

SOIL MICROBIOLOGICAL ACTIVITIES IN VEGETATIVE BUFFER STRIPS AND THEIR ASSOCIATION WITH HERBICIDE DEGRADATION

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ABSTRACT

The efficacy of vegetative buffer strips in intercepting herbicides from surface runoff is well established. A sound buffer design should also facilitate rapid degradation of deposited herbicides before they have a chance to be released to surface and subsurface flow. Experimental plots were arranged in a split-plot design with three replications and a factorial combination of three land-use treatments with five landscape positions (summit, shoulder slope, back slope, foot slope, and toe slope). Land-use treatments included a contour grass buffer watershed, a contour tree-grass agroforestry buffer watershed, and a control watershed. Watershed areas ranged from 1.65 to 4.44 ha. All land-use treatments were in a corn-soybean minimum-till production system, and the vegetative buffers were contoured strips from the summit to toe slope positions. To evaluate microbial activities and herbicide degradation potential, β -glucosidase, dehydrogenase and fluorescein diacetate hydrolytic activities were measured. The results suggested that the microbial enzymatic activities were significantly higher in grass and tree-grass buffers than in the control treatment. Among the treatments, the soils collected from the grass buffers showed the highest microbial enzymatic activities and herbicide degradation potential. Topographic position did not significantly affect soil microbial activities. A growth chamber study was also conducted to investigate the microbial mineralization rates of ^{14}C ring labeled atrazine and the activities of FDA, dehydrogenase, and β -glucosidase in the rhizosphere of seven selected forages treatments. Preliminary results suggested that the mineralization rates of atrazine, after 100 days of incubation, were more closely correlated with activities of β -glucosidase ($r = 0.857$) and dehydrogenase ($r = 0.763$) than FDA hydrolysis rates ($r = 0.494$). Efforts are currently under way to correlate the microbial activity in different land use treatments with herbicide mineralization and degradation rates using ^{14}C -labeled herbicides, such as atrazine, bentazon, pendimethalin, s-metolachlor, and glyphosate.

Keywords: atrazine, β -glucosidase, dehydrogenase, fluorescein diacetate hydrolysis

INTRODUCTION

Multi-species vegetative riparian buffers have been shown to be a cost-effective approach to intercept both dissolved and sediment-bound herbicides transported in surface runoff (Arora et al. 1996; Cole et al. 1997; Hoffman et al. 1995; Lowrance et al. 1997; Schultz et al. 1995; Schultz et al. 1991). In a watershed study conducted in central Texas, Hoffman et al. (1995) observed a 44-50% reduction in atrazine levels when a 9 m filter strip was used. The enhanced infiltration rates within vegetative buffers have been identified as the major physical mitigation process by which herbicides transported in surface runoff are intercepted (Misra et al. 1996; Zins et al. 1991).

In addition to high infiltration rates, a buffer should be designed to rapidly degrade the deposited herbicides before they have a chance to be released to surface and subsurface flow. The potential for enhanced herbicides degradation presumably results from increased soil enzyme activities and microbial biomass associated with incorporation of grass and woody species. In soils planted with Pennisetum (*Pennisetum clandestinum*), microbial biomass and soil dehydrogenase activity were stimulated by sevenfold over that in unplanted soils (Singh et al. 2004). About 45% and 52% of atrazine and simazine, respectively, were degraded in soil planted with *P. clandestinum* while only 22% and 20% of the respective herbicides were degraded in the unplanted soil within 80 days. Results of a field lysimeter study also suggested that microbial dealkylation and hydroxylation rates of atrazine in switchgrass (*Panicum virgatum*) treatments were significantly enhanced compared to a bare ground treatment (Lin et al. 2005). In the same study, the increased degradation rates of atrazine in grass treatments strongly correlated with the increased microbial biomass carbon. Rapid degradation of isoproturon was observed in soil from grass buffer strips, whereas most of the herbicide remained undegraded in cultivated soils (Benoit et al. 1999). Half-lives of isoproturon were significantly reduced from 72 days in the cultivated field to 8 days in the grass buffers (Benoit et al. 1999).

