

# Variations in soil aggregate stability and enzyme activities in a temperate agroforestry practice

# Ranjith P. Udawatta <sup>a,b,\*</sup>, Robert J. Kremer<sup>c</sup>, Brandon W. Adamson<sup>a</sup>, Stephen H. Anderson<sup>b</sup>

<sup>a</sup> Center for Agroforestry, School of Natural Resources, University of Missouri, Columbia, MO 65211, USA <sup>b</sup> Department of Soil, Environmental and Atmospheric Sciences, School of Natural Resources, University of Missouri, Columbia, MO 65211, USA <sup>c</sup> USDA Agriculture Research Service, University of Missouri, Columbia, MO 65211, USA

#### ARTICLE INFO

Article history: Received 23 May 2007 Received in revised form 16 November 2007 Accepted 5 December 2007

Keywords: Corn–soybean rotation Grass buffer Grass waterways Nonpoint source pollution Total carbon Soil DNA

#### ABSTRACT

Agroforestry and grass buffers have been shown to improve soil properties and overall environmental quality. The objective of this study was to examine management and landscape effects on water stable soil aggregates (WSA), soil carbon, soil nitrogen, enzyme activity, and microbial community DNA content. Treatments were row crop (RC), grass buffer (GB), agroforestry buffer (AG), and grass waterways (GWW). A corn (Zea mays L.)soybean (Glycine max L.) rotation under no-till management was established in a watershed in northeast Missouri in 1991; grass buffers were implemented in 1997. Grass buffers, 4.5 m wide and 36.5 m apart, consisted of a mixture of redtop (Agrostis gigantea Roth), brome grass (Bromus spp.), and birdsfoot trefoil (Lotus corniculatus L.) on contour within the watershed. Agroforestry buffers have pin oak (Quercus palustris Muenchh.) trees distributed down the center of the grass buffers on one half of the watershed. Soils were collected from two transects extending from the summit to lower landscape positions within the grass and agroforestry portions of the watershed in June 2006. Soil enzymes studied include: fluorescein diacetate hydrolase,  $\beta$ -glucosidase, glucosaminidase, and dehydrogenase. Soil DNA content was determined as an alternative for microbial biomass. WSA was significantly different among treatments and landscape positions. WSA decreased from GWW > AG > GB > RC management treatments and also decreased from lower > middle > summit landscape positions. Soil carbon and nitrogen were highest for the GWW treatment and lowest for RC. The landscape position effect was significant for RC and AG treatments. Fluorescein diacetate,  $\beta$ -glucosidase and glucosaminidase enzyme activities were significantly higher in buffers and GWW areas than RC areas. Dehydrogenase activity was different between grass (GB and GWW) and crop areas. The landscape effect was insignificant for enzyme activity. Although soil DNA may be a good indicator of microbial biomass, it did not appear to differentiate among management systems as selectively as other microbial parameters. Results of the study show that establishment of AG, GB, and GWW increased WSA, soil carbon, soil nitrogen, and enzyme activity.

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E-mail address: UdawattaR@Missouri.edu (R.P. Udawatta). 0929-1393/\$ – see front matter © 2007 Elsevier B.V. All rights reserved. doi:10.1016/j.apsoil.2007.12.002

<sup>\*</sup> Corresponding author at: Center for Agroforestry, School of Natural Resources, University of Missouri, Columbia, MO 65211, USA. Tel.: +1 573 882 4347; fax: +1 573 882 1977.

# 1. Introduction

Agroforestry, grass, and vegetative buffers reduce nonpoint source pollution (NPSP) from row crop agriculture (Schmitt et al., 1999; Udawatta et al., 2002; Abu-Zreig et al., 2003) by improving soil physical properties (Wood, 1977; Broersma et al., 1995; Bharati et al., 2002; Seobi et al., 2005) and increasing water and nutrient use by permanent vegetation (Udawatta et al., 2005). Udawatta et al. (2006, 2008) also showed that adoption of agroforestry and grass buffers improve mm- and  $\mu$ m-scale soil pore parameters. Seobi et al. (2005) stated that 6-year-old agroforestry and grass buffers improved porosity and coarse mesoporosity (diameter 60–1000  $\mu$ m) in silt loam soil by 3 and 33%, respectively, compared to soil under no-till soybean management. Results showed that most of the physical changes in soil properties occurred in the surface 0- to 100-mm depth.

