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The Impacts of Invasive Earthworms on Decompositional Enzyme Activity

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by

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Abstract

Native earthworms are believed to have been removed from the northeastern United States during the last period of glaciation, and have since been slow to recolonize. In areas that have been colonized, non-native earthworms have caused major impacts to the litter layer and soil horizons, rapidly consuming litter and homogenizing soil horizons through burrowing activity. In this study, we used colorimetric enzyme assays to observe the potential earthworm impacts on soil microbial decomposition. We hypothesized that earthworms would increase the production of oxidative enzymes, which degrade recalcitrant litter, due to preferential consumption of labile leaf litter. Through this study, we found that earthworms have impacts on the production of oxidative enzymes in the organic horizon, promoting increased phenol oxidase activity in order to decompose more recalcitrant nutrient sources. The long-term effects of non-native earthworms should be further studied, and may impact forest conservation and management programs in the future.

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Glossary of Terms

- **β-1,4-Glucosidase –** an enzyme that degrades the β-1,4-linkages between glucose molecules within cellulose, resulting in cellobioside intermediates.
- **β-D-1,4-Cellobiosidase –** a hydrolytic enzyme that degrades the linkages between cellobioside molecules, the intermediates of cellulose decomposition. These compose cellulose into glucose subunits.
- **β-N-Acetylglucosaminidase –** a hydrolytic enzyme that degrades the 1,4-β-linked N-acetylglucosamine bonds, resulting in disaccharide N,N'-diacetylchitobiose subunits (Beier and Bertilsson, 2013).
- **Hydrolytic –** a class of enzymes that degrades labile substrates.
- **Labile –** a class of substrate that is easy to decompose based on molecular structure, such as cellulose and chitin.
- **Oxidative –** a class of enzymes that degrades recalcitrant substrates, such as lignin.
- **Peroxidase –** a class of enzymes that use peroxide as an electron acceptor during decomposition of molecules (Sinsabaugh, 2010).
- **Phenol Oxidase –** a generic class of enzyme that oxidize phenols within compounds, and consume oxygen (Sinsabaugh, 2010).
- **Recalcitrant –** a class of substrate that is more difficult to decompose than labile substrates based on its molecular structure.

Acknowledgements

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1. Introduction

It is generally believed that most native earthworms were removed north of the glacial front during the last period of glaciation and glacial recession. Since then, earthworms have been slowly reintroduced from Europe and Asia via anthropogenic activity (Dempsey *et al.*, 2013), (Fahey *et al.*, 2013). The first invasions were officially documented approximately 150 years ago, but little is known about the long-term consequences of earthworms on forested ecosystems (Crumsey *et al.*, 2014).

Earthworm invasions have major impacts on soil structure and quality, by mixing soil, and by rapidly consuming leaf litter (McLean *et al.*, 2006). These physical disturbances may reduce soil carbon storage, and promote the formation of hydrophobic soil aggregates, thereby inhibiting microbial decomposition of soil organic matter (Fahey *et al.*, 2013). Earthworms also alter the pH of the soil, creating a more basic pH environment (Sackett *et al.*, 2013). These changes indirectly impact the soil nutrient and carbon pools, which alter nutrient cycling and microbial communities (Sackett *et al.*, 2013).

Earthworms are classified ecologically by three main burrowing habits: anecic, endogeic, and epigeic (Bouche, 1977). Epigeic earthworms do not form burrows, but are found in surface organic matter, where they consume leaf litter. Endogeic worms burrow horizontally within the mineral soil, and consume mainly soil organic matter. Anecic worms build deep, vertical burrows, and consume both fragmented leaf litter and soil organic matter (Huang *et al.*, 2010). In the plots used for this study, at Mohonk Preserve in the Hudson Valley region of New York, two genera of earthworms have been found (Walling *et al.*, in preparation), including *Amynthas* and *Dendrobaena*. Earthworms of

the genus *Dendrobaena* spp. are epigeic, and were transported primarily from Europe (Huang *et al*., 2010). Asian *Amynthas* spp. are also surface-dwelling, but have a larger body size, and form copious amounts of castings (Zhang *et al*. , 2010).

We hypothesized that the presence of earthworms would increase decomposition via production of oxidative enzymes, relative to decomposition by hydrolytic enzymes. We believe this to be the case because epigeic earthworms largely consume the organic matter on the forest floor, consisting of labile carbon sources, like cellulose and chitin (Crumsey *et al.*, 2014). Less preferable litter, including stems, twigs, and leaf veins, contain lignin, and are more recalcitrant.

In this study, we measured the impacts of earthworm presence on the rate of microbial enzyme production for both hydrolytic and oxidative enzymes. We analyzed soils from plots containing earthworms, and those without earthworms. Enzymatic assays were conducted using both hydrolytic and oxidative substrates, to determine whether earthworms have an effect on the amount of oxidative relative to hydrolytic enzyme expression in soil. Additionally, numerous environmental variables were measured, including temperature, soil moisture, soil organic matter content, and pH, in order to determine whether factors other than earthworm presence may be influencing microbial function within ecosystems.

2. Methods and Materials

2.1 Study Site

Research was conducted at ten plots located on the West Trapps Trail at Mohonk Preserve, in Gardiner, NY. These ten plots were previously assessed for presence or absence of invasive earthworms using mustard extraction (Hale *et al*., 2005). Of these plots, four did not have earthworms, and six had invasive earthworms in 2013.The area is mostly dominated by hemlock (*Tsuga Canadensis*) and mixed hardwood species, including maple (*Acer* spp.), black birch (*Betula lenta*), and oaks (*Quercus* spp.). The land had once been cleared for farming, but had not been disturbed in over 70 years (Thompson, personal communication, 2013).

This site was chosen for this study because it was assessed for the presence of earthworms in 2012. Plots were at least 50 meters in distance from one another.

2.2 Field Methods

The temperature of the soil was measured every two hours over the course of the experiment using ibuttons. The ibuttons were programmed to read temperatures between 0 and 20 degrees Celsius, and were wrapped in parafilm and placed in a small plastic bag prior to burial under the O horizon in each of the ten plots. After five months, the ibuttons were collected. Depth of litter on the forest floor, and the depth of the organic horizon were also measured at each of the plots.