The microbial degradation of many modern herbicides requires a group or “consortium” of microorganisms (Mandelbaum et al. 1993; Sadowsky et al. 1997). The associated biochemical processes involve a wide spectrum of enzymatic reactions, e.g., dealkylation, de-esterification, hydroxylation, dehalogenation, and oxidization (Ambus 1993; Bollag and Liu 1990; Mandelbaum et al. 1993). Some soil enzymatic parameters utilized to assess soil quality in the past might also be useful as indicators to evaluate the overall potential for herbicide biodegradation in vegetative buffers (Bergstrom et al. 1998; Zablutowicz et al. 2000). For instance, fluorescein diacetate (FDA) hydrolysis rates have been used to estimate total microbial activity (Bandick and Dick 1999; Schnurer and Rosswall 1982). FDA is a substrate for a wide variety of enzymes, including proteases, lipases, and esterases. Recent findings suggested that FDA hydrolytic activity was highly correlated with the de-esterification of fenoxaprop-ethyl (Zablutowicz et al. 2000). Triphenyl-tetrazolium chloride (TTC) reduction rates have been used to estimate the overall metabolic activity of dehydrogenases in soil. In this assay procedure, dehydrogenases utilize TTC as an electron acceptor. Dehydrogenases are required to catalyze the biological oxidation and dehalogenation of a number of herbicides and other organic compounds (Beller et al. 1996; Waarrde et al. 1993).

Various contour buffer strips designs have been prescribed in the United States to remove sediment, chemicals, and organic material transported in surface runoff (Tim and Jolly 1994; Udawatta et al. 2004). However, there is relatively little information about the microbial characteristics of these buffers and their association with the fate of the herbicides deposited within the buffers. The objectives of this study were to investigate the effect of two contour buffer designs (grass buffer and tree-grass agroforestry buffer) at five topographic positions (summit, shoulder slope, back slope, foot slope, and toe slope) on soil microbial enzyme activities and their association with the fate of herbicides in contour vegetative buffers.

MATERIALS AND METHOD

Experimental Design

The study was conducted on three watersheds ranging in size from 1.65 to 4.44 ha established during 1991-1997 at the University of Missouri-Greenley Memorial Research Center in Knox County, Missouri (40° 01'N, 92° 11'W) (Figure 1). Plots within each watershed were replicated three times in a split-plot design to accommodate the factorial combination of three land-use treatments and five landscape positions (summit, shoulder slope, back slope, foot slope, and toe slope). Land-use treatments included a contour grass strip watershed, a tree-grass agroforestry buffer watershed, and a control watershed treatment with a minimum-till corn-soybean crop production system in all three watersheds. The grass buffer strips are 4.5 m wide and consist of the following forages: redbtop (*Agrostis gigante* Roth), brome grass (*Bromus* spp.), and birdfoot trefoil (*Lotus corniculatus* L.). This pattern is repeated six times over the landscape. Agroforestry buffer treatments include pin oak (*Quercus palustris* Muenchh.), swamp white oak (*Q. bicolor* Wild), and bur oak (*Q. macrocarpa* Michx.) mixed with the same grass species. Tree buffer width is 4.5 m. The cropped width between buffers is 36.5 m in the grass and tree-grass watersheds. To evaluate the microbial degradation of the herbicide, three 25-cm deep soil cores were collected from all treatments at each of the five landscape positions in May 2003, placed in polyethylene bags, and stored in a cold room at 4°C.

