Microbial resilience as influenced by an herbicide in soils from native prairie, CRP, and row crop management

Ranjith P. Udawatta[A], Robert J. Kremer[B], Stephen H. Anderson[C], and Harold E. Garrett[D]

[A]Center for Agroforestry and Department of Soil, Environmental and Atmospheric Sciences, School of Natural Resources, University of Missouri, MO 65211, USA. UdawattaR@missouri.edu
[B]USDA-ARS Cropping Systems and Water Quality Unit, Columbia, MO 65211. USA.
[C]Department of Soil, Environmental and Atmospheric Sciences, University of Missouri, MO 65211, USA.
[D]Center for Agroforestry and Department of Forestry, School of Natural Resources, University of Missouri, MO 65211, USA.

Abstract

The objective of this study was to examine differences in soil microbial resilience as impacted by herbicide application on soils from prairie and row crop management. The treatments were: native prairie (TP), restored prairie (PF), CRP*, and corn-soybean rotation (CS) management. Surface soil (10 cm) was collected from two locations within each treatment with four subsamples per location in June 2008. Soil enzymes studied include: fluorescein diacetate (FDA) and dehydrogenase. Eleven glyphosate concentrations (0 to 2000 ppm) were applied (Roundup Original Max) and soil dehydrogenase and FDA hydrolase activities were assayed periodically over 50 days. Soil enzyme activities were lower on day one and ten irrespective of enzyme type or management. The highest enzyme activities were observed on day 5 and the activity declined thereafter. However, some soil treatments exhibited increased activity on days 30 and 50. Herbicide concentration had a smaller effect on FDA hydrolase activity while the effect of concentration was highly variable for dehydrogenase activity. Results of the study show that soils from native prairie have greater enzyme resilience while cropped soils have the lowest. Establishment of prairies may help maintain active soil processes and could help maintain or improve soil quality.

Key Words

corn-soybean, dehydrogenase, FDA, grassland ecosystems

Introduction

Herbicides are used in large quantities in modern agriculture to control undesirable plant species within a field and also to increase watershed or site productivity. The increased application of herbicides leads to increased chemical concentrations in soil, altered soil reactions, and potential adverse effects on non-target organisms (Perucci and Scarponi, 1994). For example, 10- and 100-fold higher herbicide concentrations of imazethapyr reduced microbial biomass and activity of dehydrogenase enzyme in soils (Perucci and Scarponi, 1994). In another study, respiration rates increased with higher concentrations of 2,4-D, picloram, and glyphosate (Wardle and Parkinson, 1990). Ratcliff *et al*. (2006) observed 25% increase in bacterial counts and fungal hyphal length on glyphosate treated soils.

Modes of action for most herbicides are well defined. Glyphosate is a commonly used herbicide in many agricultural row crop management practices worldwide. Glyphosate inhibits protein synthesis by blocking the shikimic acid pathway (Franz *et al*., 1997). A surfactant, polyoxyethylene tallow amine, is toxic to bacteria and protozoa (Tsui and Chu, 2003).

Biological and biochemically mediated processes in soils are significant for ecosystem functions (Zabaloy *et al*., 2008). Since microbes play a critical role in carbon (C) and nutrient transformations, any change in their population and activity may affect cycling of nutrients as well as availability of nutrients, thus indirectly affecting plant growth and other soil functions (Wang *et al*., 2008). Research suggests that repeated application of herbicides may involve a risk of reduced or altered soil microbial activities. Management practices may also indicate short-term differences in soil quality improvement and microbial diversity, soil chemical processes, mineralization rates, and organic matter accumulation. Other advantages of Research shows that fluorescein diacetate (FDA) hydrolysis and dehydrogenase activity can be used as good indicators of soil biogeochemical processes (Kremer and Li, 2003). Enzyme assays among different enzyme assays include evaluation of rapid responses to changes in management and understanding sensitivity to environmental stresses (Dick, 1997).

---

* CRP is a government incentive program in which previously cultivated land is taken out of crop production and maintained under a 15-yr continuous cool-season grass and legume pasture system with no agrichemical inputs.