2.3 *Soil Collection and Processing*

Three 10 cm soil cores were collected at each of the ten plots, and were separated into O horizon and a combined AB horizon core. The cores were manually homogenized

into a single sample. These were transported to Syracuse, NY on ice in order to be processed. One gram of each soil sample was measured for enzymatic assays, eight to ten grams were used to measure soil moisture and percentage organic using loss on ignition, and the remainder of the soil was air-dried in order to be used for pH measurements. Loss on ignition was measured by heating dried soil for 12 hours at 460 degrees Celsius, and reweighing after soils were cooled.

Measurements of pH were taken by measuring one gram of organic soil, and two grams of combined AB horizon soils. To these, four milliliters of deionized water were added, and one measurement per sample was taken using a Corning pH meter.

2.4 Microbial Enzymatic Assays

Enzymatic activity was assessed through a colorimetric assay technique developed by Frey *et al.*, 2012. The activity of hydrolytic enzymes including β-1, 4-glucosidase, β-D-1,4-cellobiosidase, β-N-acetylglucosaminidase, and oxidative enzymes including phenol oxidase and phenol peroxidase were measured. Assays for both the hydrolytic and oxidative enzymes were prepared simultaneously. Soil was homogenized, and one gram was weighed out and mixed with 75 mL of sodium acetate buffer. This was mixed in a blender for 1 minute, and an additional 125 mL of buffer was used to rinse the slurry into a beaker containing a stir bar. Soil slurry was pipetted into three black, clear-bottom 96-well microplates, and four, 2 mL deep-well plates, following a clearly defined plate map (see Appendix I). Sodium acetate buffer was prepared two days prior to use, and substrates were prepared the same day as soil processing.

Plates were incubated in the dark at room temperature until being read using a Synergy 2 microplate reader and Gen5 computer software, located at the Syracuse

University Biomaterials Institute. For hydrolytic assays, the excitation wavelength was set to 360 nm, and the emission wavelength was set to 460 nm. The fluorescence of hydrolytic enzyme plates was read at 1, 2, 3, 3.5, and 4 hours from the start time in order to achieve a standard curve for comparison. For the oxidative assays, the absorbance wavelength was set to 460 nm. Oxidative enzyme plates were pipetted into clear, 96-well plates, and the absorbance was read at 22, 24, 28, and 48 hours from the starting time. The hydrolytic standard plate, and hydrolytic and oxidative sample blanks, and substrate control plates were prepared and read simultaneously with the soil assay plates.

Hydrolytic enzyme activity was calculated using the standard plate and soil quench for comparison of enzymatic activity. The corrected fluorescence was calculated by subtracting the fluorescence of the sample control and substrate control plates. The corrected fluorescence was converted to a µM amount, and was then converted to nmol. The nmol value was multiplied by 125mL, or the total volume of soil slurry used, and was then divided by the product of the microplate well volume, incubation time in hours, and the gram amount of soil used for the assay. The final calculation yielded the activity rate of the hydrolytic enzyme in nmol/g/h.

Similarly, oxidative enzyme activity was calculated by first determining the final optical density (OD) of the oxidative enzyme absorbance reads. This was completed by subtracting the mean OD of the negative control cells from the mean OD of the oxidative assay wells. The final OD was multiplied by the total buffer volume of 125 mL, which was then divided by product of the total volume of the deep-well plates (0.5 mL), the total incubation time, and the total soil amount in grams. The final calculation yielded the final activity reading for the oxidative enzymes in µmol/g/h.

2.5 *Statistical Analyses*

In order to analyze the enzymatic activity, the mean activity rate and the standard error were calculated for all of the enzymes by month. These were plotted as strip charts using the statistical program, R version 3.0.2, and t-tests were conducted to determine if there was a difference in enzymatic activity between plots with and without earthworms.

A Shapiro test for normality was conducted for all enzyme activities and soil variable data, and any non-normally distributed data was transformed using a square root transformation. Simple linear regression was performed to determine if there was a correlation between enzymatic activity and soil conditions, including soil percent moisture, percent organic matter, pH, and temperature.

All statistics were analyzed using an alpha level of 0.1 due to *in situ* plot variation and a small sample size of n=10 per month by soil horizon. If significant at a 0.1 alpha level, sample means on the charts in figures 1-10 were marked with a single asterisk. If statistically significant at $p<0.05$, sample means were marked with two asterisks.

3. Results

Means of hydrolytic and oxidative enzyme activity were plotted with standard error bars (Figures 1-10). In general, the hydrolytic enzyme activities were not statistically significantly different between plots with and without earthworms. However, β-glucosidase was significantly different for July in the O horizon (Fig. 1), and October in the AB horizon (Fig. 2). Cellobiosidase activity was significantly different for September and October in the O horizon (Fig. 3), and was also significantly different for August in the AB Horizon (Fig. 4). The activity of N-acetylglucosaminidase was significant for October in the O horizon.

The oxidative enzyme activities were statistically significantly different between plots with, and without earthworms for the oxidative enzyme, phenol oxidase. In the organic horizon, data from July, August, September, and October were statistically significantly different for the effects of earthworms on activity (Fig. 7). June was the only month in which a statistically significant difference was detected for phenol oxidase in the AB horizon (Fig. 8). Data for the oxidative enzyme peroxidase generally did not differ statistically between soils with and without worms (Fig. 9 and 10). Peroxidase activity between plots with earthworms and without was significantly different in the organic horizon for the September assays (Fig. 10).

Analysis of soil and environmental variables revealed significant relationships for enzyme activity and soil moisture, soil organic matter, and soil pH for multiple enzymes in both horizons. There were significant correlations between β-glucosidase, cellobiosidase, and peroxidase activity and the measured soil properties. β-glucosdiase activity was positively correlated with soil organic matter and pH in the organic horizon, and with moisture, and organic matter in the AB horizon. Cellobiosidase activity was positively correlated with soil moisture and soil organic matter in the AB horizon. Peroxidase activity was positively correlated with soil organic matter and pH in both horizons, and soil moisture in the organic horizon. A table listing R-squared values, p-values, and significance is listed in Appendix II.