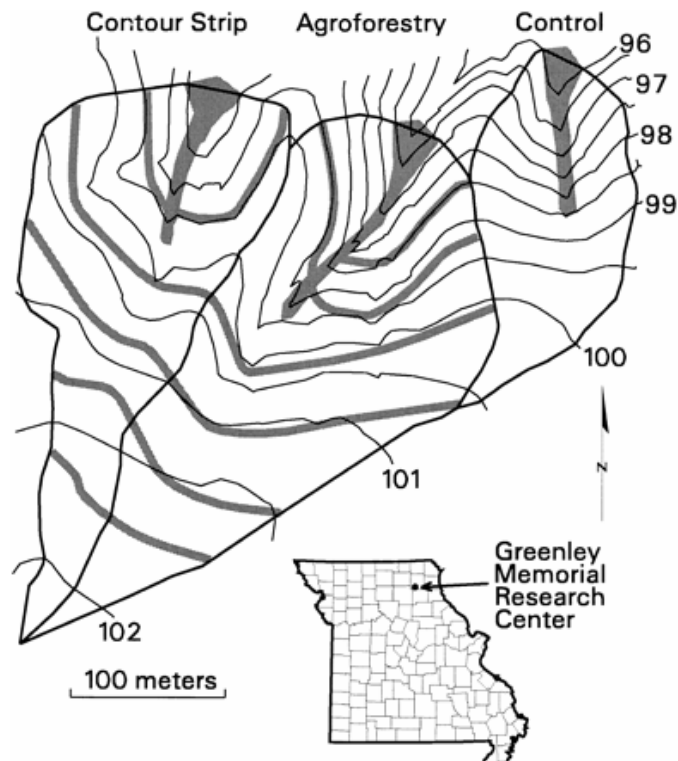


Figure 1. Study site location and 0.5-m-interval topographic maps of contour strip, agroforestry, and control watersheds. Broad gray areas represent grass strips (contour strip), trees and grass strips (agroforestry), and grass waterways (contour strip, agroforestry, and control).

Microbial Enzyme Activity

Dehydrogenase activity was measured following the procedure described by Gerba and Brendecke (1995). Briefly, this procedure utilizes 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-triphenyltetrazolium chloride (TTC) as the electron acceptor, which is reduced to the red-colored, methanol-soluble, triphenyl formazan (TPF). Six grams of moist soil from each sample was placed in a test tube. Samples were incubated statically with 1 mL of 3% TTC (3 g /100 mL DI water) and 3 mL of 0.2 M CaCO₃ buffer solution for 24 hrs at 37°C. This reaction was terminated with 10 ml of methanol, and TPF was extracted with 30 mL of additional methanol. The final extract was filtered with Whatman #42 filter paper and TPF concentration was determined spectrophotometrically at 485 nm.

To evaluate microbial carbon utilization efficiency, β -glucosidase activity was quantified according to procedures described by Tabatabai (1994). The method is based on colorimetric measurement of *p*-nitrophenol released by β -glucosidase when soil is incubated with buffered (pH 6.0) *p*-nitrophenyl- β -D-glucoside (PNG) solution. One gram of moist soil was sampled and incubated with solution containing 0.25 ml of toluene, 4 mL of 8 mM tris-hydroxymethyl-amino-methane buffer (THAM; pH 6) for 1 hour at 37°C. Then, 1 mL of 0.5 M CaCl₂ and 4 mL of 0.1 M THAM (pH 12) solution was added. This solution was vortexed and filtered through Whatman #2 filter paper. The absorbance was measured at 410 nm.

Fluorescein diacetate (FDA) hydrolytic activity was determined by the enzymatic assay procedures described by Bandick and Dick (1999). One gram of moist soil from each sample was placed into a 50 mL Erlenmeyer flask. Twenty mL of 60 mM sodium phosphate buffer (pH 7.6) and 100 μ L of 4.8 mM FDA were added into the flask. The flask was then capped with parafilm. Samples were incubated for 105 min on a reciprocal shaker at room temperature. After 105 min, 10 mL of acetone were added to terminate the hydrolysis reaction. Samples then were filtered through Whatman #4 filter paper and centrifuged for 5 min. Fluorescein (the product of FDA hydrolysis) concentration was determined spectrophotometrically at 490 nm.

