samples were from the 0 to 10 and 10 to 20 cm soil depths.

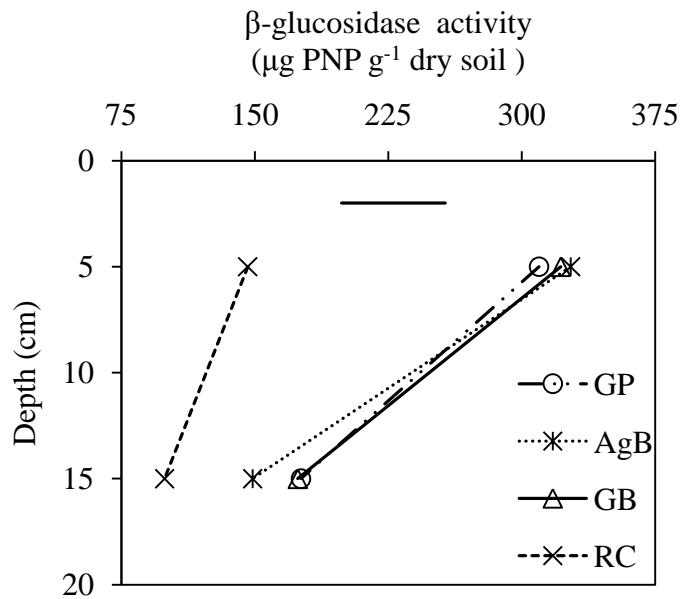
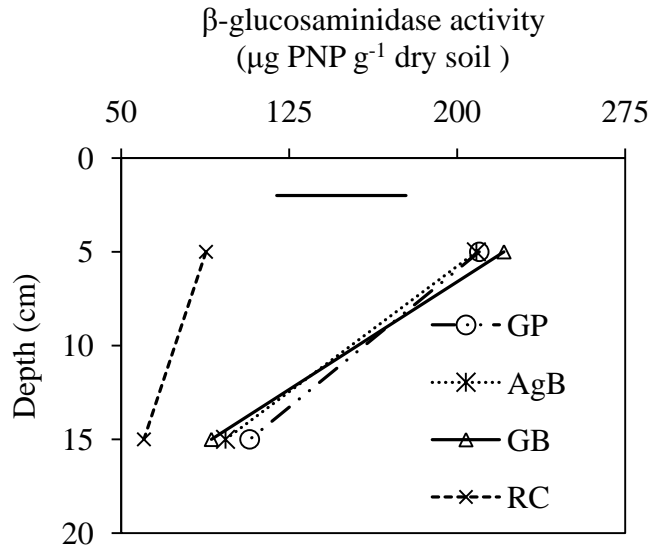
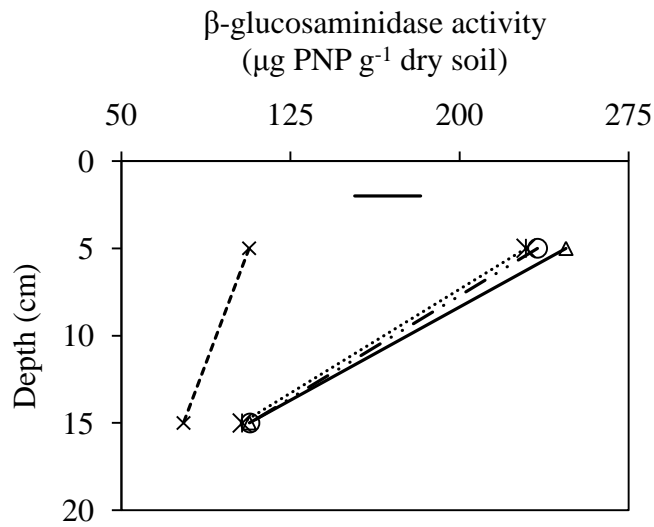


Figure 4.4. β-glucosidase enzyme activity as a function of depth for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row crop (RC) for the year 2009. Samples were from the 0 to 10 and 10 to 20 cm soil depths. The bar indicates the LSD value (58.3).



a.



b.

Figure 4.5.  $\beta$ -glucosaminidase enzyme activity as a function of depth in 2009 (a.) and 2010 (b.) for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row crop (RC) for the year 2009. Samples were from the 0 to 10 and 10 to 20 cm soil depth. The bar indicates the LSD value (57.7 and 29.2, respectively).

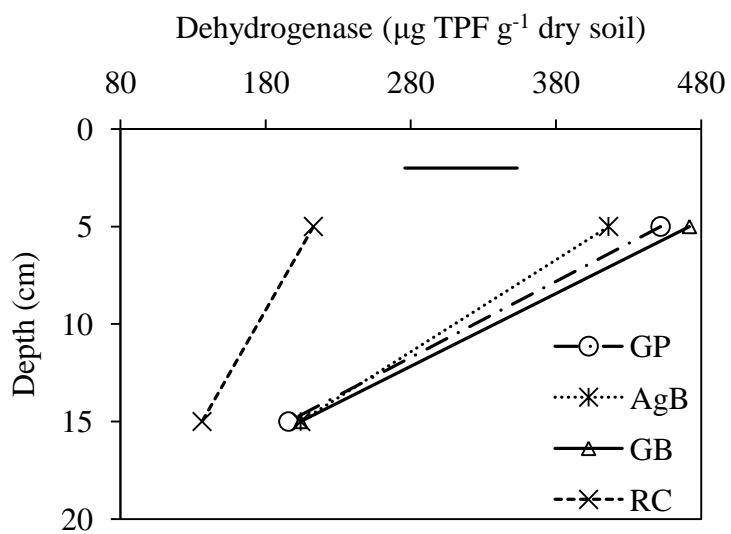


Figure 4.6. Dehydrogenase enzyme activity as a function of depth for the four study treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row crop (RC) for the year 2010. Samples were from the 0 to 20 cm soil depth. The bar indicates the LSD value (77.3)



**CHAPTER 5**  
**SOIL QUALITY INDICES FOR GRAZING PASTURE WITH BUFFERS**  
**AND ROW CROP MANAGEMENT**

**ABSTRACT**

The objective of this study was to compare the effects of soil management on soil quality indicators and generate soil quality indices for each site. In this study, agroforestry buffer (AgB), grass buffer (GB), grazed pasture (GP), and row crop (RC) management were compared with relatively undisturbed soils of Schnabel Woods (SW) on activity of selected enzymes. The samples were collected in June of 2010. The SW soils were mixed with various proportions of coarse sand and the enzyme activities of the sand-soil mixture were colorimetrically quantified in laboratory assays. The enzyme activity of SW was assigned a value of 1 and activity in each treatment was assigned a value between 0 to 1 based on the activity relative to SW. By arithmetic and geometric methods, the RC treatment obtained a lower quality index of 0.41 to 0.43 while perennial vegetation treatments obtained a soil quality index of 0.59-0.67. Results showed a lower quality index for the RC treatment and a higher index for perennial vegetation treatments. The nature of enzyme activities observed in this study support the hypothesis that perennial vegetation provides favorable conditions for higher soil quality compared to row crop management.

