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Activities of five enzymes following soil disturbance and weed control in a Missouri forest

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ABSTRACT

Forest disturbances associated with harvesting activities can affect soil properties including enzyme activity and overall soil quality. The activities of five enzymes (acid and alkaline phosphatases, beta-glucosidase, aryl-sulfatase, and beta-glucosominidase) were measured after 8 years in soil from clearcut and uncut control plots of a Missouri oak-hickory (*Quercus* L. – *Carya* Nutt.) forest. Understory treatments included subplots with and without weeds and uncut control plots. Enzyme activity was significantly ($p < 0.05$) affected by the presence or absence of weeds. Among the five enzymes measured, the activity for acid phosphatase, beta-glucosidase, and aryl-sulfatase were significantly ($p < 0.05$) lower in soil from subplots without weeds than in subplots with weeds. Activities for alkaline phosphatase and beta-glucosaminidase were higher for subplots with weeds than without weeds, but differences were not significant. Except for acid phosphatase, enzyme activity did not differ between subplots without weeds and uncut control plots. Soil phosphorus was higher in subplots with weeds than in subplots without weeds. Neither soil pH or soil C differed among understory treatments, but there were significant correlations between them and enzyme activity. Also, there were correlations among enzymes. Reduced enzyme activity conserves organically bound nutrients such as N, P, and S in soil due to the lack of mineralization processes which could lead to critical nutrient losses in forest ecosystems.

Keywords: Microbial activity, organic matter, weeds, weed control, soil carbon, soil enzymes.

INTRODUCTION

Soil carries particular importance through its storage and supply of water, nutrients, and air in ecosystem productivity. Most of the nutrients taken up for plant growth and metabolism are derived from the decomposition of vegetation produced on the site. Considerable amounts of nutrients are believed to be removed when trees are harvested. However, because soil organic matter decomposes very slowly, periodic losses of plant litter from the ecosystem appear negligible over the short term. During this time, soil organic matter is acted upon by the collective activity of saprotrophic microbial communities whose production is driven by the acquisition of nutrients released by extracellular degradation of detritus. Sinsabaugh and Moorhead (1994) concluded that the structural complexity of plant litter requires the concerted activities of many classes of enzymes for degradation and nutrient cycling. The enzymes are manufactured by a wide range of soil microbes, most often in response to both the quantity and biochemical characteristics of the organic material available for metabolism (Decker et al., 1999).

In unmanaged ecosystems, there is a strong correlation between soil enzyme activity and plant biomass production (Skujins, 1976) and an equally strong correlation between enzyme activity and microbial biomass (Eivazi and Bayan, 1996). However, in intensively managed or disturbed ecosystems, the relationship can be altered (Dick, 1994), or in well-buffered conditions, it can remain

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unchanged in the short term (Boerner et al., 2000). In intensively managed forest where weeds are controlled to maximize wood production, microbial activity in the early years could be reduced due to the lack of desirable substrate usually provided by herbaceous understory (Gallardo and Schlesinger, 1994). However in another study, long-term vegetation control in a ponderosa pine forest revealed no significant influences of vegetation control on microbial communities (Busse et al., 2001). Enzymes, once quantified, provide an integrative measure of the biological status of the soil, being a summation of enzyme stabilized by sorption and/or entrapment within the soil organo-mineral matrix. This last component confers a degree of buffering from such factors as short-term climatic events, which can cause large fluctuations in other soil biological properties, e.g., respiratory activity and microbial biomass. Consequently, soil enzyme activity is responsive to changes in land management that affect the general chemical and physical condition of the soil or the status of the organisms it supports.

In this study, we investigated the activities of five enzymes 8 years after timber harvesting and tree planting with and without weed control and an uncut control. Acid and alkaline phosphatases and aryl-sulfatase are important in cycling of some key nutrients, especially phosphorus (P) and sulfur (S). Phosphatases mediate the release of inorganic P into the soil solution. Aryl-sulfatase is believed to play an important role in the processes whereby organic soil sulfhur is mineralized and made available for plant uptake. Beta-glucosaminidase may play an important role in both carbon (C) and (N) cycling in soil (Parham and Deng, 2000). This enzyme degrades chitin (Tronsmo and Harman, 1993), one of the most abundant biopolymers on earth serving as an important transient pool of organic C and N in soils (Wood et al., 1994). Glucosidases are widely distributed in nature and their hydrolysis products are important sources of energy for soil organisms (Tabatabai, 1994). Âeta-glucosidase is the third enzyme in a chain of three that break down labile cellulose and other carbohydrate polymers. Also, the relative activities of enzymes have been shown to be regulated by substrate quality and nutrient availability: acid and alkaline phosphatases with N and/or P (Sinsabaugh et al., 1993; Sinsabaugh and Moorehead, 1994; Lizarazo et al., 2005), beta-glucosidase with N and (Ca) (Decker et al., 1999), Aryl-sulfatase with P (Tabatabai and Bremner, 1970), and beta-glucosaminidase with C and N (Ekenler and Tabatabai, 2002a). The objective of the present work was to study the activity of five enzymes in the surface (0-10 cm) soil in plots treated with and without weed control eight years after timber harvesting.

