Short and Long-Term Impact of Wildfires on Soil Enzyme Activities of the Northern Boreal Forest

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ABSTRACT

Soil microbes have an important role in the global carbon cycle. They contribute to the degradation of plant material and emit CO_2 fixed by plants back to the atmosphere. The activity of extracellular enzymes, that allow them to decompose organic plant material, may vary depending on the conditions of the soil environment. Wildfires, for example, change the soil structure and chemistry. Moreover, in places such as the northern boreal forests they are expected to increase in frequency and severity due to global climate change. This study aims to measure the activity of several enzymes involved in degradation of plant material; namely cellulases, oxidases and chitinases, in soils affected by wildfires in Alaskan boreal forests. Top soils from five wildfire and control locations were collected in July and September 2010. Activity of enzymes were tested on the basis of their ability to cleavage of a model substrate (i.e. 4-methylumbelliferone) or degrade a dedicated substrate such as L-3,4-dihydroxyphenylalanine. Overall, the control soils had the highest amount of activity of the cellulases, compared to corresponding post-fire sites in most of the sites tested. These enzymes also had lower activity in soils from recent wildfire events compared to their activity in soils from earlier wildfire events. Phenol oxidase followed this trend, however in some cases the activity of phenol peroxidase diverged. The chitinase activity differed depending on the location from which the sample was collected. The overall enzymatic activity differed depending the season and location from which it was collected. These results indicate that an aspect of wildfire is suppressing the activity of cellulases and oxidases in the soil, and the magnitude of the effect is highly variable, depending on the soil's location and season of origin.

INTRODUCTION

Extracellular enzymes involved in biodegradation of natural polymers (e.g. cellulose, lignin, chitin), play an important role in soil microbial metabolism and the global carbon cycle because they allow microorganisms to decompose organic plant material and respire, releasing CO_2 back into the atmosphere [1].This enzyme-mediated decomposition is crucial to nutrient cycling, soil structure maintenance, biological population regulation, and plant growth. These enzymatic activities are potentially highly variable to the conditions of the soil [1].

Wildfires change the carbon balance of soil [11]. An average wildfire leads to approximately 1,000-2,000 g C/m^2 released as CO_2 [8]. As global climate change progresses, fires are projected to increase in frequency and severity in many areas including the northern boreal forests. This environment is critical to the carbon cycle as it represents 30% of the world's forested area and makes up an estimated 12-42% of the global soil carbon pool [5]. Boreal ecosystems are fire-adapted, however, increased fire frequency implies a shift in the age structure of the vegetation and recovery [12]. Besides fire-mediated changes in soil and vegetation structure, changes in microbial community structure are likely to have consequences for nutrient cycling and related processes. In this study, comparisons of microbial enzymatic activities in Tanana Valley Forest and Donnelly Flats, Alaska that has been exposed to a wildfire at different years in the past will reveal its possible short and long term effects on decomposition.

This study focuses on determining the effects of wildfires on the activity of the enzymes β-1,4-glucosidase (BG), β-D-1,4-cellobiosidase (CBH), β-1,4-N-acetyl-glucosaminidase (NAG), β-1,4-xylosidase (XYL), phenol oxidase (PO), and peroxidase (POX). These enzymes are involved in degradation of different components of plant material, namely wood (Table 1). Previous findings on rates of lignin degradation in post-fire boreal soil suggest that compared to pre-fire conditions, lower levels of lignin degradation associated with lower levels of phenol oxidase activity can be observed [9]. Based on this research, we hypothesized that there will less enzymatic activity in samples that have

been exposed to wildfire than in corresponding control soils and the soils exposed to a recent fire will in turn have a lower activity than soils exposed to wildfire earlier. For each type of enzyme in each separate environment we looked for answers to the following questions:

- How different are the enzymatic activities of samples from their respective controls?
- Which enzymatic activities have the largest/smallest differences?
- Are the differences between samples and control activities similar between the seasons?
- After a wildfire event, how long does it take for the enzymatic activities to return to the same rate as the control?

Table 1: Enzyme activities measured, their functions, and substrates used in their assays.