Although several environmental benefits of restoration of prairies are reported in the literature, immediate quantifiable information on herbicide effects on these soils as compared to other soils is unavailable. This information should help explain changes in water and soil quality due to management practices, develop soil cleaning procedures, and assist in establishment of prairie vegetation establishment guidelines. The present study was designed to elucidate the effects of glyphosate (Roundup) on soil microbial resilience and potential soil enzyme activity in soils from native prairie, restored prairie, conservation reserve program (CRP), and crop management.

Materials and Methods

Soil dehydrogenase and FDA enzyme activities in response to varying concentrations of glyphosate and duration on soils from row crop and conservation reserve program (CRP[*]) treatments were compared to native prairie and restored prairie ecosystems in central Missouri, USA. The treatments for the study include the following: Tucker Prairie (TP; native prairie), Prairie Fork (PF; restored prairie), Conservation Reserve Program (CRP), and corn-soybean rotation (CS). The undisturbed Tucker Prairie site has been under native prairie vegetation and consists of big blue stem (*Andropogon gerardi* Vitman.), little blue stem (*Schizachyrium scoparium* Nash.), prairie dropseed (S*porobolus heterolepis* [A. Gray] A.Gray), and Indian grass (*Sorghastrum nutans* [L.J. Nash]) (Buyanovsky *et al*., 1987). The Prairie Fork Conservation Area was restored in 1993 with native grasses and legumes. Prior to restoration, the site was under row crop management for approximately 100 years. The study area vegetation consisted of little blue stem, side-oats gamma (*Bouteloua curtipendula var. curtipendula*), and Indian grass. Soils for the CRP and CS treatments were sampled from long-term study plots located within the USDA-ARS Agricultural Systems for Environmental Quality site near Centralia, Missouri, USA.

Five grams of soil was placed in a petri dish; moisture was maintained by adding water and keeping the lid in place in a control room at ambient temperature. Eleven glyphosate solutions (0 to 2000 ppm) were prepared by mixing the concentrated herbicide with DI water and approximately 1.5 mL of the solution was applied to the soils in the petri dish. The hydrolysis of fluorescein diacetate was colorimetrically quantified at 490 nm and indicates broad-spectrum soil enzyme activities, especially on substrates including carbohydrates, lipids, and proteins (Dick *et al*., 1996). A standard calibration curve was used to measure the concentration, expressed as μg fluorescein released g^{-1} dry soil h^{-1}. Dehydrogenase enzyme activity was determined in moist soil incubated with 2,3,5-triphenyltetrazolium chloride substrate at 37°C for 24 h (Pepper *et al*., 1995). Following incubation, a regression equation was used to determine the concentration of the triphenyl formazan (TPF) product colorimetrically (485 nm) and the enzymatic activity was expressed in μg TPF released g^{-1} dry soil h^{-1}.

Results

The dehydrogenase activity was significantly lower on day one for native prairie as compared to other three soils (Figure 1). The highest mean activity was observed in the CRP soil, which may have contained more soil carbon and may have received herbicides under previous cropping and from adjoining crop areas prior to this application; thus microbes have been less sensitive for additional herbicide application. The activity increased by almost three fold for restored prairie (PF) and CRP soils on day five. The native prairie site showed a fivefold increase from day one to day five. However, the crop soil had the lowest increment in change. The activity was the lowest on all four soils on day 10 and increased for days 30 and 50. This could possibly be due to the non-availability of organic carbon by day 10 and subsequent availability during days 30 and 50 due to increased carbon released from dying microbial biomass. A spike in microbes able to use less complex compounds derived from glyphosate after primary degradation by a more specialized, limited microbial community segment may also have contributed to the increase.