MATERIALS AND METHODS

Study site

The study site is on an area of the Missouri Department of Conservation Carr Creek State Forest in Shannon County near Ellington, MO, in the south-eastern Missouri Ozarks. The site is one location of the USDA Forest Service's long term site productivity (LTSP) study in the central USA hardwood region (Ponder and Mikkelson, 1995). The study is located on the upper north-eastern-facing side slopes (20-28%) of two parallel ridges. The soil at the site is a loamy-skeletal, mixed, mesic, Typic Paleudults (Ultisol). The soil is primarily derived from Ordovician and Cambrian dolomite with some areas of Precambrian igneous rock (Missouri Geological Survey, 1979). The soil has a mean bulk density of 1.35 g/cm^3. Average values for soil chemical properties were: pH, 5.7; C, 3.3%; total nitrogen (TN), 0.11%; P, 16.9 mg/kg; Ca , 789 mg/kg; and Mg, 61 mg/kg (Ponder et al., 2001). Mean annual precipitation at the site is 112 cm and the mean annual temperature is 13.3°C. Before harvest, the site had a well-stocked, mature, second-growth oak-hickory forest (*Quercus*- L. - *Carya* Nutt.). The oak-hickory timber is the major forest type in this central hardwood region of the USA and occurs over a variety of soils, relief, and stand conditions.

Study design

The original study is a three-factor randomized complete block design (RCBD) with three levels each of organic matter removal and soil compaction, two levels of weed control, and three replications. Half of each 0.4-ha plot was treated to control weeds. Complete information on site preparation and treatment application can be found in Ponder and Mikkelson (1995). For the present study, only the weed control or subplot treatments plus three unharvest (uncut) plots are being studied.

For the first 2 years, using a manually operated backpack sprayer, all plots were sprayed annually in late spring with a mixture of glyphosate [N-(phosphonomethyl) glycine] and simazine (6-chloro-*N',N'*-diethyl-1,3,5-triazine-2-4-diamine) at the recommended rate of 3 and 3.6 kg a.i. ha^{-1}, respectively, to control weeds and to enhance the establishment of one-year-old planted tree seedlings of white oak (*Quercus alba* L.), red oak (*Q. rubra* L.), and shortleaf pine (*Pinus echinata* Mill.). Vigorous vegetative competition from germinating seeds and sprouts of woody vines (*Vitis aestivalis* and *Parthenocissus quinquefolius*), legumes (*Desmodium nudiflorium* and *Amphicarpaea bracteata*), sedges (*Carex cephalophora* and *Scleria triglomerata*), forbs (*Cimicifuga racemosa* and *Potentilla simplex*), and trees (*Q. alba*, *Q. velutina*, *Q. marilandica*, *Cornus florida*, and *Ulmus rubra*) for growing space in newly regenerating central hardwood forests must be controlled for planted trees to become established. Ground flora in the uncut control plots was diverse, but typically dominated by a few species of woody vines, understory trees, and legumes. Common species included flowering dogwood (*Cornus florida*), sassafras (*Sassafras albidum*), Virginia creeper (*Parthenocissus quinquefolia*), summer grape (*Vitis aestivalis*), hog peanut (*Amphicarpa braceata*), and trees, primarily species of oak (*Quercus* spp.) and hickory (*Carya* spp.). Beginning in the third growing season, half of each plot was sprayed with herbicides to permit planted trees to grow freely without weeds. Weeds were not controlled in the other half of the plot.