RESULTS AND DISCUSSION

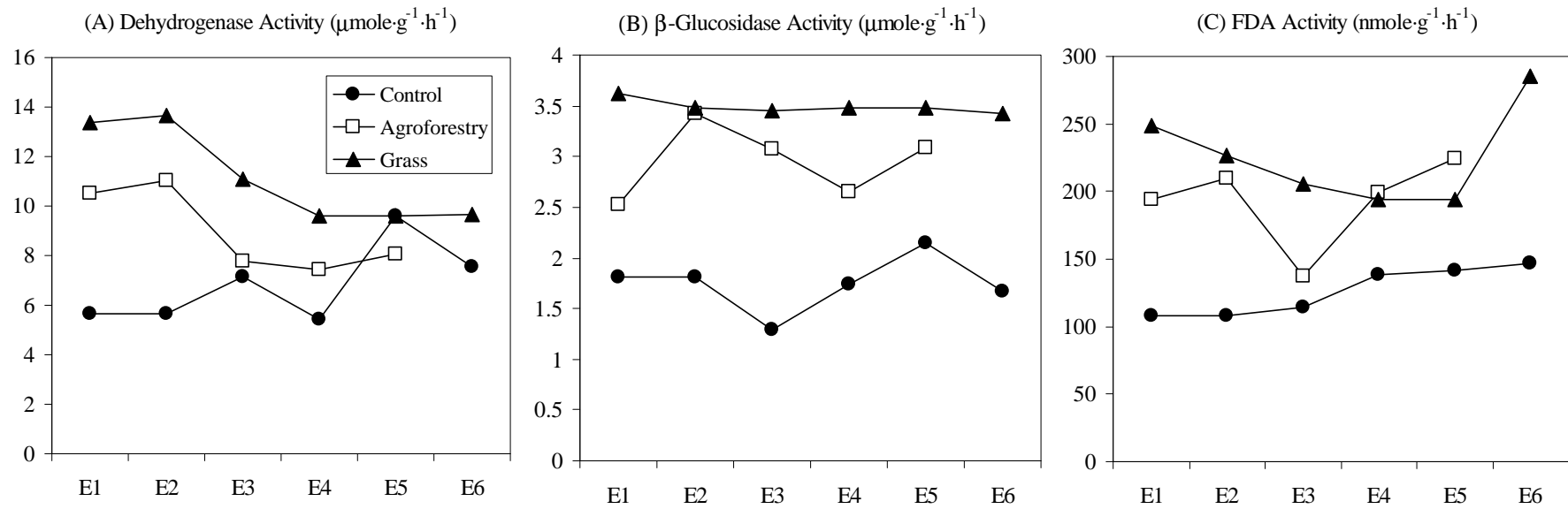
Microbial enzymatic activities within top 25 cm soils were significantly higher in both grass strip and agroforestry buffers than in the control conventional cropping treatment (Figure 2). Among the treatments, the soils collected from the grass strip treatment displayed the highest microbial enzymatic activities and herbicide degradation potential. When data from all the topographic positions was pooled, the average dehydrogenase activity was increased by 73% and 42% in grass contour and agroforestry buffers, respectively, over the control (Figure 2A). Average β -glucosidase activity was increased by 104% in grass buffers and 73% in agroforestry buffers (Figure 2B). Similarly, the average FDA activity was increased by 82% in grass buffers and 59% in agroforestry buffers (Figure 2C). The stimulated enzyme activities associated with the presence of grass and woody species is consistent with previous observations. Dick and Bandick (1999) reported significantly higher enzyme activities in a grass field than in a traditional cultivated crop field. Higher organic C and total N, microbial biomass C, basal soil respiration and activities of dehydrogenase and alkaline phosphatase have been reported in tree-crop