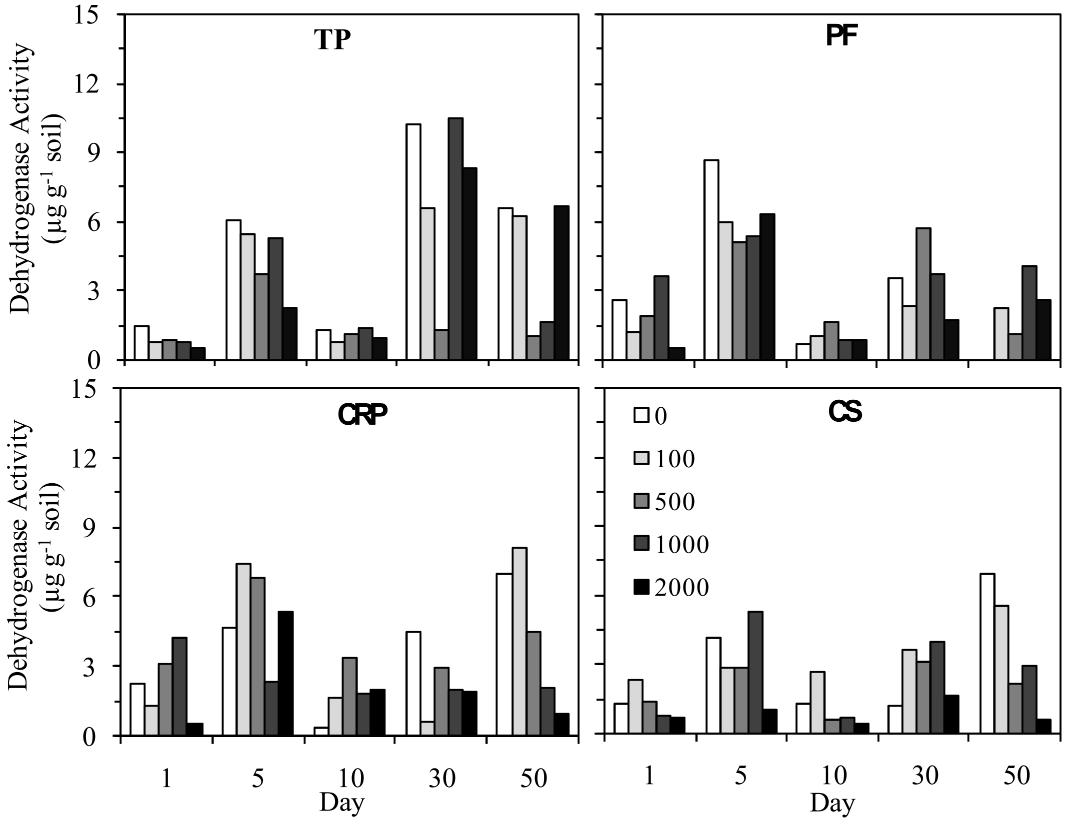

Figure 1. Dehydrogenase activity of native prairie (TP), restored prairie (PF), CRP, and corn-soybean rotation (CS) soils at 1, 5, 10, 30 and 50 days after application of 0 to 2000 PPM (selected concentrations are shown) concentrations of glyphosate.

Although the FDA enzyme activity was measured on all four soils and for several concentrations, results were presented for two selected soils (TP and CS) and five concentrations. Soils from the native prairie site exhibited the highest activity on days 1, 5, 10, 30, and 50 than the other three soils. This likely reflects the continuous presence of abundant and complex organic substances in the prairie ecosystem, which contributes to development of high diversity of enzymes required to degrade the complex organics. FDA activity of restored prairie and CRP were almost the same on day one. However, CRP soil had significantly greater activity on day five as compared to restored prairie soils. The differences were not significant between restored prairie and CRP soils for the rest of the study period. Soil from the corn-soybean rotation had the lowest activity among the four soils on all five measurement dates. The two prairie soils exhibited a 1.5 fold increase of enzyme activity from day one to day five while the CRP and CS soils exhibited two and five fold increases, respectively.

The effect of herbicide concentration on either enzyme activity within a measured date was insignificant by soil treatment. Although the highest concentration used in this study may represent an herbicide spill, results show that such high concentration did not affect enzyme activity. However, soil from the corn-soybean site had lower enzyme activity for higher herbicide concentrations. This might suggest that continuous soil disturbance and application of other agri-chemicals may have negatively affected microbial resilience for soils under crop management. Observed enzyme activities could be due to direct and/or indirect effects of pesticides which were not evaluated in this current study.

Conclusions

Results show that native prairie soils or soils with less disturbance had better enzyme resilience. This may indicate that establishment of permanent vegetation along the field crop borders may enhance microbial processes including degradation of harmful agri-chemicals, nutrient cycling, and carbon sequestration, and thus help protect soil and water quality.