Sampling and enzyme assay methods

Five surface soil samples (0 to 10 cm) were taken randomly across the midsection of each subplot in early summer and composited for a total of 27 samples each with and without weed control and nine from the uncut plots. Samples were divided into two groups. One group was sieved (4 mm), and sent by overnight delivery to a commercial laboratory (Agriculture Diagnostic Laboratory, University of Arkansas, 276 Altheimer Drive, Fayetteville, AR 72704) for pH, C, and soil extractable P and S analyses. Soil C was measured by combustion and pH in 1:2 (soil to water, w/v) suspension after 1 h by electrode. Soil P and S were extracted using Mehlich 3 extractable solution and determined by ICP (Perkin-Elmer 1983).

The other group of samples was withdrawn for enzyme analyses. Samples were sieved (4 mm), divided into duplicates, and stored in plastic bags in a laboratory refrigerator at 5°C until analyzed for enzyme activity. Acid and alkaline phosphatase activities were determined using a method developed by Eivazi and Tabatabai (1977). Beta-glucosidase activity was estimated by a method described by Eivazi and Tabatabai (1990). The method of Tabatabai and Bremner (1970) was used for assaying arylsulfatase activity. The assay of beta-glucosaminidase activity was done using the method described by Parham and Deng (2000). These standard procedures involve colorimetric determination of *p*-nitrophenol (PNP) released when soil is incubated with toluene and the respective buffered substrate for 1 h at 37°C. Enzyme activities were expressed as mg *p*-nitrophenol released g^{-1} soil h^{-1}.

Data analysis

Data for duplicate samples were averaged and treatment means were analyzed by analysis of variance with the PROC GLM procedures in SAS Version 8.2 (SAS Institute, Cary, NC) using organic matter removal (OMR), soil compaction (SC), and weed control as treatments. Because there were no differences for enzymes between OMR

treatments and only one difference for SC treatments, data were then analyzed as a randomized complete block design for three weed control treatments. Mean separation was tested using the Tukey's Studentized Range test. Pearson correlation was used as a simple correlation analysis.

RESULTS

Enzyme activities

Except for the activity of acid phosphatase, which was significantly ($p < 0.05$) increased by SC, enzyme activity was not significantly affected by either OMR or SC. Activity for the five enzymes measured was affected by the presence or absence of weeds; it was higher with weeds than without weeds, but only acid phosphatase, beta-glucosidase, and aryl-sulfatase were significantly ($p < 0.05$) different between subplots with weeds and subplots without weeds (Table 1). Except for beta glucosidase, activity differences between treatments with weeds and uncut control were not significant.

Soil pH, C, and S, although higher for subplots with weeds compared to subplots without weeds, were not significantly ($p < 0.05$) different between weed treatments (Table 1). Only extractable P was significantly ($p < 0.05$) different between weed and control treatments; lower for subplots with weeds than for subplots without weeds and for the uncut control plots.

Table 1. Enzyme activity for five soil enzymes, soil pH, C, P and S in an eight-year-old tree planting with and without weed control.

	Treatments		
Enzyme	Control	Weeds	No weeds
	mg pNP kg^{-1} soil h^{-1}		
Acid phosphatase	125.0a[1]	120.5a	83.5b
Alkaline phosphatase	31.0a	43.8a	35.8a
Beta glucosidase	74.5a	111.0b	55.1a
Aryl-sulfatase	274.2ab	319.4b	204.9a
Beta glucosaminidase	50.0a	47.9a	37.9a
Soil properties			
C (%)	2.7a	4.1a	2.9a
pH	5.6a	5.9a	5.7a
P (mg/kg)	24.0a	21.8b	28.1a
S (mg/kg)	26.7a	37.0a	29.2a

[1]Values followed by different letters are significantly different at the 0.05 level of significance.

Enzyme activity correlation with soil pH, C, P, and S

Soil properties consistently correlated with the activity for a number of enzymes (Table 2). Both acid phosphatase and beta glucosaminidase activity were positively correlated with pH, C, and P. For acid phosphatase pH, C, and S, r values were +0.31*, +0.34*, and +0.34* and were +0.37*, +0.29*, and +0.31* for beta glucosaminidase, respectively. Soil pH was also positively correlated (r = 0.37*) with

Table 2. Correlation matrix (r value) between soil enzyme activities, soil pH, C, P, S, and weed control.