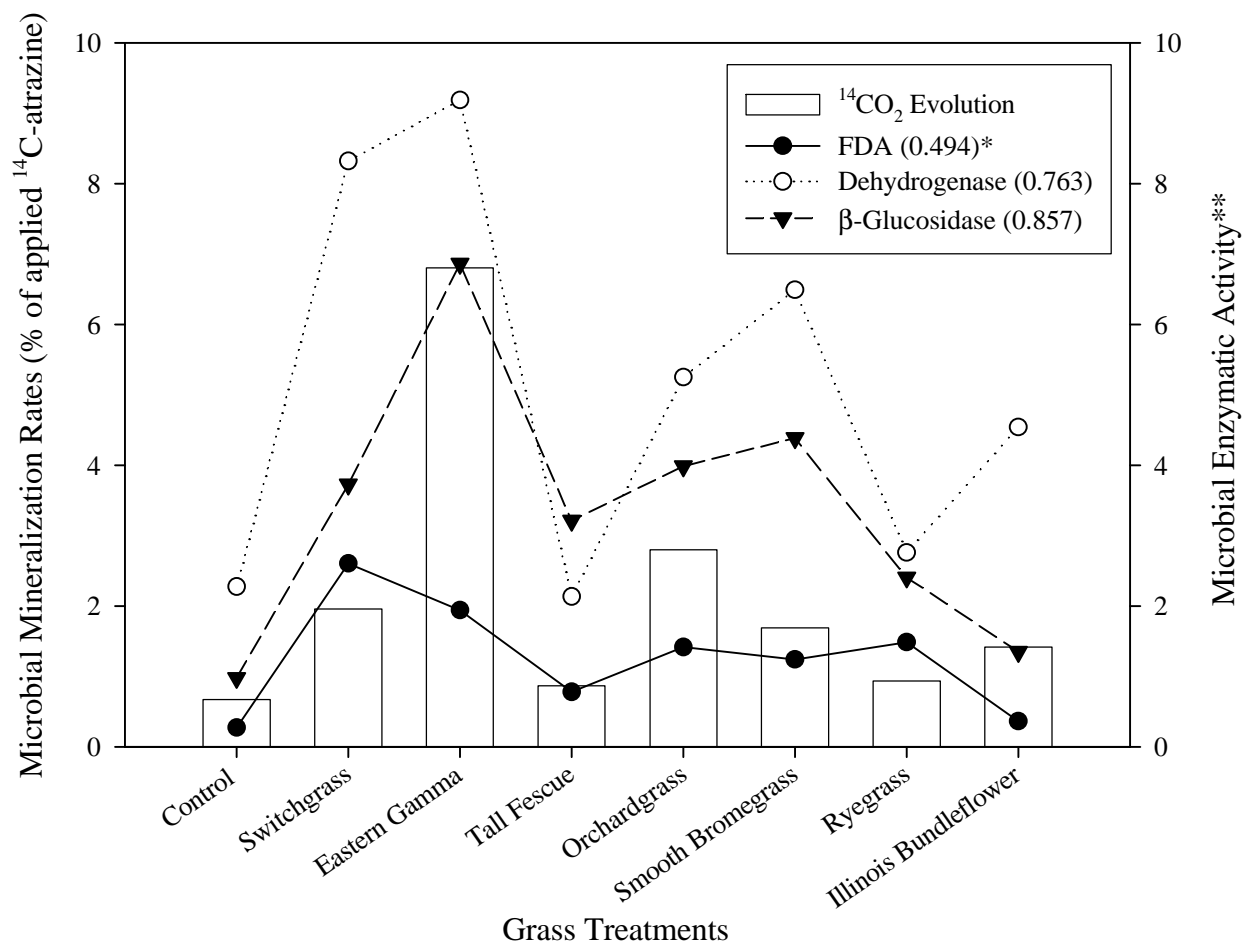
agroforestry practices when compared to crop cultivation without trees (Chander et al. 1998). Enhanced microbial enzyme activities measured in the vegetative contour buffer can be mainly attributed to the increased nutrient pool (Deng and Tabatabai 1996; Staddon et al. 2001). Lower soil enzymatic activities in the agroforestry contour buffers may result from the lower understory vegetation coverage compared to the grass contour buffer (Lin—visual field observation). Weed control management practices at the Greenley Research Center, such as implementation of weed mats and herbicide application around trees and microclimatic stress due to shading and moisture competition from trees, appeared to be the major factors reducing the understory coverage area.