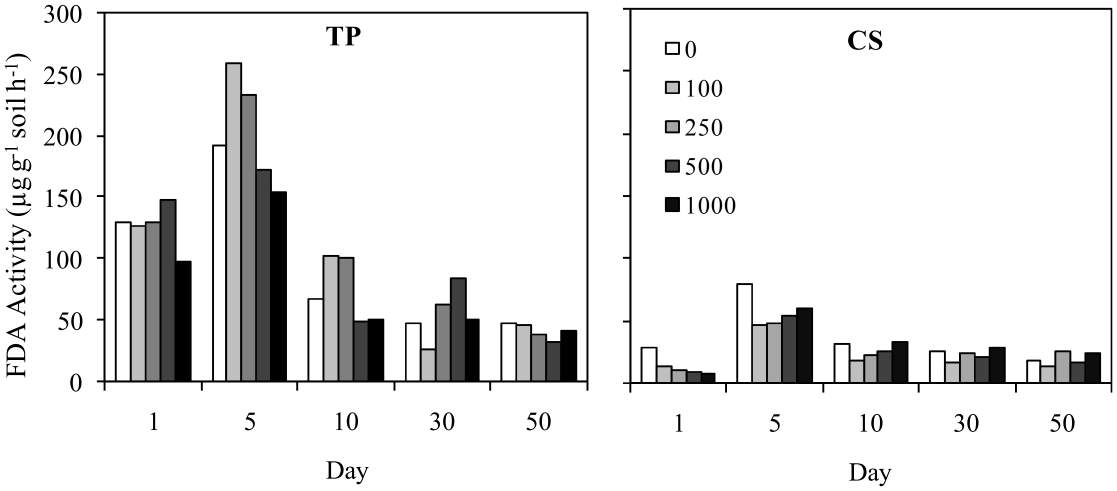

Figure 2. Fluorescein diacetate (FDA) activity of native prairie (TP) and corn-soybean rotation (CS) soils at 1, 5, 10, 30 and 50 days after application of 0 to 1000 PPM concentrations of glyphosate. Only two sites and five selected concentrations are shown.

References

Buyanovsky GA, Kucera CL, Wagner GH (1987) Comparative analyses of carbon dynamics in native and cultivated ecosystems. *Ecology* **68**, 2023-2031.

Dick RP, Breakwell DP, Turco RF (1996) Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In Methods of assessing soil quality. SSSA special publication 49 (Eds. Doran JW, Jones AJ), pp. 247-271. (Soil Science Society of America, Madison, WI, USA).

Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In. Biological indicators of soil health. (Eds. Pankhurst CE, Doube BM, Gupta VVSR), pp. 121-156. (CAB International, Wallingford, UK).

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide: In American Chemical society monograph 189, American Chemical Society, Washington, DC, USA.

Kremer RJ, Li J (2003) Developing weed-suppressive soils through improved soil quality management. *Soil & Tillage Research* **72**, 193-202.

Perucci P, Scarponi L (1994) Effects of the herbicide imazethapyr on soil microbial biomass and various enzyme activities. *Boil. Fertil. Soils* **17**, 237-240.

Pepper IL, Gerba CP, Brendecke JW (1995) Dehydrogenase Activity of Soils. Environmental Microbiology: A Lab Manual, pp 51-56. (Academic Press, San Diego, CA, USA).

Ratcliff AW, Busse MD, Shestak CJ (2006) Changes in microbial community structure following herbicide (glyphosate) addition to forest soils. *Applied Soil Ecology* **34**, 114-24.

Tsui MTK, Chu LM (2003) Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* **52**, 11891197.

Wang QK, Wang SL, Liu YX (2008) Responses to N and P fertilization in a young Eucalyptus dunnii plantation: microbial properties, enzyme activities, and dissolved organic carbon. *Applied Soil Ecology* **40**, 484-490.

Wardle DA, Parkinson D (1990) Effects of three herbicides on soil microbial biomass and activity. *Plant and Soil* **122**, 21-28.

Zabaloy MC, Garland JL, Gomez MA (2008) An integrated approach to evaluate the impacts of the herbicides glyphosate, 2, 4-D, and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina, *Applied Soil Ecology* **40**, 1-12.