Establishment of agroforestry and grass buffers also contributes to greater soil carbon sequestration and landscape diversity (Palma et al., 2007). Soil organic matter is considered the key soil quality factor that promotes aggregate stability including larger diameter aggregates. The percentage of water stable aggregates (WSA) is an indication of resistance to breakdown by water and mechanical manipulation and also is an indicator for improved soil water and air movement. Macroaggregates (diameter  $>250 \,\mu$ m) are considered as a secondary soil structure associated with pores, microbial habitat, and physical protection of organic matter (Christensen, 2001; Carter, 2004). Aggregates provide spatially differentiated habitats for microorganisms and are important for biogeochemical soil processes (Park and Smucker, 2005). More oxygen demanding microorganisms tend to concentrate near the external regions of the soil aggregates while less oxygen demanding organisms remain within aggregates.

Enzyme assays provide quantitative information on soil chemical processes, nutrient mineralization rates, and organic matter accumulation. However, enzyme activities and microbial communities are different in agroforestry alley cropping practices compared with row crop areas due to differences in litter quality and quantity, as well as root exudates (Gomez et al., 2000; Myers et al., 2001; Mungai et al., 2005). Enzyme assays among different management practices may also indicate short-term differences in soil quality improvement, and can be used to evaluate rapid responses to changes in management and in understanding sensitivity to environmental stresses (Dick, 1997). Research shows that fluorescein diacetate (FDA) hydrolysis,  $\beta$ -glucosidase, glucosaminidase, and dehydrogenase activities are good indicators of soil biogeochemical processes.

Fluorescein diacetate hydrolysis represents a broad spectrum of enzymatic activities such as proteases, lipases, and esterases involved in the decomposition of complex organic compounds (Dick et al., 1996). The activity of FDA is distributed among a variety of primary decomposers and has been correlated with soil organic matter and carbon contents (Dick et al., 1996; Gasper et al., 2001). Mungai et al. (2005) found that FDA activity was significantly higher in tree rows in a temperate alley cropping practice and the differences were attributed to tree age and soil water content.

 $\beta$ -Glucosidase enzyme is involved in the degradation of cellulose, the main component of plant polysaccharides and

other carbohydrate polymers (Turner et al., 2002). In a temperate alley cropping practice, Mungai et al. (2005) observed increased  $\beta$ -glucosidase activity in tree areas compared with crop alleys. The treatment difference was related to differences in soil organic matter content.

The substrate for glucosaminidase includes chitobiose and higher proteins (Parham and Deng, 2000). Chitin is the second most abundant biopolymer on Earth and serves as an important transient pool of organic C and N in soils (Parham and Deng, 2000; Ekenler and Tabatabai, 2003). Chitin-hydrolyzing enzymes are widely distributed in nature resulting in the release of amino sugars – about 5–10% of organic N in soil (Stevenson, 1994) – due to degradation of chitin. Nitrogen acquisition by microorganisms is also mediated by this enzyme (Sinsabaugh and Moorhead, 1995). Dehydrogenase activity is directly related to the oxidation of organic matter (Dick et al., 1996). Research has shown that dehydrogenase activity does not consistently correlate with soil biological activities but may be used as an indicator of viable microbial populations (Stevenson, 1959; Dick et al., 1996).

Marstorp et al. (2000) demonstrated that DNA extracted directly from soil was apparently correlated with traditional measures of soil microbial biomass. Measurements of soil DNA content are useful in differentiating the effects of management practices on soil quality and productivity (Releeder et al., 2006). Thus, soil DNA extraction and quantification may be an effective alternative measure of microbial biomass for evaluating impacts of management on the soil ecosystem.