	Soil enzyme activity					Soil properties				Weed control
Variables	Acid phosphatase	Alkaline phosphatase	Beta-glucosidase	Aryl-sulfatase	Beta-glucosaminidase	Soil pH	Soil C	Soil P	Soil S	Weed control
Acid phosphatase	___	0.19 ns	-0.27 ns	-0.32 *	0.94***	0.31*	0.34*	-0.07 ns	0.34*	0.32*
Alkaline phosphatase		___	-0.25 ns	-0.09 ns	0.44**	0.37**	-0.06 ns	-0.39**	0.00 ns	0.22 ns
Beta-glucosidase			___	0.43**	-0.21 ns	0.00 ns	-0.21 ns	0.37**	-0.17 ns	-0.44***
Aryl-sulfatase				___	-0.25 ns	0.06 ns	-0.16 ns	0.29*	-0.19 ns-	0.76***
Beta-glucosaminidase					___	0.37*	0.29*	-0.13 ns	0.31*	0.31*
Soil pH						___	0.42**	0.08 ns	0.65***	0.12 ns
Soil carbon							___	0.21 ns	0.94***	0.16 ns
Soil P								___	0.22 ns	-0.31*
Soil S									___	0.14 ns
Weed control										___

*, **, ***Significant at 0.05, 0.01, and 0.001 probability levels, respectively; Ns=Non-significant

alkaline phosphatase while soil P was negatively correlated (r = -0.39**) with alkaline phosphatase. Beta-glucosidase was correlated (r = +0.37**) only with soil P. Among soil properties, there were correlations between soil pH and soil C (r = +0.42**) and between soil pH and soil S (r = +0.65***). Also, the correlation (r = +0.94) between soil C and soil S was significant (p<0.001). Neither beta glucosidase nor aryl-sulfatase activities were significantly related to soil pH or soil C. In generally, subplots with weeds can be characterized as having less soil P, more soil C and soil S (not significantly higher), slightly higher pH, and higher, but not always significantly higher enzyme activities than subplots without weeds.

Enzyme activity correlation with weed control

Both acid phosphatase and beta glucosaminidase were positively correlated (r = +0.32* and r = +0.31*) to weed control while beta-glucosidase and aryl-sulfatase were negatively correlated (r = -0.44*** and r = -0.76***) to weed control. Aryl-sulfatase was positively correlated (r = +0.29*) to soil P while alkaline phosphatase was negatively (r = -0.39**) related. Soil P, which was lower in subplots with weeds than in subplots without weeds, was negatively correlated (r = -0.31*) with weed control. These correlations indicate that while there appears to be a clear difference between weed and no weed subplots for enzyme activity, the activity is further affected by the soil environmental factors including soil chemistry associated with differences between weed treatments.

The activity of one or more enzymes was also significantly corrrelated to each other (Table 2). Acid phosphatase was negatively correlated (r = -0.32*) with aryl-sulfatase and positively (r = 0.94***) correlated with beta-glucosaminidase. Alkaline phosphatase was also positively (r = +0.44**) correlated with beta-glucosaminidase. Beta-glucosidase was positively correlated (r = +0.43**) with aryl-sulfatase.

DISCUSSION

Microorganisms are a major source of soil enzyme activity (Skujins, 1976), and conditions such as soil temperature and moisture affect soil microorganism populations causing changes in enzyme activity. Previous work on this study site showed that soil temperature and moisture for plots without weeds between June and September averaged 20.6˚C and 37% while soil temperature and moisture in plots with weeds averaged 15.1˚C and 15.5%, about 6˚C warmer and 22% wetter without weeds than with weeds (Ponder, 2004).

Soil properties such as organic matter content and pH have been shown to influence soil enzyme activity (Bunzl et al., 1976; Gadd and Griffiths, 1978; Sinha et al., 1978); affected by some soil factors more than others, and individual enzymes may respond differently to the same soil factors. However, soil enzyme activity measurements, as recorded in this study, are only a measure of the potential activity of an enzyme in soil and not the *in-situ* activity in the natural soil environment, where activity may be impaired by soil properties (Gianfreda and Bollag, 1994; Naseby and Lynch, 1997). Also, enzyme activity in the present report represents measurements replicated in 27 treatment and 3 control plots during a single sampling time.

The exact reason for the higher acid phosphatase activity for the uncut control than for the no weeds subplots is not known. However, the reason is likely due to the better composition and amount of understory vegetation in the uncut control plots compared to the scarcity of vegetation in no weed subplots except for planted trees.