Enzyme	Function	Substrate
β-1,4-glucosidase (BG)	Hydrolyzes terminal, non reducing 1,4-β-D-glucose residues from short chain cellulose oligomers, releasing β-D-glucose	4-methylumbelliferone (MUB)-β-D-glucoside
β-D-1,4-cellobiosidase (CBH)	Hydrolyzes 1,4-β-D-glucosidic linkages in cellulose and cellotetraose, releasing cellobiose	4-MUB-β-D-cellobioside
β-1,4-N-acetyl-glucosaminidase (NAG)	Hydrolyzes N-acetyl-β-D-glucosaminide 1,4-β-linkages in chitin and chitodextrin	4-MUB-N-acetyl-β-D-glycosaminide
β-1,4-xylosidase (XYL)	Degrades short xylan (plant structural polymer) chains into xylose	4-MUB-β-D-xyloside
phenol oxidase (PO)	Oxidizes benzenediols (an aromatic carbon compound with two hydroxy groups substituted to the benzene ring) to semiquinones (the resulting free radical) with O_2.	L-3,4-dihydroxyphenylalanine (L-DOPA)
peroxidase (POX)	Reduces H_2O_2 to catalyze oxidation reactions	L-3,4-dihydroxyphenylalanine (L-DOPA)

MATERIALS AND METHODS

Study Sites

To study wildfire's effect on enzyme activity, samples were collected in two sampling campaigns in July and September 2010 from two different regions in the upland boreal forests of Alaska. In the first region, Donnelly Flats (638559 N, 1458449 W), top soils of four different locations that were burned in 1956, 1987, 1999, and control location were sampled. In the second location, Tanana Valley Forest, three different soils were collected from control and locations that were burned in

2004 and 2010.

Table 2: Measures of percent carbon, moisture content, soil pH, and fungal to bacteria ratio in Donnelly Flats samples.

	July-C (%)	Sept-C (%)	July-Moisture Content (%)	Sept-Moisture Content (%)	Soil pH	Fungal/bacteria ratio
Control	22.8 ± 3.3	25.2 ± 2.0	49 ± 6.0	61 ± 5.0	5.05	July: 0.51 September: 0.84
1956 Burn	15.8 ± 2.3		48 ± 2.0			July: 0.62
1987 Burn	12.9 ± 5.2	5.4 ± 1.6	34 ± 3.0	33 ± 2.0	5.1	July: 0.55 September: 0.70
1999 Burn	12.9 ± 2.1	8.1 ± 2.3	33 ± 2.0	34 ± 1.0	5.05	July: 0.57 September: 0.58

Table 3: Measures of percent carbon, moisture content, soil pH, and fungal to bacteria ratio in Tanana Valley Forest samples.

	July-C (%)	Sept-C (%)	July-Moisture Content (%)	Sept-Moisture Content (%)	Soil pH	Fungal/bacteria ratio
Control	23.6 ± 3.9	22.9 ± 2.5	47 ± 1.0	64 ± 2.0	5.03	July: 0.96 September: 0.64
2004 Burn	8.2 ± 1.2	11.5 ± 1.9	34 ± 2.0	41 ± 4.0	5.04	July: 0.53 September: 0.55
2010 Burn	25.3 ± 1.0	14.1 ± 4.3	45 ± 4.0	52 ± 6.0	5.2	July: 0.89 September: 0.48

Buffer and Substrate Preparation

Soil extraction buffer contained 50 mM sodium acetate and 25 mM EDTA at pH =5.0 and stored at room temperature. Fresh buffer was prepared every week. When testing the enzymatic activity of the enzymes BG, CBH, NAG, and XYL, 4-methylumbelliferone (MUB)-linked model substrate was used (Table 1). For these experiments, 100 μM stock solution of each substrate was prepared weekly and stored at 4 °C covered from the light. Also, in order to establish a standard curve for MUB-linked substrates, solutions of MUB at concentrations of 100 μM, 75 μM, 50 μM, 37.5 μM, 25 μM, 12.5 μM, 5 μM and 2.5 μM were made daily (Appendix A).

When testing the activity of PO and POX enzymes, L-3,4-dihydroxyphenylalanine (L-DOPA)

was used as the substrate. Twenty-five mM solution of L-DOPA was prepared in fresh extraction buffer. On the day of experimentation, a solution of 30% hydrogen peroxide was mixed and 50 mg/uL tyrosinase, a phenol oxidase, was used as positive control and standard for the reaction (Appendix B).