*Keywords:* arithmetic method, enzyme activity, perennial vegetation, reference soil, soil quality index.

## **Introduction**

Soil quality has been defined as "*the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health*" (Soil Science Society of America, 1997). Soil quality reflects the biological, chemical, and physical properties and processes and their interactions within each soil resource (Karlen et al., 2001). It also correlates to the dynamic nature of soil as influenced by management practices (Mausbach and Seybold, 1998). Carter et al. (1997) stated that the concept of soil quality is relative to a specific soil function or use. The soil functions include serving as a medium for biomass production, an environmental buffer, and a habitat of flora and fauna (Schroeder and Blum, 1992; Brady and Weil, 2002).

Indicators of soil quality should be responsive to management practices, integrate ecosystem processes, and be sensitive to soil management. Optimum ranges for soil quality parameters need to be defined for different climates and uses. These indicators must be estimated to record the improvement or degradation of soil quality (Larson and Pierce, 1994). Quantifying these variables through comprehensive studies may lead to a better understanding of the effects of land management practices and natural or human-caused disturbances on the soil. Many studies have used undisturbed or native soil as a benchmark for comparison with different management systems (Rasmussen et al., 1989). Periodic assessment of soil quality with known indicators, thresholds and other criteria for evaluation will make it easier to quantify these parameters. Assessment of soil quality and health should be achieved most efficiently using a modeling framework based on collecting and synthesizing an array of soil quality indicators (Harris et al., 1996;

Udawatta and Henderson, 2003). However, each function may differ in its response to changes in certain aspects of soil quality, and these differences may change with plant species and age, management, and environment (Ryan et al., 1997). Therefore, selection of parameters for soil quality assessment may vary with management, soil, and function being evaluated.

Numerous quantitative properties are potential indicators of changes in soil quality (Smith and Mullins, 1991; Trasar-Cepeda et al., 2000; Miguens et al., 2007). These include microbial biomass, diversity, and activity; carbon and nitrogen content and dynamics; fertility status and nutrient availability; soil structure; and water infiltration. To serve as good indicators, the selected soil properties should be sensitive, easy to measure, verifiable, and well correlated with soil management and the effect of environmental (Carter et al., 1997; Seybold et al., 2001).

Of the many parameters singled out as potential indicators of soil quality, organic matter is one of the most important indices. According to Rasmussen and Collins (1991), it is the most universal indicator of soil quality presently available. Depletion in soil organic matter has been linked to the decline of soil quality as a whole and is highly inclined to management strategies (Ding et al., 2002). Agricultural practices result in a significant decline of soil organic matter mainly as a consequence of intensive tillage, inadequate residue management, and an over-reliance on inorganic fertilizers (Rasmussen and Collins, 1991; Ding et al., 2002). In contrast, establishment of agroforestry and other types of perennial vegetation has been shown to improve soil organic matter in agricultural soils (Kremer Li, 2003; Udawatta et al., 2008, 2009).

Biological indicators represent different functions of soil quality in ecosystems (Elliott, 1997). Among the microbial parameters, enzyme activities have been identified as possible indicators of the quality of soil because of their rapid responses to changes in soil management (Bandick and Dick, 1999; Nannipieri et al., 2002). To evaluate the impact of management practices on the quality of soil, and thus to predict their consequences on the environment, studies have attempted to determine soil quality by using microbial parameters as indicators (Schloter et al., 2003). Enzyme activities have been identified as measurable soil quality indicators for early responses to changes in soil management (Elliott, 1997; Bandick and Dick, 1999).

Soil quality indices (SQI) have been proposed in recent years as tools for assessing effects of soil management practice on soil productivity and health. The use of SQI to integrate or summarize soil properties is a relatively new concept. Only a few studies have proposed and developed such indices for agricultural systems (Doran and Parkin, 1996; Wienhold et al., 2009). The objective of this study was to compare the effects of land management on soil quality where grazed pasture, agroforestry buffer, grass buffer, and row crop management were compared with relatively undisturbed soils from a long-term, undisturbed and wooded area on activity of selected enzymes and thereby generating soil quality indices for each management.

## **Materials and Methods**

### **Study Area**

The experimental site was located at the Horticulture and Agroforestry Research Center (HARC) of the University of Missouri in New Franklin, MO (92°74' W and 37°2'

N; 195 m above sea level). This experiment site consists of four management treatments, grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB) and row crop (RC). The grazed pastures were in four small watersheds which include replicate watersheds with agroforestry buffers (AgB; tree-grass buffers) and grass buffers (GB). This area was under rotational grazing since 2005. Soils for the row-crop (RC) treatment were sampled from an adjacent corn field on the north side of the pasture area and this area was under a corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation.

The reference soil was sampled from Schnabel Woods, Missouri, twelve miles southwest of Columbia. Schnabel Woods is a natural conservation area within the loess bluffs adjacent to the Missouri River floodplain with an 80-acre old-growth tract in the River Hills region of Missouri consisting of a variety of hardwood stands. Species composition in Schnabel Woods includes relatively mesophytic conditions. Vegetation growing on these sites is predominantly sugar maple (*Acer saccharum* Marsh.), walnut (*Juglans nigra* L.), and northern red oak (*Quercus rubra* L.) (Udawatta and Henderson, 2003). Soils for study treatments and reference site were classified as Menfro silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalfs).

### Soil Sampling

The sampling of soil was performed during June of 2010 in both the study treatments and the reference site. For the study treatments, the soil samples were collected from three sub-sample locations for each treatment at two depths with two replications. The soil samples for the GB buffer treatment were taken from the center of the buffer. Samples for the AgB buffer treatment were sampled about 40 cm from the

base of a tree trunk. The soil samples for the row crop were taken from the crop area in the north side of the grazing area.

The soil in the Schnabel Woods reference site (SW; Table 5.1) was sampled from a relatively undisturbed area of the site. Three sampling locations were selected within each of two plots and soils were collected at two depths (0-10 and 10-20 cm) at each location.