Although several environmental benefits of agroforestry practices are reported in the literature, immediate quantifiable information is unavailable that could be used to evaluate management at the field scale. Loveall and Sullivan (2006) stated that more research is needed to improve understanding of buffer effects on overall water quality and environmental quality. Improved understanding of buffer influences on WSA, soil microbial diversity, and soil enzyme activity is needed. Because microbial communities influence the environment through the production of various metabolites such as organic acids, polysaccharides, polypeptides, bases, and ligands, the composition of air and water in soil is altered (O'Donnel et al., 2007). Thus, information on microbial activities and soil aggregates should help explain changes in water quality due to management, assist in developing model parameters, and assist in the creation of vegetative buffer establishment guidelines. We hypothesized that adoption of grass and agroforestry buffer practices would improve soil properties and functional diversity of microbial populations. The objective of this study was to compare differences in water stable aggregates, soil carbon, soil nitrogen, enzyme activities, and microbial DNA among row crop, grass buffer, agroforestry buffer, and grass waterway areas at three landscape positions for an agroforestry and grass buffer alley watershed under row crop management.

## 2. Materials and methods

#### 2.1. Experimental site description

The study was conducted in the north-facing experimental watershed located at the University of Missouri, Greenley



Fig. 1 – Topographic map of the grass buffer and the agroforestry watersheds with 0.5 m elevation interval contour lines (black), and grass (grass only) and agroforestry (grass + trees) buffers (gray). Grass waterways (wide black) are located at the outflow ends of the watersheds. The three bold numbers (1, 3, and 5) on buffers denote sampled buffers. Black squares represent approximate sampling locations for buffer and crop areas. White squares show sampling locations within the grass waterways. The inset map shows the location of watershed in Knox County, MO.

Memorial Research Center near Novelty, Missouri (40°01'N, 92°11′W; Fig. 1). The watershed has been under a corn (Zea mays L.)-soybean [Glycine max (L.) Merr.] rotation, with no-till land preparation and contour planting since 1991 (Udawatta et al., 2002). The 3.16 ha grass buffer and 4.44 ha agroforestry watersheds consist of 4.5 m wide buffer strips at 36.5-m spacings (22.8 m at lower slope positions). The grass-legume combination planted throughout the buffer strips included redtop (Agrostis gigantea Roth), brome grass (Bromus spp.), and birdsfoot trefoil (Lotus corniculatus L.) established in 1997. Pin oak (Quercus palustris Muenchh.) trees were planted in the center of the buffer strips at 3-m spacing in the agroforestry watershed. Grass waterways on both watersheds consist of Kentucky 31 tall fescue (Festuca arundinacea var. genuina Schreb.). Details on watershed management, parent material, soils, and climatic data can be found in Udawatta et al. (2002, 2006).

In brief, the soil parent materials are glacial till and windblown Peorian loess (Unklesbay and Vineyard, 1992). Soils on the two watersheds include, Putnam silt loam (fine, smectitic, mesic Vertic Albaqualf), Kilwinning silt loam (fine, smectitic, mesic Vertic Epiaqualf), and Armstrong loam (fine, smectitic, mesic Aquertic Hapludalf; Watson, 1979). About 600 mm of the 920 mm long-term average annual precipitation falls from April through September in the area (Owenby and Ezell, 1992). Mean annual air temperature is approximately 11.7 °C with an average monthly low of -6.6 °C in February and an average monthly high of 31.4 °C in July (Owenby and Ezell, 1992). Snow can stay on the ground for extended periods and snowfall averages about 590 mm per year.