4. Discussion

In our plots, phenol oxidase enzyme activity was significantly different between plots with and without earthworms in the organic horizon soil. Plots with worms had consistently higher phenol oxidase production, than plots that did not have worms (Fig. 7). This finding supports our initial hypothesis that the presence of earthworms would increase production of oxidative enzymes. A previous study found that low *in situ* oxidative enzyme activity contributes to soil organic matter storage (Sinsabaugh, 2010). In this study, we found higher phenol oxidative enzyme activity in plots with earthworms, which may reflect decreased carbon and nutrient storage. These findings are in agreement with Fahey *et al.*, 2013, which found that soil disturbances, including those caused by invasive earthworms, decrease carbon and nutrient storage. These results suggest that there may be long-term consequences for plant nutrient uptake and microbial carbon-use efficiency. Previous studies have suggested that a rapid release of nutrients caused by earthworm invasion may be beneficial at first, releasing important nutrients for use by plants and soil fauna. However, after long-term invasion, microbial biomass is known to decrease, indicating a change in the microbial community to a smaller, more active group of microorganisms (Eisenhauer *et al.*, 2011).

Hydrolytic enzyme activity was generally not significantly different in this study. Hydrolytic enzymes are thought to be more stable within the environment than are oxidative enzymes due to dynamic changes within their activity in previous studies, making a change to hydrolytic enzyme production less likely (Sinsabaugh, 2010). The presence of earthworms has also been associated with an alteration in the bacterial to fungal organism ratio, thereby increasing the presence of bacteria relative to fungal soil

organisms, again, creating a smaller, but more active microbial community (Eisenhauer, *et al*., 2011). This decrease in bacteria relative to fungi may contribute to the lack of statistically significant findings between hydrolytic enzyme activities in plots with and without earthworm. Fungi are mainly responsible for oxidative enzyme production, and a decrease in bacterial organisms may reduce hydrolytic enzyme production proportional to oxidative, but may not have a significant effect on overall hydrolytic activity.

Each of the enzyme figures follows a similar temporal pattern, with low activity in June, increasing in July, decreasing greatly in August, increasing in September, and finally decreasing in October. This temporal trend may be due to seasonal fluxes in leaf litter input, moisture, and temperature. Enzymatic activity could be expected to increase in July, as activity is a function of soil variables, including temperature, moisture, and pH, and temperature is naturally highest in July in our study region (Sinsabaugh, 2010). Our October sampling date occurred prior to leaf senescence, which may explain why we found the lowest enzyme activity this month. Much of the leaf litter from the previous season had already been consumed, and current season leaves usually fall later in October after sampling was completed. The significant correlations between β-glucosidase, cellobiosidase, and peroxidase activity, and the measured variables of moisture, organic matter, and pH, support these findings from previous studies. These correlations may account for some of the variability in enzyme activity, as these enzymes were generally not significantly different for the effects of earthworms on enzyme activity.

Few experiments have been conducted to determine the impacts of non-native earthworms on soil microbial communities and enzyme expression. Sinsabaugh, 2010 estimates that only about 150 studies have been published in this area. However, studies

of the impacts of non-native earthworms on soil nutrient storage are well-documented. Earthworms are responsible for much of the litter consumption on the forest floor, and homogenize the soil horizons (Sackett *et al*., 2013). It appears that changes in microbial production of phenol oxidase in the organic horizon can be attributed to these earthworm-mediated changes. Changes in organic horizon enzyme activity is of particular interest, as epigeic earthworms have drastic impacts on the surface litter and organic soil. At our site, these epigeic Earthworms may not be having as large an effect on the mineral horizons. However, the effects we are seeing may be further impacted by time since invasion, and the previous soil carbon pool composition (Fahey *et al*., 2013). Studying this site for an increased time interval since initial invasion may elucidate the impacts earthworms may have on microbial community composition, and ultimately the roles of these communities in a changing soil environment.

There is still much to learn about the effects of invasive earthworms on soil enzymatics and litter decomposition. Future studies should conduct soil assays more than once monthly. Future assays should also take into account spatial variability across plot areas, including microtopography, moisture gradients, and differences in canopy cover. Plot-to-plot variability can contribute to enzyme activity variation, and if accounted for we may be able to get a clearer picture of the effect of invasive earthworms on decomposition activity within forest ecosystems. Additionally, presence of oxygen within soil should be considered because enzymes, such as peroxidase, are produced in response to certain reactive oxygen molecules that result from variations in nutrient and oxygen availability (Sinsabaugh, 2010).

5. Conclusions

Non-native earthworms drastically impact the soil environment within forest ecosystems, by homogenizing soil horizons, decreasing soil organic carbon storage, and decreasing the diversity of soil microbial communities. The data analyzed in this study revealed that these earthworms impact the phenol oxidase activity within the organic horizon, indicating that earthworms do play a large role in the carbon storage and litter decomposition in the organic soil, but may not have a large impact on mineral horizon soil dynamics. It is important that we continue to study these impacts long-term to obtain knowledge about the consequences of earthworm invasion in terms of litter loss and microbial decomposition. This information may be essential for future forest conservation efforts. As the consequences of non-native earthworms become scientific knowledge, conservationists and forest managers must properly educate the public about how to prevent the spread of earthworms further into forested lands.