Soils were collected with a soil auger and were placed in labeled plastic bags. The sampling bags were sealed and transported to the laboratory in a cooler. All samples were maintained at field moist conditions and were stored at 4°C until analyzed. The gravimetric soil moisture content was measured for all samples.

#### Mixing of Schnabel Woods (SW) Soil with Sand

Composite bulk soil samples ( $\approx 600$  g) were prepared by mixing about 100 g of soil from each of six surface soil and sub-surface soil samples separately. Sand (Table 5.1) was washed three times with DI water and oven dried before mixing with soil. Eleven soil-sand combinations of 100 g each, thoroughly mixed, were prepared by mixing Schnabel Woods (SW) bulk soil with coarse sand. The eleven mixed samples were as follows:

- |                            |                            |
|----------------------------|----------------------------|
| a. 100% sand.              | g. 60% soil with 40% sand. |
| b. 10% soil with 90% sand. | h. 70% soil with 30% sand. |
| c. 20% soil with 80% sand. | i. 80% soil with 20% sand. |
| d. 30% soil with 70% sand. | j. 90% soil with 10% sand. |
| e. 40% soil with 60% sand. | k. 100% soil               |

- f. 50% soil with 50% sand.

### Laboratory Analysis

Each mixing sample was analyzed in triplicate according to standard procedures (Table 5.2). Soil organic carbon (SOC) and total nitrogen (TN) contents were determined by dry combustion analysis at 950°C using LECO TruSpec CN analyzer based on methodology of Nelson and Sommers (1996).

### Soil Quality Index for Each Soil for Each Enzyme

A soil quality index of for each soil for each enzyme was calculated using the enzyme curves of sand-soil mixtures (Table 5.3).

For example: the dehydrogenase activity of surface SW soil was 847  $\mu\text{g TPF g}^{-1}$ . A soil quality index of 1 was assigned for a soil with this activity. The soil quality indices for all management treatments for this enzyme were estimated using a simple linear relationship. The AgB soil had dehydrogenase activity of 416  $\mu\text{g TPF g}^{-1}$  soil. Hence the soil quality index for AgB surface soil was 0.49. Soil quality indices for all enzymes and for all management treatments were determined in a similar manner.

After estimating the soil quality index for each soil for each enzyme, a single index was developed for each soil by combining the values of each enzyme (Fig. 5.1).

Three methods were used to calculate soil quality for a soil:

*Arithmetic method:* In this method, the sum of all the index values was divided by the number of enzymes to obtain a single value for each soil.

Example: AgB soil,

$$\text{Index} = (0.49+0.98+0.85+0.52)/4 = 0.71$$

*Geometric method:* In this method, the product of all the index values was raised to the exponent of the reciprocal of the number of enzymes.

Example: AgB soil,

$$\text{Index} = (0.49*0.98*0.85*0.52)^{1/4} = 0.68$$

*Multiplicative method:* In this method, the product of all the values was used to obtain a single index value for each soil.

Example: AgB soil,

$$\text{Index} = (0.49*0.98*0.85*0.52) = 0.25$$

After estimating the indices for each surface and sub-surface soil, the values were averaged across depths to obtain a value for each soil management system.

## **Results**

### Dehydrogenase Activity

The dehydrogenase activity of mixture of Schnabel Woods soil and sand showed a high correlation with the soil percent in the sand-soil mixture (Fig. 5.2). Curves for surface and sub-surface soil were linear (surface soil activity =  $8.31 * \text{percent soil} - 10.25$ ; sub-surface soil activity =  $3.54 * \text{soil percent} + 22.41$ ) with  $r^2$  values  $> 0.98$ .

The maximum activities were 847 and 389  $\mu\text{g TPF g}^{-1}$  soil in the surface and sub-surface soils, respectively. The AgB, GB, GP and RC treatments had enzyme activity of 310, 338, 324, and 175  $\mu\text{g TPF g}^{-1}$  soil averaged across surface and sub-surface respectively (Table 5.4). Soil quality index for AgB, GB, GP, and RC treatments were



0.51, 0.40, 0.38, and 0.21, respectively, for this enzyme with respect to the reference soil (Table 5.3).

#### FDA Hydrolase Activity

The FDA activity increased linearly with the increase of soil percent in the mixture. However, the rate of increase was low at the beginning up to 40% soil (Fig. 5.3). The equations (surface activity =  $20.88 * \text{percent soil} - 137.2$ ,  $r^2 = 0.97$ ; sub-surface activity =  $11.62 * \text{soil percent} - 138.90$ ,  $r^2 = 0.90$ ) explained greater than 90% of the variations in activities.

The maximum activities were 1953 and 1235  $\mu\text{g fluorescein g}^{-1}$  soil in the surface and sub-surface soils, respectively. The AgB, GB, GP and RC treatments had FDA activity of 805, 811, 760, and 705  $\mu\text{g fluorescein g}^{-1}$  soil, respectively (Table 5.4). For this enzyme, the soil quality indices were 0.50, 0.51, 0.48, and 0.44 for AgB, GB, GP, and RC treatments, respectively (Table 5.3).

#### $\beta$ -glucosidase Activity

The  $\beta$ -glucosidase activity of Schnabel Woods soil mixture with sand was quadratically correlated with the percent soil in the sand-soil mixture (Fig. 5.4). The equations (surface activity =  $0.013 * (\text{percent soil})^2 + 0.151 * \text{percent soil} + 29.94$ ,  $r^2 = 0.96$ ; sub-surface activity =  $-0.007 * (\text{soil percent})^2 - 2.3 * \text{percent soil} + 23.74$ ,  $r^2 = 0.91$ ) described over 93% of the variations in the enzyme activity.

The maximum activities were 346 and 190  $\mu\text{g PNP g}^{-1}$  soil in the surface and sub-surface soils, respectively. The average  $\beta$ -glucosidase activity in the AgB, GB, GP and

RC treatments was 246, 237, 241, and 190  $\mu\text{g PNP g}^{-1}$  soil, respectively (Table 5.4). Hence, soil quality indices of 0.88, 0.86, 0.86, and 0.63 were assigned to management treatments AgB, GB, GP, and RC, respectively (Table 5.3).

### $\beta$ -glucosaminidase Activity

The  $\beta$ -glucosaminidase activity of Schnabel Woods soil mixture with sand was also quadratically correlated with the soil percent in the sand-soil mixture in the surface soil, however the sub-surface soil activity was linearly correlated (Fig. 5.5). The equations for surface (activity =  $0.029 * (\text{percent soil})^2 - 0.307 * \text{percent soil} + 14.29$ ) and sub-surface soils (activity =  $1.35 * \text{soil percent} - 10.49$ ) described significant variations of enzyme activities with  $r^2 > 0.93$ .