#### 2.2. Sampling and analysis procedures

The experimental design for the study assumed a completely randomized design with two treatment factors, management and landscape positions. Management included four treatments: row crop (RC), grass buffer (GB), agroforestry buffer (AG), and grass waterway (GWW). Landscape position treatments included upper, middle, and lower landscape positions. The study included two replications. Soils were sampled in two transects extending from the summit to the lower backslope landscape positions in June 2006. Buffers 1, 3, and 5 (counting from south; Fig. 1) were sampled for GB and AG treatments representing upper, middle, and lower landscape positions, respectively. Soil samples for the GB were taken from the center of the buffer. Soil for the AG treatment was sampled about 40 cm from the base of the tree trunk. For the RC treatment, soils were collected about 2 m south of the buffer edge in the crop area. For the GWW treatment, soils were collected from three landscape positions (south, middle, and north). At each sampling location, surface 0-10 cm soils were collected in duplicate with a soil auger and soils were placed in labeled plastic bags and composited. Sampling bags were sealed and transported to the laboratory in a cooler and stored at 4 °C prior to analysis.

Water stable aggregates were determined using a 10-g soil sample with two replications using the wet-sieving method on aggregates  $>250 \,\mu$ m diameter (Kemper and Rosenau, 1986; Angers and Mehuys, 1993). The aggregate content was corrected for soil moisture and expressed on an oven dry weight basis. Total carbon (TC) and nitrogen concentrations were determined by combustion analysis at 950 °C using a LECO TruSpec CN Analyzer.

The hydrolysis of fluorescein diacetate was colorimetrically quantified at 490 nm to measure enzyme activity (Dick et al., 1996). A sieved 1-g soil sample was shaken for 15 min with 20 mL of sodium phosphate buffer and subsequently shaken with 4.8 mL 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  $\mu$ g fluorescein released g<sup>-1</sup> dry soil h<sup>-1</sup>.

β-Glucosidase enzyme activity was determined according to Dick et al. (1996). Sieved (2 mm) 1-g samples of air-dried soil were used in the analysis with two replications. Soil was incubated with the *p*-nitrophenyl-β-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 µg *p*-nitrophenol released g<sup>-1</sup> dry soil h<sup>-1</sup>.

Glucosaminidase enzyme activity was determined as described by Parham and Deng (2000). Air-dried soil samples

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(A)

(1 g) were used in this analysis with four replications. 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  $\mu$ g *p*-nitrophenol released g<sup>-1</sup> soil h<sup>-1</sup>.

Dehydrogenase enzyme activity was determined as described by Pepper et al. (1995). Moist soil samples (6 g) were used in this analysis with two replications. Soil was incubated with 2,3,5-triphenyltetrazolium chloride substrate at 37 °C for 24 h. Following incubation, a previously created regression equation was used to determine the concentration of the triphenyl formazan (TPF) product colorimetrically (485 nm) and the enzymatic activity was expressed in  $\mu$ g TPF released g<sup>-1</sup> dry soil h<sup>-1</sup>.

Total DNA was extracted from 0.50 g (dry weight) soil using the Soil Isolation DNA Kit (MoBio Laboratories, Carlsbad, CA). Soil samples added to the bead solution were vortexed for 10 min followed by centrifugation at 10,000 × *g* for 30 s; the supernatant was re-centrifuged after protein precipitation and then purified by passing through a spin column by centrifuging at 10,000 × *g* for 30 s. The DNA concentration in each purified extract was quantified by UV spectroscopy at 260 nm and expressed as  $\mu$ g DNA g<sup>-1</sup> dry soil. The extracted DNA was stored at -60 °C.

#### 2.3. Statistical analyses

The data were analyzed assuming a completely randomized design with four management levels and three landscape positions with two replications. Analysis of variance was conducted with SAS using the GLM and MIXED procedures to test differences among treatments and landscape positions (SAS Institute, 1999). Least significant differences (Duncan's LSD) were calculated to find significant differences between treatments for each measured parameter and differences were declared significant at the  $\alpha = 0.05$ . Proc CORR in SAS was used to examine correlation relationships between variables.