Sample Preparation

Each of the 13 Alaskan soil samples were frozen and stored in individual packets at -80 °C. Two gram of each soil sample was mixed and homogenized in the soil extraction buffer with a stir bar for eight minutes. For BG, CBH, NAG, and XYL detection, a dilution factor of 1/20 (g soil/mL buffer) was used. For PO and POX detection, a dilution factor of 1/25 (g soil/mL buffer) was used.

Experimental design

Assays of BG, CBH, NAG, and XYL were performed in 96-well flat bottom, black polystyrene Corning Assay Plates (costar 3915). Figure 1 summarizes the experimental layout. First, 200 µL of the 100 µM MUB-linked model substrate was added to the wells followed by 50 µL of homogenized soil slurry. Each individual sample had 16 replicates. In order to create a standard curve, 200 µL of MUB at the concentrations listed above were added to wells followed by the addition of 50 µL of the same sample. Each concentration of MUB had three replicates. Finally, to account for background fluorescence, 200 µL of extraction buffer was added to wells followed by 50 µL of sample. Soil background fluorescence was measured with eight replicates per sample. Each plate only contained one type of MUB-linked substrate and so a total of four different plates were made in this way to measure the activity of two different soils at a time.

Next, within the first few minutes of the sample addition, the plates were centrifuged at 3000 rpm for 90 seconds and then the fluorescence emitted was measured using a Molecular Devices Spectra Max Gemini XPS fluorometric spectrometer. The fluorometric spectrometer excites the samples with 365 nm light and reads the amount of light emitted at 460 nm. After each reading, the solutions were mixed with a pipette and allowed to react at room temperature, covered from light, for 15 minutes. This process was repeated every 15 minutes for two hours to produce an activity curve for each enzyme in

each soil sample.

<table>
<tr><th></th><th colspan="2">1</th><th colspan="2">3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>a</td><td colspan="2" rowspan="8">200 µL of 100 µM MUB-linked substrate + 50 µL soil sample</td><td colspan="4">100 µM MUB + 50 µL soil sample</td><td rowspan="8">200 µL sodium acetate buffer + 50 µL soil sample</td><td colspan="6" rowspan="8">Same as columns 1-6 except with a different soil sample.</td></tr>
<tr><td>b</td><td colspan="4">75 µM MUB + 50 µL soil sample</td></tr>
<tr><td>c</td><td colspan="4">50 µM MUB + 50 µL soil sample</td></tr>
<tr><td>d</td><td colspan="4">37.5 µM MUB + 50 µL soil sample</td></tr>
<tr><td>e</td><td colspan="4">25 µM MUB + 50 µL soil sample</td></tr>
<tr><td>f</td><td colspan="4">12.5 µM MUB + 50 µL soil sample</td></tr>
<tr><td>g</td><td colspan="4">5 µM MUB + 50 µL soil sample</td></tr>
<tr><td>h</td><td colspan="4">2 µM MUB + 50 µL soil sample</td></tr>
</table>

Figure 1: BG, CBH, NAG, XYL assay plate design.

To test the activity of PO and POX enzymes, a slightly different procedure and plate design were used (Figure 2). PO activity was determined by adding 750 µL of 25 mM L-DOPA to the wells of 2 mL 96-well Corning polypropylene assay blocks (corning 3370) followed by 750 uL of soil slurry. Sixteen replicates per sample were prepared. Next, 750 µL of buffer as well as 750 µL of soil sample were added to four replicate wells to account for any background. In two wells, besides L-DOPA and sample, 50 µL of 50 mg/µL tyrosinase was added to serve as standard. Also, another two wells contained 750 µL of L-DOPA, 750 µL of buffer, and 50 µL of tyrosinase to serve as reaction (positive) control. Similar set-up with the addition of 15 µL of 30% H_2O_2 was used for POX detection. The difference between the activity in the wells with the added H_2O_2 and without H_2O_2 describes the activity of POX.