The maximum activities were 270 and 144  $\mu\text{g PNP g}^{-1}$  soil in the surface and sub-surface soils, respectively. The  $\beta$ -glucosaminidase activities for study treatments were 167, 177, 171, and 92  $\mu\text{g PNP g}^{-1}$  soil for AgB, GB, GP, and RC, respectively (Table 5.4). The soil quality index for the soils of the treatments AgB, GB, GP, and RC estimated as 0.79, 0.83, 0.80, and 0.47, respectively (Table 5.3).

### Soil Organic Carbon (SOC) and Total Nitrogen (TN)

The soil organic carbon and total nitrogen of the Schnabel Woods soil mixture with sand exhibited a linear relationship with the increase in the soil percent in the mixture (Fig. 5.6 a. and b.) in both surface and sub-surface soils. These were expected results as the presence of sand in the mixture diluted the carbon amount; the sand virtually contained 0% carbon and nitrogen.

## Soil Quality Indices

By the arithmetic method, the soil quality indices for AgB, GB, GP and RC soils were found to be 0.67, 0.65, 0.63, and 0.45, respectively (Fig. 5.1). For the respective soils, soil quality indices were 0.65, 0.61, 0.59 and 0.41 by the geometric method. However, the values were significantly smaller by the multiplicative method as compared to the arithmetic and geometric methods. The indices obtained by this method were 0.18, 0.15, 0.14, and 0.03 for AgB, GB, GP, and RC treatments, respectively (Fig. 5.1). The AgB soil had the highest and RC soil had the lowest soil quality index by all three methods.

## Discussion

The activities of selected soil enzymes for the reference soil mixture with sand provided a rationale to compare the management treatments of HARC. Considering Schnabel Woods soil as a reference soil was also justifiable as this site represented a relatively undisturbed soil of the same sub-order of Udalfs. The soil-sand mixture demonstrated a significant correlation between the enzyme activity and the percent of soil in the mixture. The activities increased with the increase in the soil percent either linearly or exponentially. Hence, we compared the activities of managed soils with the various enzyme curves of the sand-soil mixture.

Our results showed that perennial vegetation treatments had soil quality indices between 0.63 and 0.67 while the RC treatment had soil quality index of 0.43 with respect to the reference soil by the arithmetic method. The perennial vegetation treatments

revealed a soil quality index of 0.59 to 0.65 and the RC treatment had an index of 0.41 by the geometric method. Similarly, according to the multiplicative method, the soil quality indices ranged from 0.14 to 0.18 in the perennial vegetation treatments while the RC treatment had an index of 0.03. In all three methods, the AgB treatment had the highest index and the RC treatment had the lowest index. The RC treatment had a lower soil quality index due to lower activities among all treatments (chapters 3 and 4) which satisfies the general hypothesis that soil quality degrades as a result of soil disturbance through periodic cultivation practices, effects of agrochemicals and reduction in organic carbon.

The results indicated that the soil quality index of AgB was six times greater than that of RC soil according to the multiplicative method. However, the soil quality indices of AgB were about 1.5 times greater compared to that of RC soil by arithmetic and geometric methods. It could be interesting to interpret which methods demonstrated variation of soil quality status in RC and other vegetation treatments the most accurately. However, further consideration might be necessary to identify the most representative indicator to estimate soil quality. The results of this study also enabled us to compare different soil management practices. Our results showed that perennial vegetative cover and incorporation of trees and shrubs led to an increasing trend in soil quality indices. These observations reflect the positive changes in soil quality with less soil disturbance and greater organic matter accumulation. The results also imply that the grazed pasture did not reveal significantly higher soil quality than buffers in the short duration.

Other quality indicators could also be considered and the responses of indicators with stresses and disturbances of the soil could be useful for better index development.

Nevertheless, the soil quality index is a simple index that indicates the variation of soil characteristics under different management systems. According to Karlen et al. (2001), it is relative and conditional. They also emphasized the need of better accuracy of indices estimated for a specific soil.

Using only soil biological indicators to rank soil quality is difficult. As soil quality is composed of many variables, an exact integration method is necessary to develop a soil quality index. More importantly, the soil quality indexing methods should reflect suitability of a particular soil for a specified function. Greater soil quality should improve soil productivity. Other studies have also reported an increase in crop productivity with improvement in soil quality (Lee et al., 2006). The current study demonstrated a general trend of soil quality index in perennial vegetation treatments and row crop management and the results of this study could be used to compare soils to explain effects of land management.

## **Conclusions**

In this study, we estimated soil quality indices for various management practices with respect to a reference soil. The soil enzyme activities were selected as potential soil quality indicators. Firstly, an index was obtained for each enzyme and each soil separately. The indices of four enzymes were then combined with arithmetic, geometric and multiplicative methods to estimate a single value. The soil quality indices estimated in this study showed that RC soils had a lower quality index and perennial vegetation treatments had a higher soil quality index. The nature of enzyme activities observed in

this study also support the hypothesis that perennial vegetation provides favorable conditions for greater enzyme activities compared to row crop management.

The arithmetic, geometric and multiplicative methods showed a similar trend of soil quality indices. However, the multiplicative method revealed relatively lower indices for management soils. According to this method, the AgB soil had six times greater soil quality compared to RC soil.

It is justifiable to assign a lower quality index to the RC treatment and a higher index to perennial vegetation treatments assuming the reference soil represents an ideal soil. The methods used in this study and the results obtained will certainly be informative for future research. Conclusions can be made that assessing enzyme activity as soil quality indicators are useful to estimate a soil quality index.

### **Acknowledgements**

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Table 5.1. Particle size distribution of Schnabel Woods (SW) soils and sand (<2mm fraction).

Sample	Texture			Silt			Sand			Textural class	
	Clay	Silt	Sand	Fine	Coarse	Very fine	Fine	Medium	Coarse		Very coarse
SW (0-10 cm)	17.8	76.9	5.3	29.5	47.4	4.5	0.5	0.1	0.1	0.1	Silt loam
SW (0-20 cm)	16.1	78.1	5.8	29.8	48.3	5.1	0.4	0.1	0.1	0.1	Silt loam
Sand	0.0	0.9	99.1	0.3	0.6	0.2	17.0	35.8	44.0	2.1	Coarse sand

Table 5.2. Standard methods of enzyme assays.