# 3. Results

The percentage of WSA was significantly different between soils from the buffer and crop treatments (Fig. 2A). The GB and AG areas had nearly twice (1.94) the stable aggregates compared with crop areas. The proportion of WSA among the four treatments was highest in GWW with 19.97  $\pm$  1.75, which was significantly different from the other three treatments; the crop area had the lowest WSA at 7.68  $\pm$  1.24. The difference between GB and AG was not significant. The percentage of WSA was significantly different among all three landscape positions (Fig. 2B). The lower and middle positions contained higher percentages of WSA with 17.30%  $\pm$  1.53% and 13.03%  $\pm$  1.53%, respectively. The upper landscape position, with the lowest percentage of WSA, contained 8.76%  $\pm$  1.54%.

Total carbon concentrations (TC) in the surface soil were significantly higher for buffer and GWW treatments compared with RC (Fig. 3A). The TC content for the RC treatment was less



Fig. 2 – Water stable aggregates by management treatment (A) and landscape position (B). Bars with the same lower case letters were not significant at p < 0.05.

than 2% whereas it was greater than 2.25% for the other three treatments. The GWW treatment had the highest TC among the four treatments. However, the difference was not significant among the permanent vegetative practices. The effect of landscape position on TC concentration was significant for the RC treatment (Fig. 3B); however, the landscape effect was not significant for the GB treatment. Among the three landscape positions sampled, the lower position within the AG system had the highest TC content. Nitrogen distribution also followed a trend similar to TC. The RC treatment values were lowest in TN content, which was significantly different from the other three treatments.

The analysis of FDA activity revealed significant differences between RC area and the remaining three treatment areas (Table 1). FDA activity was lowest in the RC treatment which released an average of  $8.49 \pm 0.61 \,\mu g$  fluorescein  $g^{-1}$  dry soil  $h^{-1}$  under assay conditions. Of the remaining three treatment areas, the GB contained the greatest enzyme activity releasing, on average,  $13.55 \pm 0.86 \,\mu g$  fluorescein  $g^{-1}$  dry soil  $h^{-1}$ . The FDA activity was significantly greater in GB compared with the other three treatments. The AG and GWW treatments



Fig. 3 – Total soil carbon and nitrogen by management treatment (A) and total soil carbon by landscape position (B) for row crop, grass buffer, and agroforestry buffer treatments. Bars with different upper case letters (B) denote significant differences within a landscape position among management treatments and bars with different lower case letters (B) denote significance difference among landscape positions within a management treatment, at p < 0.05.

contained similar amounts of enzyme activity releasing an average of 11.64  $\pm$  0.86 and 10.53  $\pm$  0.86  $\mu g$  fluorescein  $g^{-1}$  dry soil  $h^{-1}$ , respectively. The landscape position from which soil samples were taken had no significant influence on FDA activity within the sampling area (results not presented).

The RC area showed significantly lower  $\beta$ -glucosidase activity compared with the GB, AG, and GWW treatments (Table 1). Analysis of RC revealed an average of 129.1  $\pm$  14.8  $\mu$ g

p-nitrophenol released  $g^{-1}$  dry soil  $h^{-1}$ . The GB exhibited the highest enzyme activity with 228.8  $\pm$  20.6  $\mu g$  p-nitrophenol released  $g^{-1}$  dry soil  $h^{-1}$ . With only slight differences, the AG and GWW treatments contained 204.3  $\pm$  19.3 and 199.3  $\pm$  20.6  $\mu g$  p-nitrophenol released  $g^{-1}$  dry soil  $h^{-1}$ , respectively.

Analysis of glucosaminidase activity revealed significant differences between the RC area and the remaining treatment areas (Table 1). Grass areas contained the highest glucosaminidase activity with GWW and GB yielding  $135.0 \pm 7.80$  and  $133.8 \pm 8.34 \,\mu g \, p$ -nitrophenol released  $g^{-1}$  dry soil  $h^{-1}$ , respectively. Glucosaminidase activity decreased only slightly under AG conditions, which had an average of  $124.9 \pm 6.65 \,\mu g \, p$ -nitrophenol released  $g^{-1}$  dry soil  $h^{-1}$ . Glucosaminidase activity was lowest within the RC treatment, which contained an average of  $73.5 \pm 5.51 \,\mu g \, p$ -nitrophenol released  $g^{-1}$  dry soil  $h^{-1}$ .