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Figures

Hydrolytic Enzyme Results

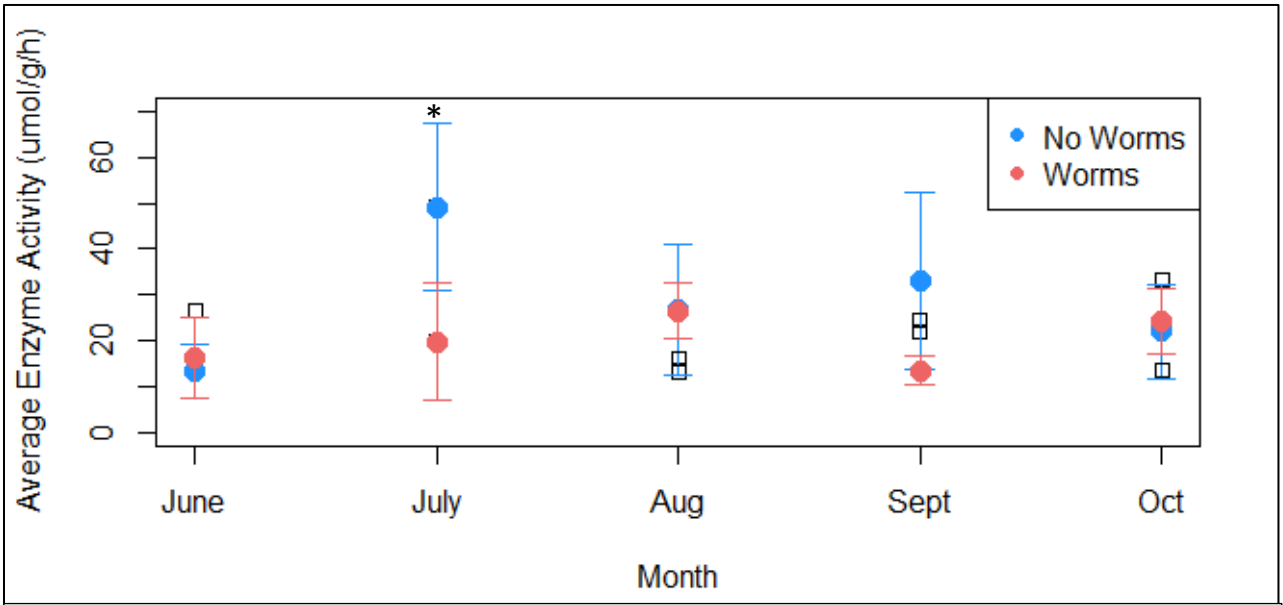

Fig. 1 Average β-Glucosidase Activity in Organic Horizon Soil. Data is presented as a strip chart, where blue represents the mean and standard error of plots without worms, and red represents plots with worms. Significance with a 0.1 alpha level is represented by *, where n=10 per month. The mean activity of soils with no worms is statistically different than the plots with worms.

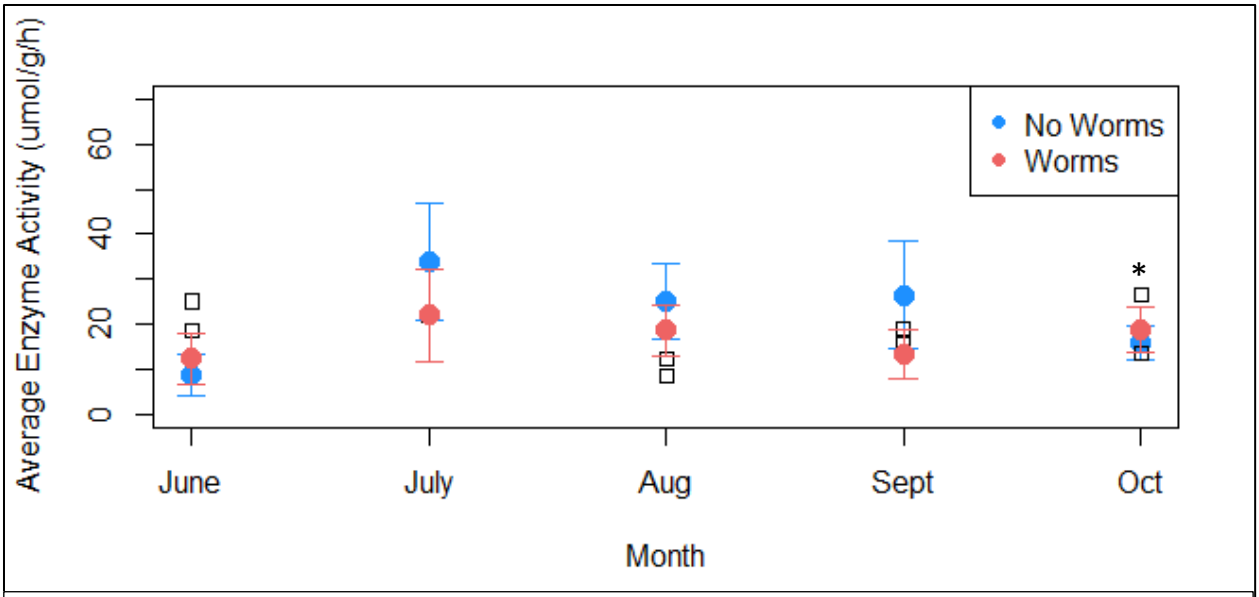

Fig. 2 Average β-Glucosidase Activity in AB Horizon Soil. Mean activity of β-Glucosidase is significant for October in the AB horizon based on a 0.1 alpha level, where n=10 per month.

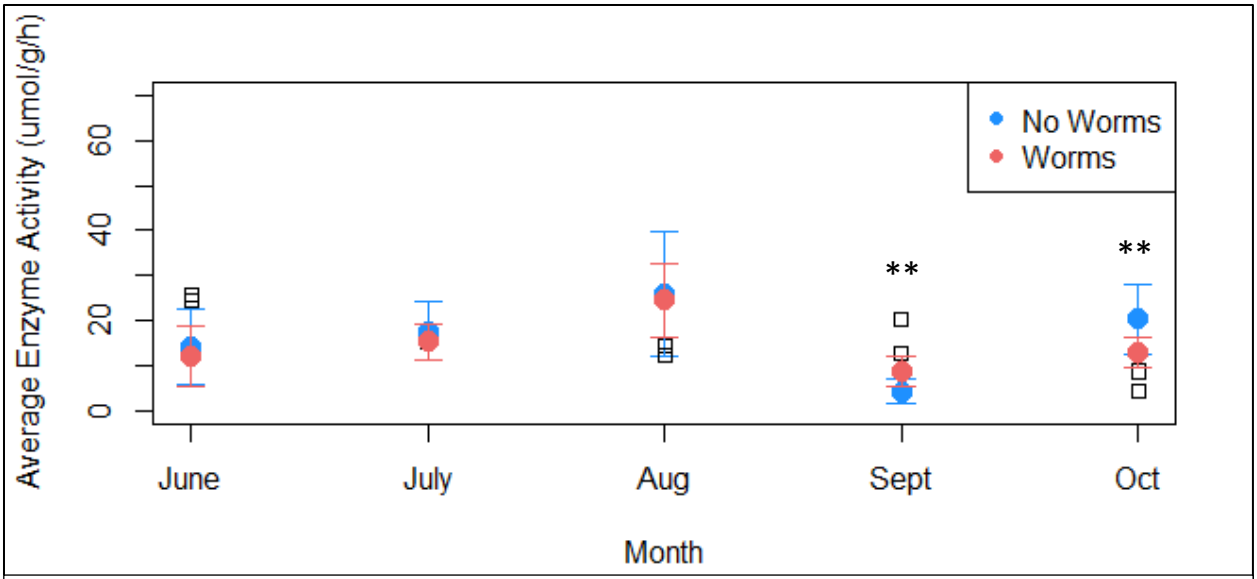

Fig. 3 Average Cellobiosidase Activity in O Horizon Soil. Mean activity of cellobiosidase is significant for September and October in the O horizon based on a 0.1 alpha level, where n=10 per month. Very significant p-values are noted with **.