Enzymes	Weight	Substrate	Incubation /shaking	Spectrophotometer		Reference
				Wavelength	Unit	
Dehydrogenase	6g	TTC	37°C, 24 h	485 nm	$\mu\text{g TPF g}^{-1}$ dry soil	Tabatabai, 1994
$\beta$ -glucosidase	1g	PNG	37°C, 1 h	410 nm	$\mu\text{g PNP g}^{-1}$ dry soil	Dick et al., 1996
$\beta$ -glucosaminidase	1g	PNNG	37°C, 1h	405 nm	$\mu\text{g PNP g}^{-1}$ dry soil	Parham and Deng, 2000
FDA	1g	FDA	Shaking, 105 min	490 nm	$\mu\text{g F g}^{-1}$ dry soil	Dick et al., 1996

Abbreviations:

TTC: 2, 3, 5-triphenyltetrazolium chloride TPF: triphenyl formazan PNG: p-nitrophenyl- $\beta$ -D-glucoside

PNNG: p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide PNP: p-nitrophenol FDA: Fluorescein diacetate F: fluorescein

Table 5.3. Soil quality index for surface and sub-surface soils of agroforestry buffer (AgB), grass buffer (GB), grazed pasture (GP), and row crop (RC) for dehydrogenase (DH),  $\beta$ -glucosaminidase (GS),  $\beta$ -glucosidase (GC), and fluorescein Diacetate (FDA) hydrolase activities.

Treatment	Depth	DH	GC	GS	FDA	Arithmetic	Geometric	Multiplicative
	-cm-							
AgB	0-10	0.49	0.98	0.85	0.52	0.71	0.68	0.21
	10-20	0.52	0.78	0.72	0.48	0.63	0.61	0.14
GB	0-10	0.55	0.92	0.91	0.51	0.72	0.70	0.23
	10-20	0.24	0.80	0.74	0.50	0.57	0.52	0.07
GP	0-10	0.53	0.93	0.86	0.48	0.70	0.67	0.23
	10-20	0.23	0.82	0.74	0.47	0.56	0.51	0.07
RC	0-10	0.25	0.57	0.39	0.48	0.42	0.41	0.03
	10-20	0.16	0.69	0.54	0.39	0.44	0.40	0.02

Table 5.4.  $\beta$ -glucosaminidase (GS),  $\beta$ -glucosidase (GC), dehydrogenase (DH) and fluorescein Diacetate (FDA) hydrolase enzyme activities, soil organic carbon (SOC), and total nitrogen (TN) for grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), row crop (RC), and Schnabel Woods (SW) treatments (n=12).

Treatment	GS	GC	DH	FDA	SOC	TN
			$\mu\text{g g}^{-1}$ dry soil			%
GP	170.8b	240.7b	323.8b	759.7b	1.60ab	0.18a
AgB	166.5b	246.2b	310.2b	804.6b	1.92ab	0.22a
GB	177.0b	236.6b	337.9b	811.4b	1.88ab	0.20a
RC	92.2c	190.3b	174.6c	705.4b	1.26b	0.16a
SW	236.1a	299.3a	665.6a	1702.5a	2.45a	0.23a

Data followed by the same letter within a column were not significantly different at  $p \leq 0.05$ .

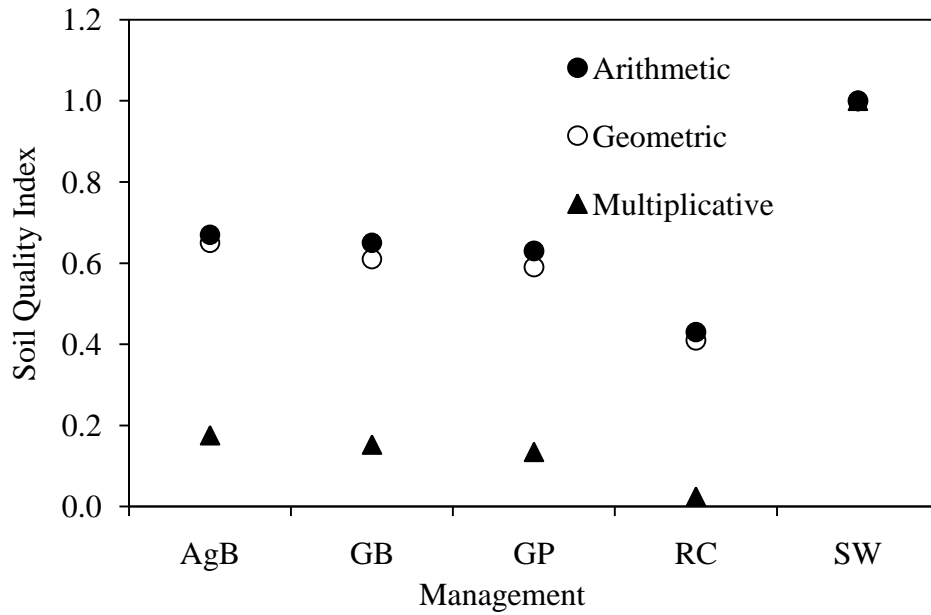


Figure 5.1. Soil quality indices for agroforestry buffer (AgB), grass buffer (GB), grazed pasture (GP), and row crop (RC) estimated by arithmetic, geometric and multiplicative methods.