Enzyme activity in other studies, especially for phosphatases and beta-glucosidase, has been shown to be higher when P levels are low compared to when levels are high (Saa et al., 1993; Tadano et al., 1993). Earlier reports also showed beta-glucosidase activity to be either inhibited or reduced by inorganic salts containing P and K

(Eivazi and Tabatabai, 1990, Decker et al., 1999). The activity of aryl-sulfatase has been shown to be reduced by increases in soil organic matter (Tabatabai and Bremner, 1970). Ekenler and Tabatabai (2002b) also reported that beta-glucosaminidase activity was reduced by increased levels of P and N fertilization, leading these authors to suggest that beta-glucosaminidase plays a major role in N mineralization in soils. Nitrogen was not investigated in our study.

Allison and Vitousek (2004) concluded that the production of enzymes reflects microbial nutrient demands, which allow microbes to acquire limiting nutrients from complex substrates in the soil. Thus, when a resource such as P is limiting, microbes may benefit from producing enzymes to obtain it, but microbial activity could be constrained by the availability of some other resources such as C and N. Sinsabaugh et al. (1993) explained the process in an ecological context, explaining that this regulatory condition is commonly manifested as inverse relationships between activity and nutrient availability.

While most correlations reported here between soil properties and enzymes were moderate, the relationships are supported by previous work where both pH and soil carbon were correlated with enzyme activity (Frankenberger and Tabatabai, 1991). Neither are the correlations between enzymes unusual because similar relationships have been reported for other enzymes (Frankenberger and Tabatabai, 1991; Speir and Ross, 1976). Although soil pH was correlated with the activity of some enzymes, given the insignificant difference between pH among weed treatments in the present study; it is likely that the significant correlations in Table 2 do not reflect any meaningful relationship. According to Burns (1978), a much wider range in soil pH is required in order for enzyme activity to be affected. Positive correlations between enzyme activity and soil factors suggest that enzyme activity may have been limited by increasing values of soil factors associated with weed control, and the negative correlations between weed control and enzyme activity suggest that controlling weeds reduce enzyme activity.

Immobilized enzymes are relatively stable until the soil is disturbed (Naseby and Lynch, 1997). This makes soil enzyme activity a strong indicator of soil health. It has been demonstrated that soil nutrient concentration changes associated with losses in organic matter due to decomposition (Sinsabaugh and Moorhead, 1994) or soil depth (Naseby and Lynch, 1997) can have a significant effect on enzyme activity. Blazier et al. (2005) demonstrated that forest microbial populations are dependent on C supplied by living vegetation, as declines in microbial biomass and activity in response to brush control and tree removal treatments in their study. In our study, except for trees, vegetation has been excluded for eight years. Living vegetation provides soil organisms with root exudates such as simple carbohydrates, amino acids, and fatty acids (Tate et al., 1991). Suppression of herbaceous vegetation led to reduction of soil C in no weed subplots compared to weed subplots (Table 1). The decline in soil C, although not significantly different between treatments, in response to weed control in our study were consistent with those reported in similar studies of forest vegetation suppression (Blazier et al., 2005; Busse et al., 1996; Périé and Munson, 2000).

Weed control chemicals can affect soil microorganism populations, but the outcome may be only short term with little noticeable long-term effect on soil health and quality (Ponder, 2002). According to information on the herbicides used in our study, neither glyphosate nor simazine has been shown to be toxic to soil microorganisms under field conditions (Ponder, 2002; Busse et al., 2001). Simazine can be utilized by certain soil microorganisms as a source of energy and mineralization. Also, Busse et al. (2001) reported that all measures of soil microbial activity tested revealed no detrimental effect of glyphosate when applied at manufacturer's recommended rates. Increasing glyphosate rates up to 100 times above the recommended rate led to increasing microbial activity as measured by soil respiration.

CONCLUSIONS

Excluding weeds from the growing site reduced enzyme activity. It appears that the lower enzyme activity in weed control subplots is due to reduced microbial activity associated with the soil environment created in the absence of weeds and their root substrates (Skujins, 1978). Although there were some significant correlations with soil factors and enzymes, they were generally low. In most cases, they explained less than 40% of the variation. The lower enzyme activity in weed-free subplots indicates that there is likely lower organic C accumulation with lower microbial activity, of which the long-term consequences are not known, but deserves further attention especially for this low nutrient soil (King, 1997; Ponder et al., 1999). Reduced enzyme activity conserves organically bound nutrients such as N, P, and S in soil due to the lack of mineralization processes which could lead to critical nutrient losses in forest ecosystems.

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