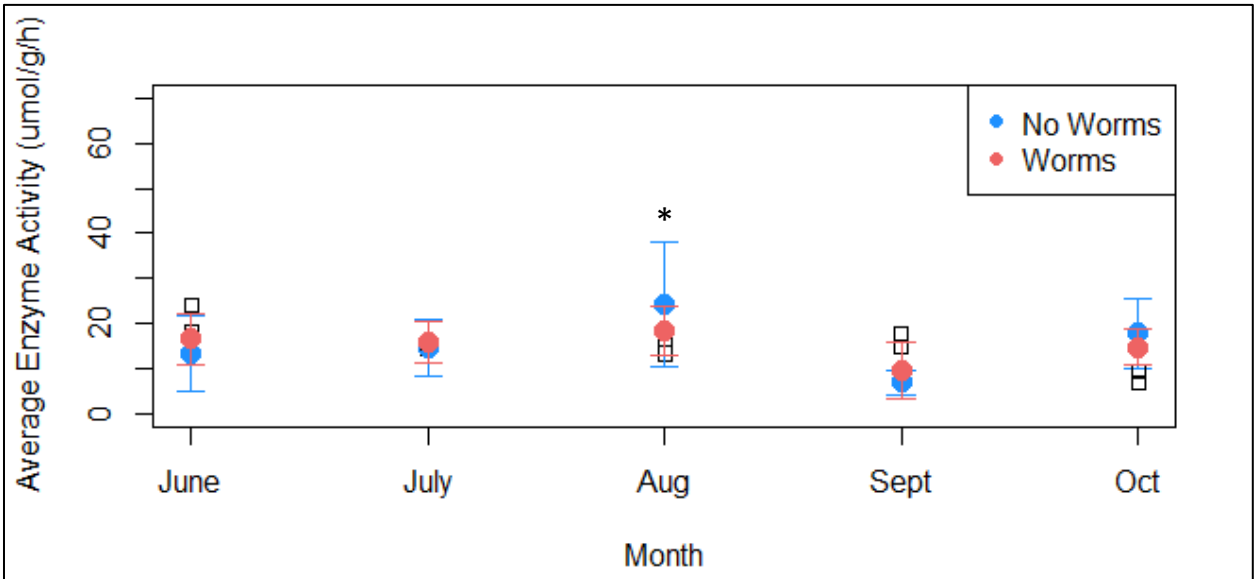

Fig. 4 Average Cellobiosidase Activity in AB Horizon Soil. Mean activity of cellobiosidase is significant for August in the O horizon based on a 0.1 alpha level, where n=10 per month.

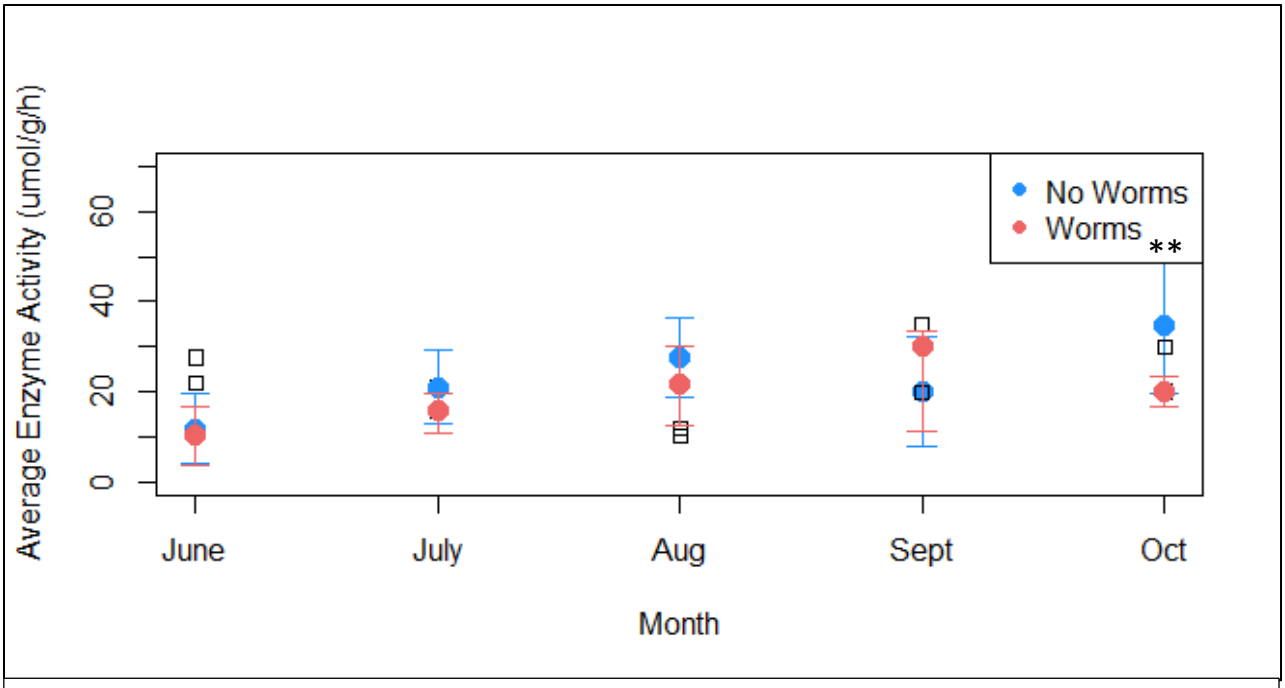

Fig. 5 Average N-acteylglucosaminidase Activity in O Horizon Soil. Mean activity of N-acetylglucosaminidase was significant for October based on a 0.1 alpha level, where n=10 per month.