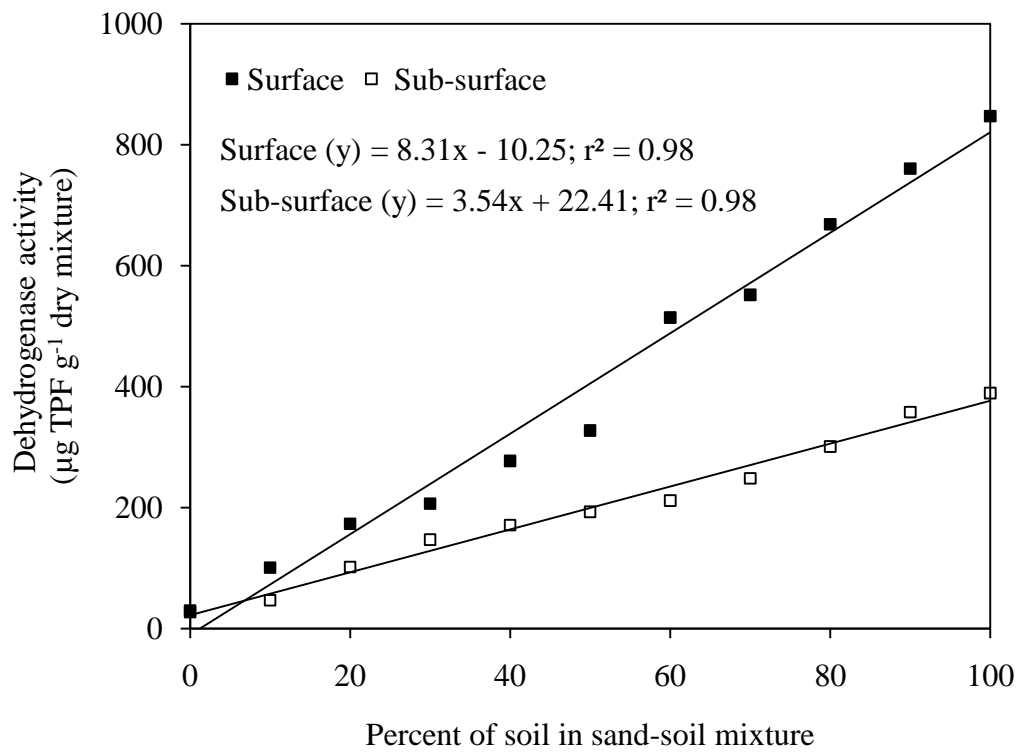


Figure 5.2. Dehydrogenase enzyme activities in surface and sub-surface soil of Schnabel Woods mixed with various proportions of sand (n=3).

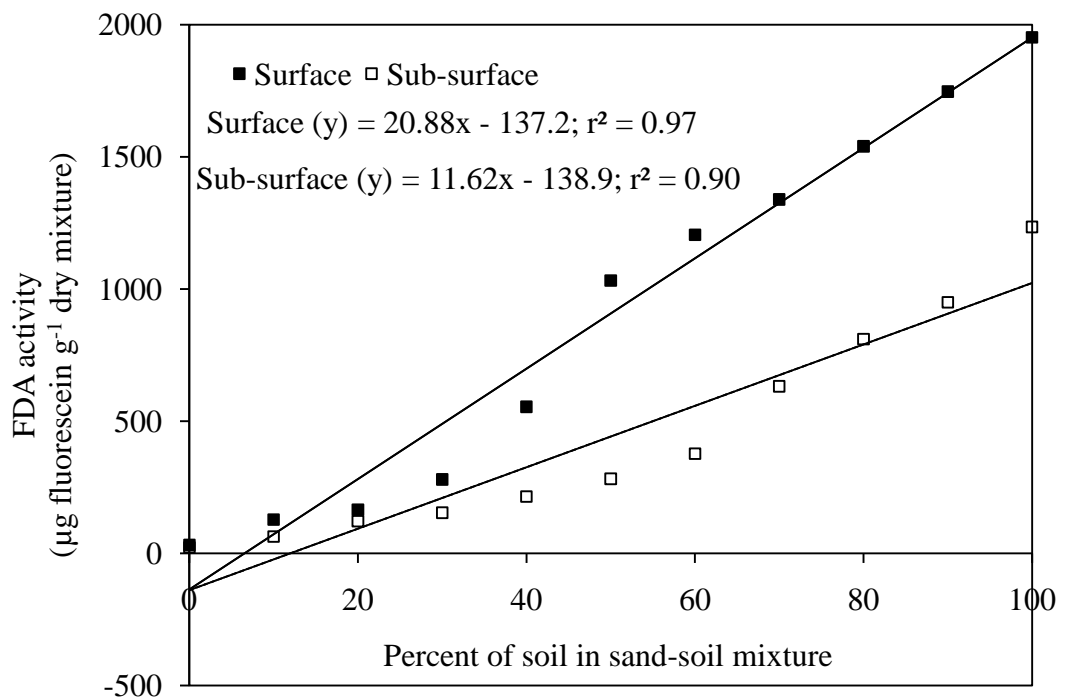


Figure 5.3. FDA hydrolase enzyme activities in surface and sub-surface soil of Schnabel Woods mixed with various proportions of sand (n=3).



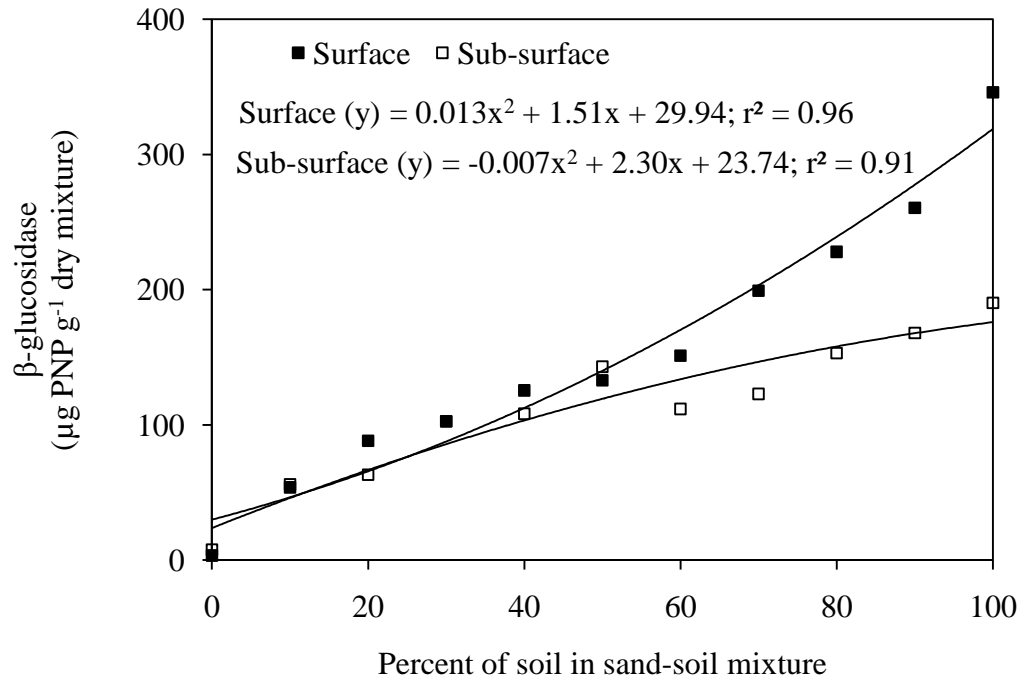


Figure 5.4.  $\beta$ -glucosidase enzyme activities in surface and sub-surface soil of Schnabel

Woods mixed with various proportions of sand (n=3).