Immediately after sample addition, the assay blocks were centrifuged at 3000 rpm for 90 seconds to separate the soil chunks from solution. One hundred µL of the upper phase was transferred

from the assay block to 96-well Corning (corning 3370) reading plate and color development was then detected by a Molecular Devices Spectra Max 340 PC colorimetric spectrometer. After each reading, solutions in the assay block were mixed with a pipette and allowed to react, covered from light, for 15 minutes. This process was repeated every 15 minutes for two hours and once after the third hour to produce an activity curve for each enzyme in each soil sample.

<table>
<tr><th></th><th colspan="2">1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>a</td><td colspan="2" rowspan="8">750 µL L-DOPA + 750 µL soil sample</td><td rowspan="4">750 µL buffer + 750 µL soil sample</td><td colspan="3" rowspan="8">Same as columns 1-3 plus the addition of 15 µL of 30% H_2O_2</td><td colspan="6" rowspan="8">Same as columns 1-6 except for a different soil sample can be used.</td></tr>
<tr><td>b</td></tr>
<tr><td>c</td></tr>
<tr><td>d</td></tr>
<tr><td>e</td><td rowspan="2">750 µL L-DOPA + 750 µL soil sample + 50 µL tyrosinase</td></tr>
<tr><td>f</td></tr>
<tr><td>g</td><td rowspan="2">750 µL L-DOPA + 750 µL buffer+ 50 µL tyrosinase</td></tr>
<tr><td>h</td></tr>
</table>

Figure 2: PO and POX assay plate design.

CALCULATIONS

To calculate the concentration of converted substrate (µM) at each time point a for an MUB-linked substrate assay (1):

(1) (sample average at time point A*slope of MUB standard curve at time point A+ y-intercept of MUB standard curve at time point A)- background concentration at time point A

To calculate uM of converted substrate/g soil* hour or relative absorbency/ g soil*hour:

- Choose a time point y at the start of the observed activity and a time point x at the end of the activity .
- Use points to calculate the slope (2):

(2) ((µM at time point x)-(µM at time point y))/((time point x)-(time point y))

To calculate the relative absorbency of enzymatic reaction at each time point a for colorimetric assays with L-DOPA (3):

(3) (absorbency at time point A)-(background absorbency at time point A)
To calculate ratio of enzymatic activity of soils exposed to wildfire compared to control soils (4):

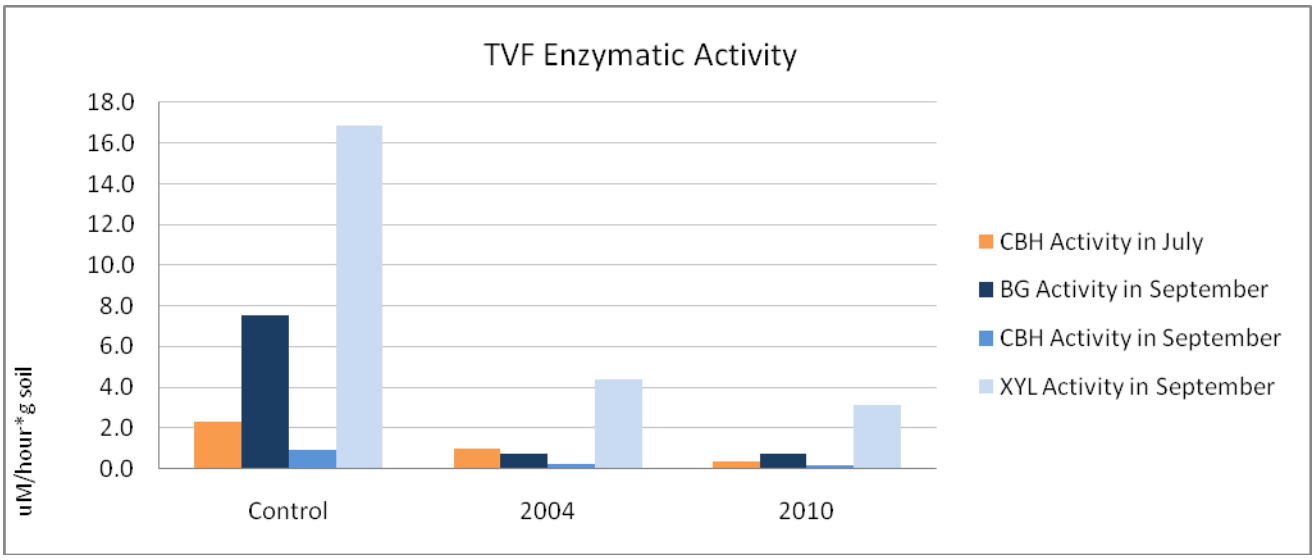
(4) (Points calculated in (2) that represent wildfire samples)-(points calculated in (2) that represent control samples)