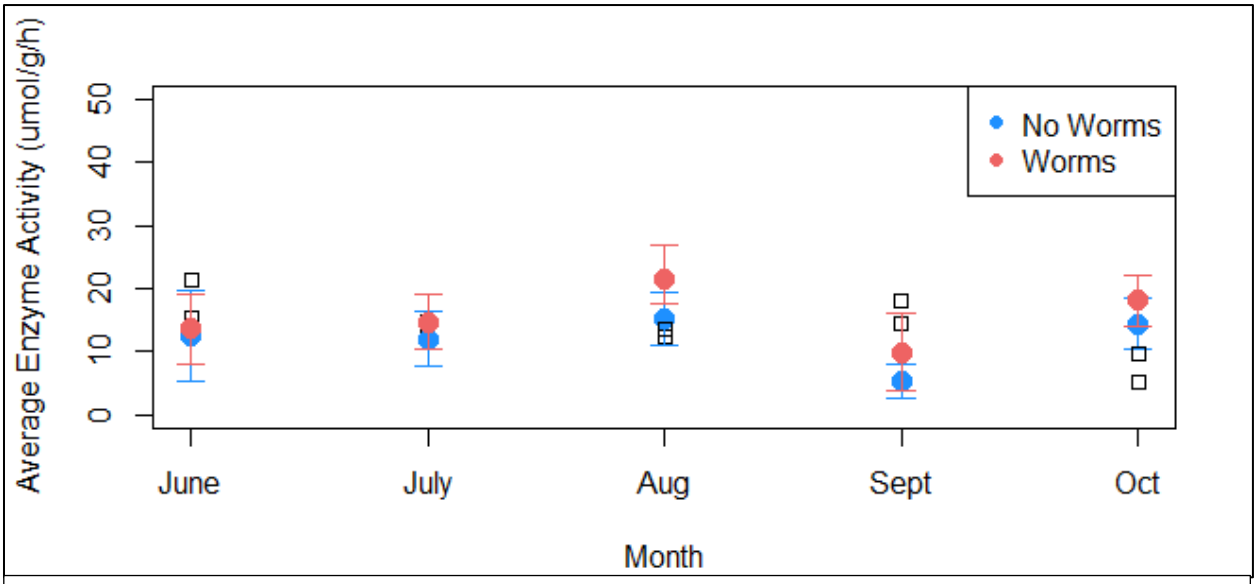

Fig. 6 Average N-acteylglucosaminidase Activity in AB Horizon Soil. Mean activity of N-acetylglucosaminidase is not significant for any of the five months based on a 0.1 alpha level, where n=10 per month.

Oxidative Enzyme Results

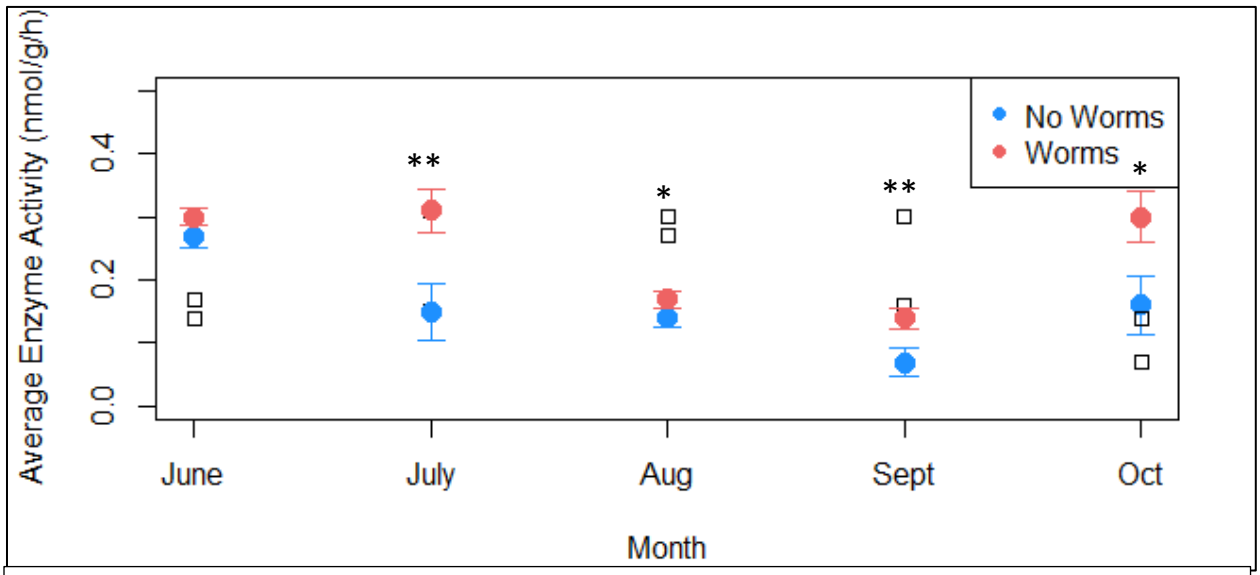

Fig. 7 Average Phenol Oxidase Activity in O Horizon Soil. Mean activity of phenol oxidase is significant for July, August, September, and October based on a 0.1 alpha level, where n=10 per month. Highly significant p-values are notated with **.