Although significant differences in microbial enzyme activities were observed between land-use treatments, the effect of topographic position on the soil microbial activities was not significant ($p > 0.05$) (Figures 2A-C). Soils from toe-slopes tend to have greater total N concentrations, lower carbon-to-nitrogen (C:N) ratios, greater potential net nitrification, and greater microbial activity than soils from the summit position (Garten et al. 1994). Greater activities of β -glucosidase, glutaminase, phosphatase and arylsulfatase, and higher organic content in coarse-textured soils at a lower slope-position compared to fine-textured soil at an upper slope-position have also been reported (Bergstrom et al. 2000). The less pronounced trends in microbial activities at the landscape scale suggests that heterogeneity of the topographical features in the research site are not sufficient to contribute to variability of nutrients pool distribution across the landscape (McCulley and Burke 2004). Land management effects contributed more than spatial effects to the overall variation in microbial enzyme activity.

The significantly higher microbial enzymatic activities in the contour vegetation buffers than in the cultivated field may imply higher bioremediation potential for herbicide degradation. A higher degradation rate of metolachlor reported in a vegetative buffer strip soil coincided with 1.9 to 3.8 times higher dehydrogenase and FDA activities (Staddon et al. 2001). FDA hydrolytic activity has also been observed to be highly correlated with the de-esterification of fenoxaprop-ethyl (Zablotowicz et al., 2000). A growth chamber study was conducted in 2004 to investigate the microbial mineralization rates of ^{14}C ring labelled atrazine and the activities of FDA, dehydrogenase and β -glucosidase in the rhizosphere of seven selected forages treatments. The preliminary results suggested that the mineralization rates of atrazine, after 100 days of incubation, are more closely correlated with activities of β -glucosidase ($r = 0.857$) and dehydrogenase ($r = 0.763$) than the activity of FDA hydrolysis ($r = 0.494$) (Figure 3). However, before any of these microbial parameters can be recommended as an indicator to assess the overall bioremediation potential, more information regarding the relationship between enzyme substrate specificity and herbicide chemical structure as well as the composition of degradation products in the soils is required. Current efforts are focused on correlating the microbial parameters obtained from this paired watershed study with herbicide degradation rates and metabolite composition ratios in the different land-use treatments using ^{14}C -labeled herbicides, such as atrazine, bentazon, pendimethalin, s-metolachlor, and glyphosate.

Figure 2. Microbial enzyme activities of dehydrogenase (A), β -glucosidase (B), and fluorescein diacetate hydrolysis (C) in control, grass and tree-grass buffers on six topographic positions (E1 and E2 - summit; E3 - shoulder slope; E4 - back slope; E5 - foot slope, E6 - toe slope) at the University of Missouri-Greenley Memorial Research Center (n=3).





*Values in parentheses represent the Pearson coefficient of correlation between mineralization rates of ^{14}C -atrazine and enzymatic activity.

**Dehydrogenase and β -glucosidase activities are expressed in $\mu\text{mole g}^{-1} \text{h}^{-1}$. FDA hydrolytic activity is expressed in $(\text{nmole g}^{-1} \text{h}^{-1}) * 0.01$.

Figure 3. The total mineralization rates of atrazine and microbial enzyme activities in soils with forage treatments (n=3). Control treatment is bare ground.

CONCLUSION

Contour vegetative buffers implemented across the landscape showed significantly increased enzyme activities compared to the cropped control treatment. Land use treatment was more important to enhanced enzyme activities than landscape position for these small watersheds. The incorporation of woody components in the contour buffer system may reduce surface runoff volume, erosion, and sediment-bound herbicide transport; however, the higher microbial enzymatic activity in the grass counter buffer system suggests a higher potential for degrading herbicides deposited in the rhizosphere. Further information is required to optimize the contour

buffer design and management regimes so the desired mitigation performance can be achieved. Microbial parameters widely used for assessment of soil quality, e.g., dehydrogenase and β -glucosidase activities, showed promise as useful tools for evaluating the overall bioremediation potential of various vegetative buffer designs.

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