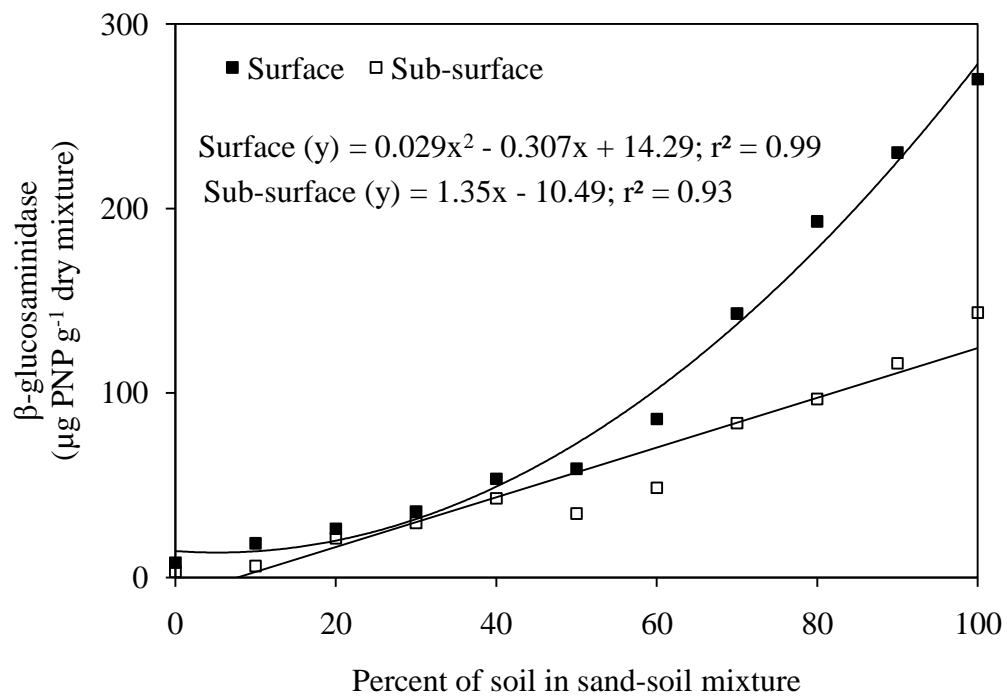
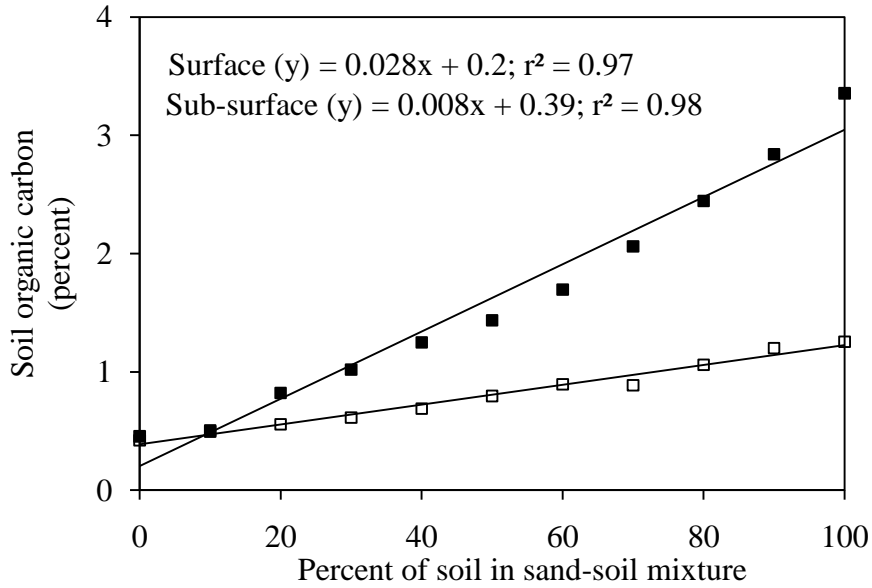
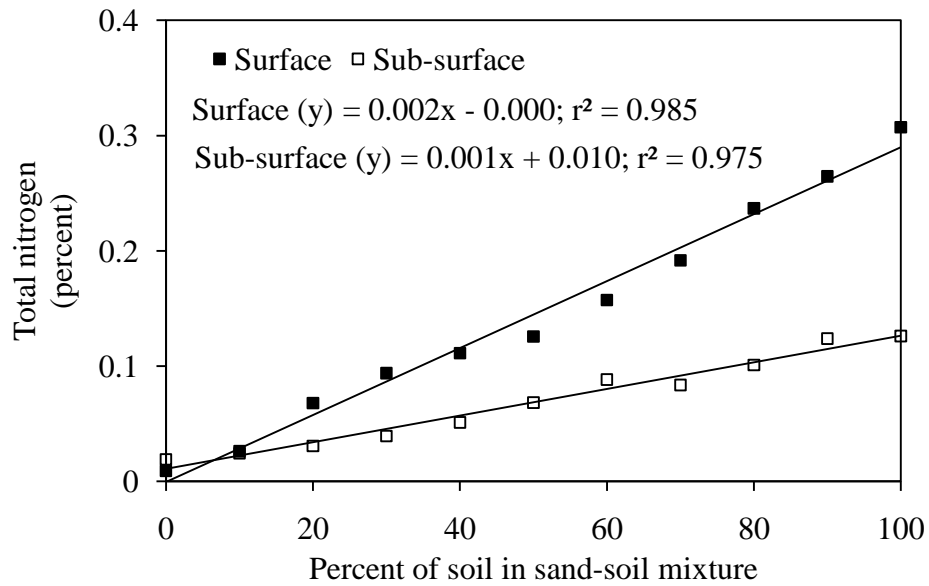


Figure 5.5.  $\beta$ -glucosaminidase enzyme activities in surface and sub-surface soil of Schnabel Woods mixed with various proportions of sand (n=3).



a.



b.

Figure 5.6. Soil organic carbon (a) and total nitrogen (b) in surface and sub-surface soil of Schnabel Woods (SW) mixed with various combinations of sand (n=3).

## CHAPTER 6

### CONCLUSIONS

The objective of this study was to evaluate soil quality indicators as influenced by permanent vegetative buffers in grazed pasture management compared with row crop management and to develop a soil quality index to compare soils. In addition, landscape effects for the row crop (RC) and grazed pasture (GP) treatments and soil depth effects for all treatments were evaluated. Soil enzyme activities (dehydrogenase, fluorescein diacetate,  $\beta$ -glucosidase, and  $\beta$ -glucosaminidase), water stable aggregates (WSA), soil organic carbon (SOC), total nitrogen (TN), and bulk density under grazed pasture (GP), agroforestry buffer (AgB), grass buffer (GB), and row crop (RC) areas were studied during 2009-2010. The experimental site is located at the Horticulture and Agroforestry Research Center in New Franklin, Missouri. Grazed pasture and grass buffer areas consist of red clover (*Trifolium pretense* L.) and lespedeza (*Kummerowia stipulacea* Maxim.) planted into fescue (*Festuca arundinacea* Schreb.). The agroforestry buffers contain eastern cottonwood trees (*Populus deltoids* Bortr. ex Marsh.) planted into fescue. The row crop area was at the north side of grazed pastures which was under a corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation. In addition, Schnabel Woods, Missouri had been chosen as a reference site which is a relatively undisturbed natural forest. Soils at the sites were Menfro silt loam (fine-silty, mixed, superactive, mesic Typic Hapludalfs).