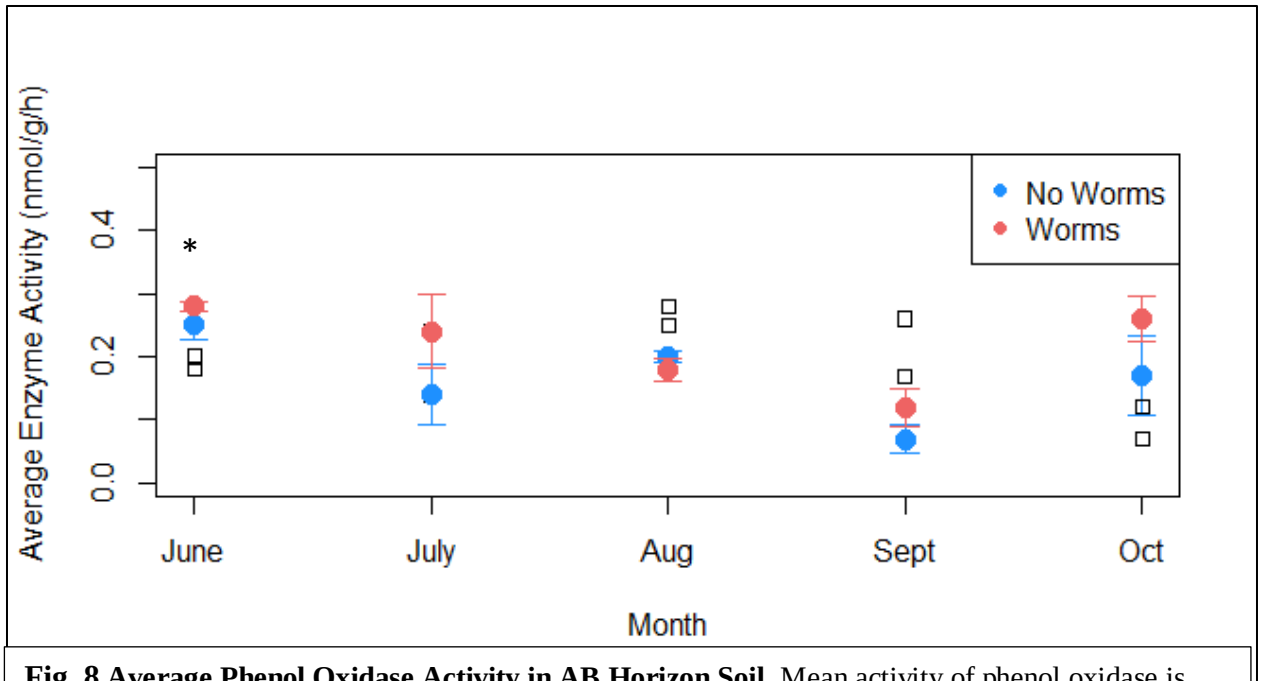

Fig. 8 Average Phenol Oxidase Activity in AB Horizon Soil. Mean activity of phenol oxidase is significant for June based on a 0.1 alpha level, where n=10 per month.

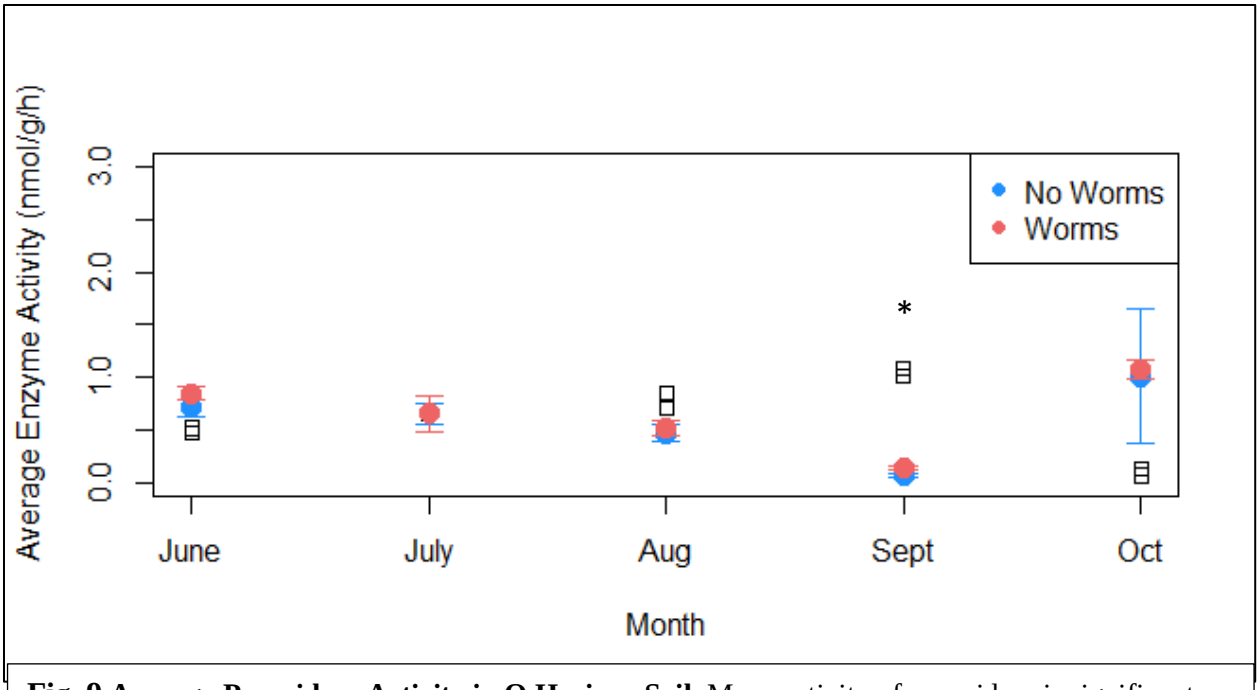

Fig. 9 Average Peroxidase Activity in O Horizon Soil. Mean activity of peroxidase is significant for September based on a 0.1 alpha level, where n=10 per month.