RESULTS

Tanana Valley Forest (TVF)

Majority of the enzymes had lower activity in fire affected locations than in controls (Figure 3). However, some notable differences were also observed. For example the activity of POX in July samples (Figure 4a), a loss in activity was observed only in 2010 fire location. Conversely, in September a higher POX activity was detected for 2004 fire location (Figure 4b). For enzymes BG, XYL (both in soil collected in July) and POX (in soil collected in September), control and soils exposed to wildfire in 2010 exhibited lower activities compared to soil exposed to wildfire in 2004 (Figure 5). Remarkably, the activity of NAG, responsible for the enzymatic degradation of chitin, peaked in soil exposed to wildfire in 2010 while the control and 2004 wildfire soils both exhibited lower activities (Figure 6).

(a)



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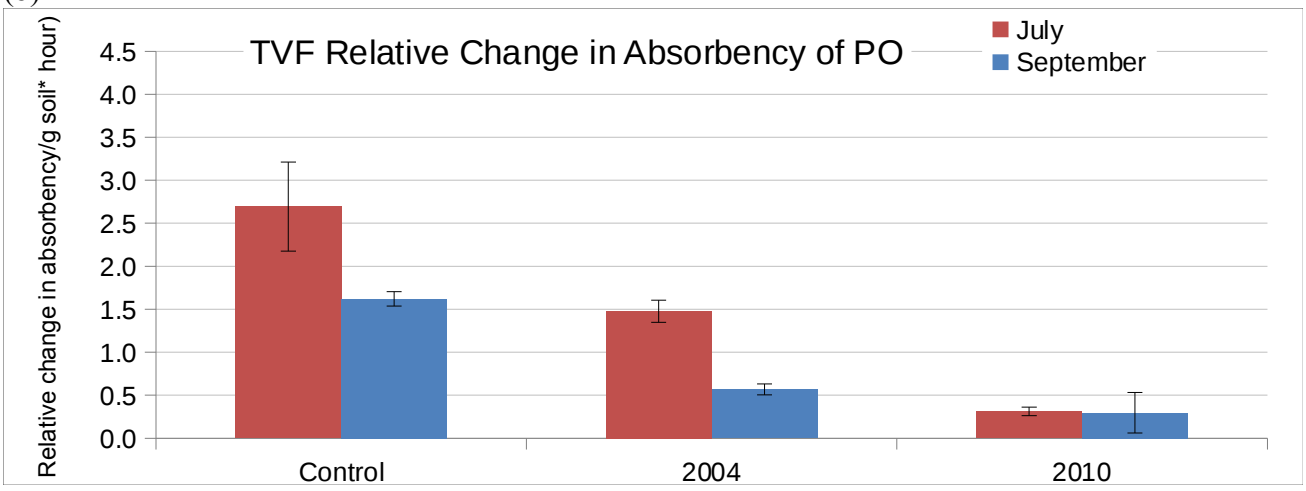
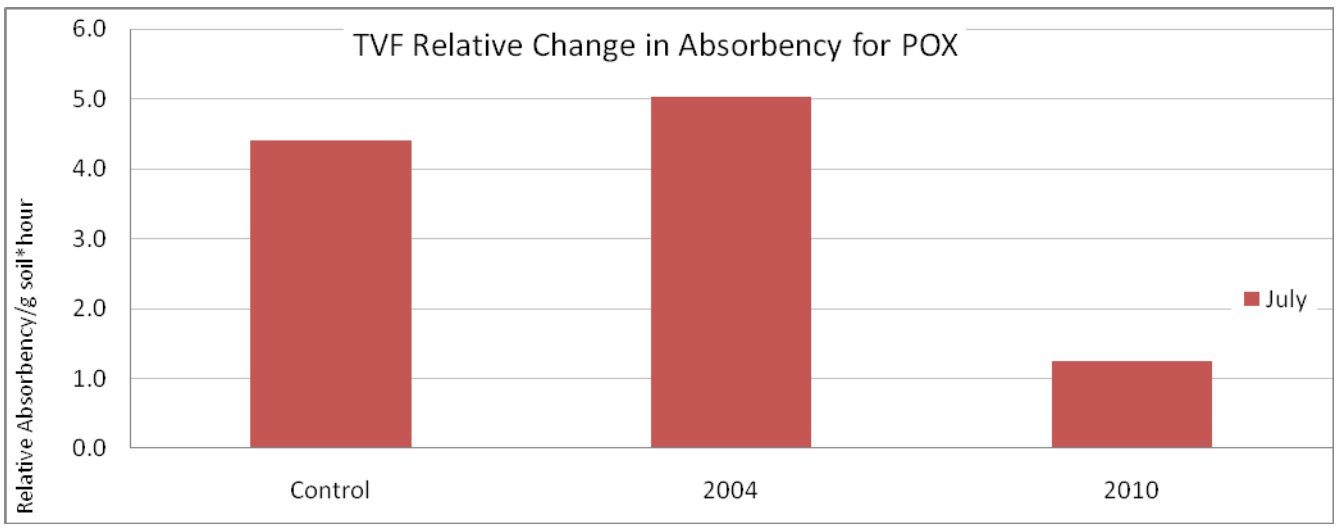