Most of the soil quality indicators were significantly greater in perennial vegetation treatments compared to row crop. The dehydrogenase activity in the GP

treatment was 323.8  $\mu\text{g TPF g}^{-1}$  dry soil while it was 174  $\mu\text{g TPF g}^{-1}$  dry soil in RC treatment. Similarly, the GB treatment showed an activity of 811.4  $\mu\text{g fluorescein g}^{-1}$  dry soil in 2010 for FDA enzyme. However, the RC treatment had an FDA activity of 705.4  $\mu\text{g fluorescein g}^{-1}$  dry soil in 2010. Most importantly, the soil quality indicators were consistent during the two year study although there were numerical differences. The  $\beta$ -glucosidase and  $\beta$ -glucosaminidase enzymes were most consistent during two years, which were significantly higher in AgB, GB, and GP treatments compared to the RC treatment. The  $\beta$ -glucosaminidase activity increased slightly from 155.6 to 177.0  $\mu\text{g PNP g}^{-1}$  dry soil while  $\beta$ -glucosidase activity slightly decreased from 248.0 to 236.6  $\mu\text{g PNP g}^{-1}$  dry soil in GB treatment from 2009 to 2010. Water stable aggregates improved from 17.8 to 31.4% in row crop while all other treatments had similar values during the two-year study. Soil enzyme activities were significantly correlated with soil organic carbon content and nitrogen ( $r=0.78$  to  $0.94$ ;  $P<0.0001$ ). Based on water stable aggregates, enzyme activities, soil organic carbon, nitrogen, and bulk density, it is obvious that regular disturbance has significantly reduced soil quality in row crop agriculture. These results showed that organic matter additions from vegetation as well as soil disturbance from cultivation practices undoubtedly hold a strong influence over various soil enzyme activities.

A separate analysis was conducted to assess whether landscape positions influenced enzyme activities, water stable aggregates, soil carbon, and total nitrogen. Data comparisons were only made with treatments that contained landscape positions; the GP and the RC treatments. Landscape effects were not significant for the measured

parameters within the treatments. Also, treatment by landscape interactions were not significant.

In this study, soils were sampled from the surface (0-10 cm) and sub-surface (10-20 cm) horizons which are believed to express the greatest biological activity and are most likely to reflect changes due to management and vegetation. Depth effect was significant for all indicators and there were significant treatment by depth interactions for  $\beta$ -glucosidase,  $\beta$ -glucosaminidase and dehydrogenase enzyme activities. The  $\beta$ -glucosidase activity was 324.3  $\mu\text{g PNP g}^{-1}$  soil in the surface while it reduced to 157.2  $\mu\text{g PNP g}^{-1}$  soil in the sub-surface in the GP treatment. Similarly, the  $\beta$ -glucosidase activity reduced to 131.8 in sub-surface from 198.8  $\mu\text{g PNP g}^{-1}$  in the surface of RC treatment. All enzymes exhibited a similar pattern between the surface and subsurface soils. This supports the hypothesis that greater microbial activities and functional diversity exist in perennial vegetation areas compared to row-crop areas.

Higher soil enzyme activities may lead to increases in other soil quality parameters such as organic matter content, aggregation, soil sustainability and productivity, and consequently soil and ecosystem functions. The perennial vegetative cover also leads to greater carbon accumulation and consequently increases in selected soil quality indicators. The soil quality indices for the perennial vegetation treatments ranged from 0.63 to 0.67 and 0.59 to 0.65 by arithmetic and geometric methods respectively. The AgB had the highest index and RC had the lowest index by all three methods. The RC treatment had the indices of 0.43 and 0.41 by arithmetic and geometric methods respectively. Hence in this study, it is justifiable to assign a lower quality index

to the RC treatment and a higher index to perennial vegetation treatments with respect to the reference soil with a soil quality index of one.

The nature of enzyme activities observed in this study support the hypothesis that perennial vegetation provides favorable conditions for greater enzyme activities and microbial diversity compared with soils under row crop management. Conclusions can be made that assessing changes in selected enzyme activities can be a useful tool to determine land degradation when reference values for similar systems are available. Results strongly support that the establishment of agroforestry and grass buffers in grazed pasture will enhance soil quality and help maintain ecosystem sustainability.

## APPENDIX

General soil properties of the soil samples used in the study.

Description	pH	SOC	TN	Ca	K	Mg
		----- % -----		----- ppm -----		
AgB-2009	5.32	1.70	0.20	1510.3	136.1	248.6
AgB-2010	5.73	1.92	0.22	1480.7	117.8	204.5
GB-2009	5.28	1.70	0.19	1240.3	96.8	238.5
GB-2010	5.36	1.88	0.20	1522.3	109.6	292.7
GP-2009	5.37	1.80	0.20	1228.5	91.3	173.2
GP-2010	5.47	1.60	0.18	1840.7	122.4	275.5
RC-2009	4.47	1.2	0.13	959.1	189.1	80.6
RC-2010	4.79	1.26	0.16	901.9	214.6	122.4
SW-2010	5.64	2.31	0.22	1279.4	96.8	128.9

Abbreviations: AgB= Agroforestry Buffer; GB= Grass Buffer; GP= Grazed Pasture; RC= Row crop

SW= Schnabel Woods. SOC= Soil organic carbon; TN= Total nitrogen; Ca= Calcium; K= Potassium

Mg= Magnesium; ppm= parts per million



## VITA

Bodh R. Paudel was born in Nepal on July 1, 1982 to Mr. and Mrs. Rishi Ram Paudel. He received his bachelor degree in agriculture in 2006 from Tribhuvan University, Institute of Agriculture and Animal Science, (Nepal). He joined the University of Missouri-Columbia in 2009 for his masters degree and received this degree in Soil Science in 2011. He worked under the advice of Drs. Ranjith P. Udawatta, Stephen H. Anderson and Robert J. Kremer. He has been accepted for a doctoral position in Washington State University to begin by Fall 2011.