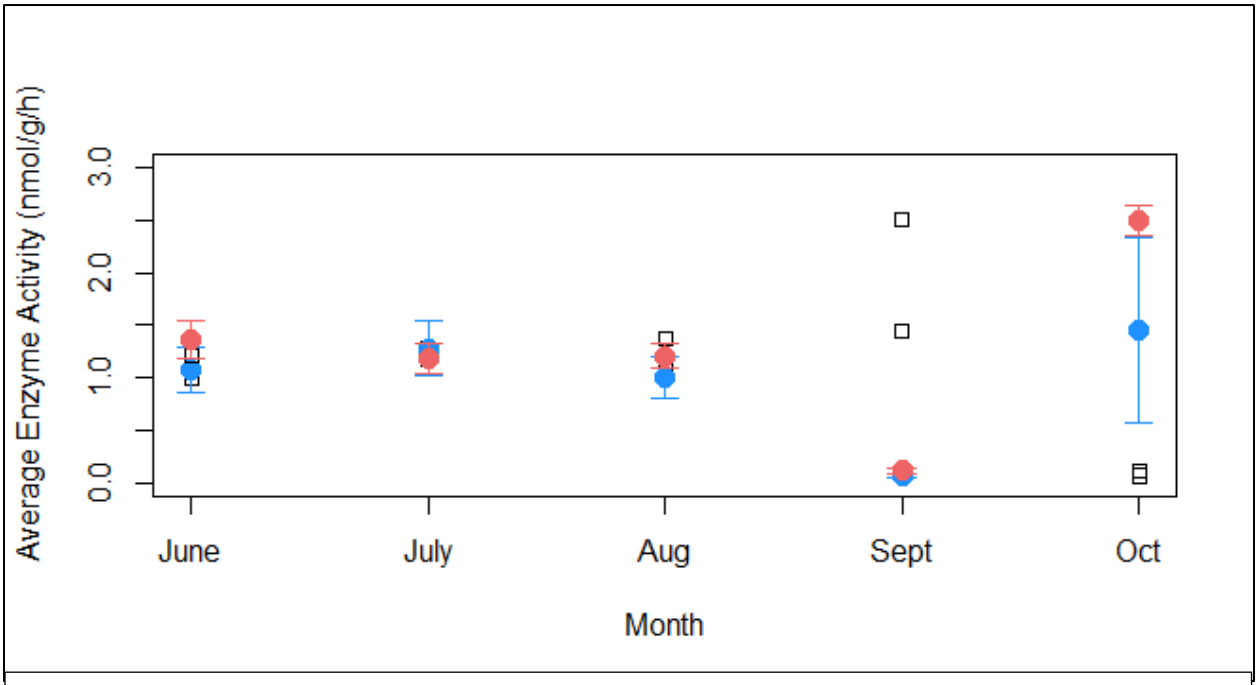

Fig. 10 Average Peroxidase Activity in AB Horizon Soil. Mean activity of peroxidase is not significant for any of the five months of sampling based on a 0.1 alpha level, where n=10 per month.

Appendices

Appendix I: Plate Maps

Table 1. Hydrolytic Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
	Soil 1 quench	Soil 1 Assay		Soil2 quench	Soil 2 Assay		Soil3 quench	Soil 3 Assay		Soil4 quench	Soil 4 Assay	
	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry
	50 µL MUB			50 µL MUB			50 µL MUB			50 µL MUB		
		50 µL sub	50 µL sub		50 µL sub	50 µL sub		50 µL sub	50 µL sub		50 µL sub	50 µL sub
A												
B												
C												
D												
E												
F												
G												
H												

Table 2. Oxidative Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
	Blank	Ref Std	Empty	Empty	Soil 1 Assay		Soil 2 Assay		Soil 3 Assay		Soil 4 Assay	
	1000 µL Buff	500 µL Buff										
					500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry
		500 µL DOPA			500 µL DOPA	500 µL DOPA	500 µL DOPA	500 µL DOPA	500 µL DOPA	500 µL DOPA	500 µL DOPA	500 µL DOPA
A												
B												
C												
D												
E												
F												
G												
H												

Table 3. Hydrolytic Substrate Blank Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
	50µL MUB	BG Substrate		50µL MUB	CBH Substrate		50µL MUB	NAG Substrate		50µL MUB	No Substrate (Empty	
	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer	200 µL Buffer
	50 µL MUB			50 µL MUB			50 µL MUB					
		50 µL BG sub	50 µL BG sub		50 µL CBH sub	50 µL CBH sub		50 µL NAG sub	50 µL NAG sub			
A												
B												
C												
D												
E												
F												
G												
H												

Table 4. Hydrolytic Sample Blank Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
	3.PH.DC.O	1.PH.H.O	5.PH.DC.O	6.PH.H.O	9.PH.DC.O	8.PH.H.O	10.PH.DC.O	12.PH.H.O	13.PH.DC.O	15.PH.H.O	17.PH.DC.O	16.PH.H.O
	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry	200 µL slurry
	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer	50 µL buffer
A												
B												
C												
D												
E												
F												
G												
H												

Table 5. Oxidative Sample Blank Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
	3.PH.DC.O	1.PH.H.O	5.PH.DC.O	6.PH.H.O	9.PH.DC.O	8.PH.H.O	10.PH.DC.O	12.PH.H.O	13.PH.DC.O	15.PH.H.O	17.PH.DC.O	16.PH.H.O
	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry	500 µL slurry
	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer	500 µL buffer
A												
B												
C												
D												
E												
F												
G												
H												

Table 6. Hydrolytic Standard Plate Map

MUB standards

	1	2	3	4	5	6	7	8	9	10	11	12
	Blanks		Standards					Specs				
			1 µM	3 µM	5 µM	7 µM	10 µM	0.5 µM	2 µM	4 µM	6 µM	8 µM
A												
B												
C												
D												
E												
F												
G												
H												

Appendix II: Linear Regression Analysis

Table 7. Summary Statistics for Linear Regression. Table provides data summary for linear regression between enzyme activity and the variables of soil moisture, soil organic matter, and soil pH. Both adjusted R-squared values and p-values are listed. Statistics are based on an alpha level of 0.1, where n=50 per soil horizon.

Enzyme	Soil Horizon	Variable	Adjusted R^2	p-value
BG	O	Moisture	-0.08757	0.4521
BG	O	SOM	0.03055	0.1173*
BG	O	pH	0.02862	0.1246*
BG	AB	Moisture	0.04857	0.06742*
BG	AB	SOM	0.02619	0.1345*
BG	AB	pH	-0.008261	0.4429
CBH	O	Moisture	-0.007662	0.4322
CBH	O	SOM	-0.01881	0.7591
CBH	O	pH	-0.01182	0.5162
CBH	AB	Moisture	0.03078	0.1164*
CBH	AB	SOM	0.04459	0.07608*
CBH	AB	pH	-0.01804	0.7185
NAG	O	Moisture	-0.01263	0.5358
NAG	O	SOM	-0.01842	0.7376
NAG	O	pH	-0.01731	0.6854
NAG	AB	Moisture	-0.01177	0.5151
NAG	AB	SOM	-0.003651	0.3692
NAG	AB	pH	-0.006978	0.4204
PhenOx	O	Moisture	-0.01927	0.7872
PhenOx	O	SOM	-0.01197	0.5197
PhenOx	O	pH	0.03567	0.1
PhenOx	AB	Moisture	-0.01008	0.4782
PhenOx	AB	SOM	0.004796	0.2718
PhenOx	AB	pH	-0.02046	0.8949
PerOx	O	Moisture	-0.0007476	0.3313
PerOx	O	SOM	0.1514	0.003043**
PerOx	O	pH	0.1621	0.002194**
PerOx	AB	Moisture	0.0377	0.09397*
PerOx	AB	SOM	0.1511	0.003073**
PerOx	AB	pH	0.2657	7.6e-05**