Dehydrogenase activity was higher in grassed areas compared with crop treatments (Table 1). Grass buffers and grass waterways exhibited an average production of  $79.1\pm7.5$  and  $65.5\pm7.2~\mu g$  TPF  $g^{-1}$  dry soil  $h^{-1}$ , respectively. The lower levels of dehydrogenase activity within cropped areas differed slightly between AG and RC treatments, which contained an average TPF production of  $51.0\pm7.5$  and  $48.0\pm5.2~\mu g$  TPF  $g^{-1}$  dry soil  $h^{-1}$ , respectively.

DNA analyses further supported differences among the treatments as indicated by enzyme activities. DNA concentration was highest in the grass waterway ( $16.5 \pm 2.4 \ \mu g g^{-1}$ ) and was significantly different from the other three treatments (Table 1). The DNA concentration in the GWW was more than twice (2.2) that of the RC and GB treatments. The differences in DNA concentrations among the AG, GB, and RC treatments were not significant.

#### 4. Discussion

Results show that WSA percentages within soils under RC management were lower as compared to the GB, AG, and GWW treatments which closely parallel previous findings. Kremer and Li (2003) found WSA percentages to be 18% within Missouri soils planted with various cool season grasses and legumes at sites managed under the "Conservation Reserve Program" (CRP). The CRP is an incentive program of USDA that supports farmers in converting erodible cropland into long-term vegetative cover such as the established meadow sampled for the study in Kremer and Li (2003). The same study concluded that soil planted with corn, under conventional tillage exhibited a significantly lower WSA content (10%). WSA in this study was

treatments	Table 1 – FDA, $\beta$ -glucosidase, g	lucosaminidase, dehydrogenas	e enzyme activities and DNA	concentrations for the four
	treatments			

Treatment	FDA	$\beta$ -Glucosidase	Glucosaminidase	Dehydrogenase	DNA
$(\mu g g^{-1} \text{ soil } h^{-1})$				$(\mu g g^{-1} \text{ soil})$	
Crop	$8.49\pm0.61~\text{a}$	$129.09 \pm 14.82$ a	$73.53 \pm 5.51 \ a$	$48.02 \pm 5.21 \text{ a}$	$7.6\pm3.4~\text{a}$
Grass buffer	$13.55\pm0.86~c$	$228.81 \pm 20.59 \; b$	$133.85\pm8.34\ b$	$79.15\pm7.52~b$	$7.2\pm3.4$ a
Agroforestry	$11.64\pm0.86~b$	$204.29 \pm 19.26 \ b$	$124.92\pm6.65~b$	$50.97 \pm 7.52 \ a$	$10.3\pm3.4~\text{a}$
Grass waterway	$10.53\pm0.86\ b$	$199.29\pm20.58\ b$	$135.05\pm7.79~b$	$65.49\pm7.23\ b$	$16.5\pm2.4\ b$

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

also affected by the type of management, as soil under GWW management with fescue had significantly higher WSA when compared with AG and GB treatments. In a previous study, on the same watersheds, Seobi et al. (2005) and Udawatta et al. (2006) observed increased soil porosity and reduced soil bulk density under the buffer areas compared with the crop areas. This may suggest that continuous organic matter additions of fibrous root systems, leaves, twigs, and branches associated with the perennial AG and GB buffer systems increased the WSA contents and improved other soil physical properties at much greater rates than can be achieved with no-till cropping practices.

Landscape position significantly affected WSA which increased from the summit to the lower landscape positions. This likely is attributed to the correlation (r = 0.68, p < 0.05) between organic matter buildup and increased WSA as organic matter is deposited on the upper slopes and transported to lower landscape positions due to water and other displacing forces. This result could also be due to higher water content at the lower landscape positions which promotes more organic matter buildup and thus more soil aggregate development. These factors may contribute to a greater percentage of WSA in the lower landscape position than the other two positions.

The proportion of water stable aggregates closely followed the distribution of soil C (r = 0.31, p < 0.05), soil N (r = 0.26, p < 0.05), bulk density (r = 0.61, p < 0.001) and glucosaminidase activity (r = 0.41, p < 0.001). A good correlation between organic matter and increased WSA was also reported by Kremer and Li (2003) who found that soils under grass vegetation held greater amounts of organic matter along with higher WSA levels when compared with traditionally cropped areas. A higher percentage of WSA improves soil air and water movement and increases surface area as well as smaller aggregates as a secondary soil structure for microbial populations (Carter, 2004).

Previous research indicates that different tillage systems, residue management practices, and cropping practices influence microbial populations and enzyme activity due to changes in substrate supply, soil moisture, and temperature (Doran et al., 1998; Mungai et al., 2005). Udawatta et al. (2005) evaluated soil water dynamics using reflectometer soil moisture sensors and observed lower volumetric soil water under AG and GB areas than RC areas from March to November. Inferring results from this study and the study of Udawatta et al. (2005), it appears that permanent vegetation leads to carbon accumulation but does not maintain high volumetric soil water content compared with crop areas.

Soil managed to enhance organic matter production provides a healthy environment for diverse and competitive microbial populations and greater enzyme activity (Doran, 1980). Other studies have shown that cover crops, crop rotation and organic matter addition increase microbial population and diversity (Kirchner et al., 1993). In this study, soil carbon originated from three different sources: crop, grass, and trees. Therefore, the quantity and biochemical characteristics of the organic material available for decomposition varied among the management practices, which may have contributed to differences in enzyme activities. Furthermore, diverse plant communities in AG and GB likely support functionally distinct microbial communities through production of root exudates that differ from row crops in monoculture or short rotation systems (Lupawayi et al., 1998).

The patterns of enzyme activities observed in this study support the hypothesis that permanent vegetative cover would provide favorable conditions for greater enzyme activity and associated microbial communities compared with soils under row crop management. Enzyme activity was consistently lower within the RC treatment under no-till management than within the other three treatments. The reduction in enzyme activity under frequently disturbed soils is similar to previous findings (Dick et al., 1996). Soil disturbance and conventional tillage consisting of separate treatments of chisel plow or moldboard plow significantly reduced enzyme activity, specifically  $\beta$ -glucosaminidase activity (Ekenler and Tabatabai, 2003).

Our results show a correlation between high enzyme activity and increased levels of organic matter provided by the continuous vegetative groundcover similar to previously published research (Myers et al., 2001; Kremer and Li, 2003; Mungai et al., 2005). The cessation of intensive tillage could have positively affected enzyme activities in the permanent vegetation areas. Unlike the RC treatment, the other three treatments had continuous vegetative cover, would have been cooler, and would have continuously supplied substrate for the microbial population. In a mature agroforestry site in northeast Missouri, Mungai et al. (2005) observed greater enzyme activities under the trees and less activity in the crop areas. They attributed these differences to organic matter quality and quantity. Similar findings reported by Kremer and Li (2003) in Missouri showed greater FDA and dehydrogenase activity under soils receiving organic matter compared with conventionally tilled areas. In forested ecosystems, Myers et al. (2001) found a direct correlation between microbial communities and litter quality and quantity. The results of the current study indicate that establishment of AG buffers, GB buffers, and GWW in row crop watersheds may help increase degradation of agrochemicals due to increased enzyme activities. Increases in enzyme activities will increase nutrient flow within the system and play a role in balancing C and N cycles and conversely increase nutrient availability, which favors root growth, promotes microbial activity, and eventually increases the total soil carbon pool. With a few exceptions, the largest differences were always found between the RC and GB or GWW treatments. Between the two grass treatments, the GWW treatments had significantly lower FDA activity. The mature fescue in the GWW appears to support a lower overall enzyme activity compared with the GB treatment.

Agroforestry management systems generally enhance organic matter accumulation in soils through the inclusion of cover crops and permanent vegetation, which would be expected to increase soil microbial populations and, consequently, increase the soil DNA. Only the GWW in our temperate agroforestry system provided significantly higher DNA compared with the other management components. It is not clear why soil DNA content in GB and AG were similar to that in RC, in contrast to the differences observed for other biological parameters. A more accurate method for using soil DNA to differentiate effects of management on the soil microbial community would be to quantify specific sequences via PCR or hybridization, as suggested by Taylor et al. (2002). This approach will be pursued in subsequent studies. Widmer et al. (2006) observed no relationship between farming systems and DNA content and suggested that the magnitude of management effects on microbial properties strongly depends on plant species, composition of the plant population, and the proportion of rhizosphere to bulk soil in the sample. Thus, GWW treatment may reflect well-established vegetation with dense root systems compared with more recently established GB and AG treatments and the RC treatment with annual plant components. Further, microbial community structure resolved through molecular analyses or "fingerprinting" will yield more information on specific consortia that may contribute to the various processes and properties in the soils under different vegetation.

The effect of landscape position on enzyme activities was not significant. This suggests that factors influencing enzyme activity, such as organic matter quality and litter quantity, were not significantly influenced at various landscape positions within a management system. Decker et al. (1999) observed similar findings in which no significant change was apparent across various landscape positions in a soil enzyme study conducted in Ohio. Soil samples for the current study were collected in 2006 during which the precipitation was 82% of the long-term (962 mm) average precipitation. The soil moisture content was low at the end of the growing season for the site. The lower soil water content conditions may have contributed to insignificant landscape effects on enzyme activities.

Soil organic matter accumulation occurs over several years; and measurement of soil organic matter for estimating soil quality reflects long-term impacts. Estimation of enzyme activity can be performed within a short period to understand the effects of management practices. Such information can be used to develop base line data for development of management strategies including modeling. Once a large data pool is available, indicator enzymes and microbial populations can be identified to forecast influences of various management practices. Until such data are available, information that is available can be used to compare different management options. Alternatively, 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.

# 5. Conclusions

The purpose of the study was to examine changes in water stable soil aggregates, soil carbon, soil nitrogen, functional diversity of microbial populations, and enzyme activity as influenced by permanent vegetative buffers compared with row crop agriculture. In this study, only the top 10 cm soil was sampled which contains the highest biological activity and most likely exhibits short-term changes in response to plant communities. Based on soil carbon content, nitrogen content, and enzyme activities, it is obvious that continuous disturbance has significantly reduced soil quality in the crop areas. The study showed that establishment of agroforestry buffers on previously cultivated agricultural areas has a significant effect on the measured soil quality indicators. The buffers were established in 1997 and therefore, the changes reported here occurred in less than 10 years. Soil disturbance from tillage practices as well as organic matter input from vegetation clearly holds a strong influence over the various activities taking place within our soils and therefore, may aid in the future evaluation of soil enzyme activity and soil quality in various settings. The extent of the improvement is partially determined by the vegetation type. These improved properties and other associated changes due to establishment of buffers may help to reduce NPSP from row crop agricultural lands.

Further studies are needed to understand the temporal variations and to quantify the influence of buffer age on these parameters. This will help to determine whether microbial populations take a shorter time period to reach a steady state under these management systems as compared to soil organic matter. Additional research is also needed to understand substrate composition and its chemical quality on soil enzyme activity and microbial diversity in relation to agroforestry and grass buffer conservation management practices. Future research goals should include identifying the most suitable indicator/s as influenced by buffers that can be used to estimate soil quality in the shortest possible time.

### Acknowledgements

This work was funded through the University of Missouri Center for Agroforestry under cooperative agreements 58-6227-1-004 with the USDA-ARS. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture. We thank Ashley Schlichenmayer and Jim Ortbals for excellent technical assistance.

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