Figure 3: (a). The enzymatic activity of CBH (in July soil) and BG, CBH, and XYL (in September soil) calculated in uM/hour*g soil. (b). The enzymatic activity of PO in soil from both seasons calculated in relative change in absorbency/g soil*hour. Both of these represent a decreasing trend in activity in post-fire soils.

(a)



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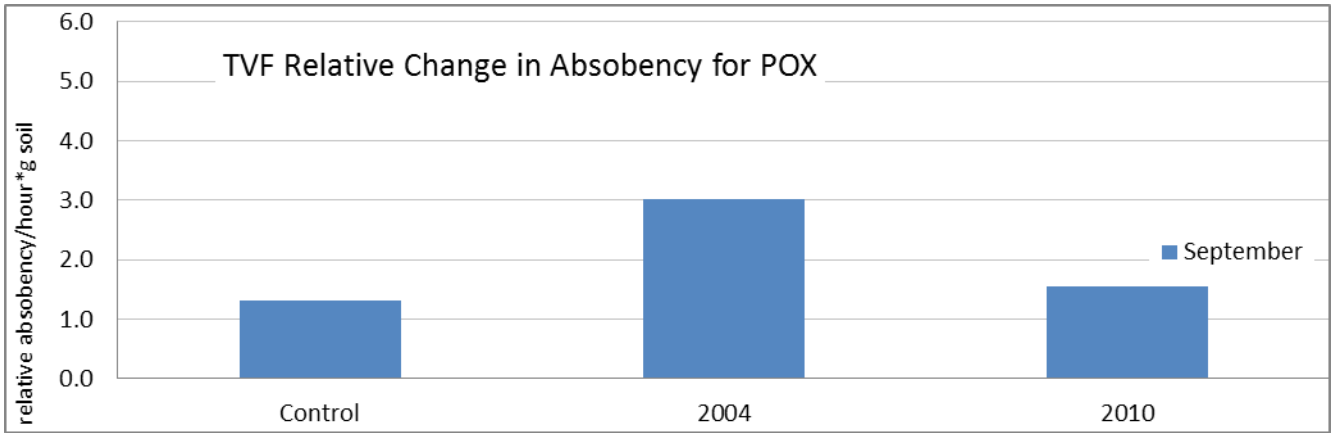


Figure 4: (a). The enzymatic activity of POX in July soil calculated in relative absorbency/g soil*hour. This graph illustrates consistent activity in both control and soil exposed to wildfire in 2004 with a decrease in activity in soil exposed to wildfire in 2010. (b) The enzymatic activity of POX in September soil calculated in relative absorbency/g soil* hour.

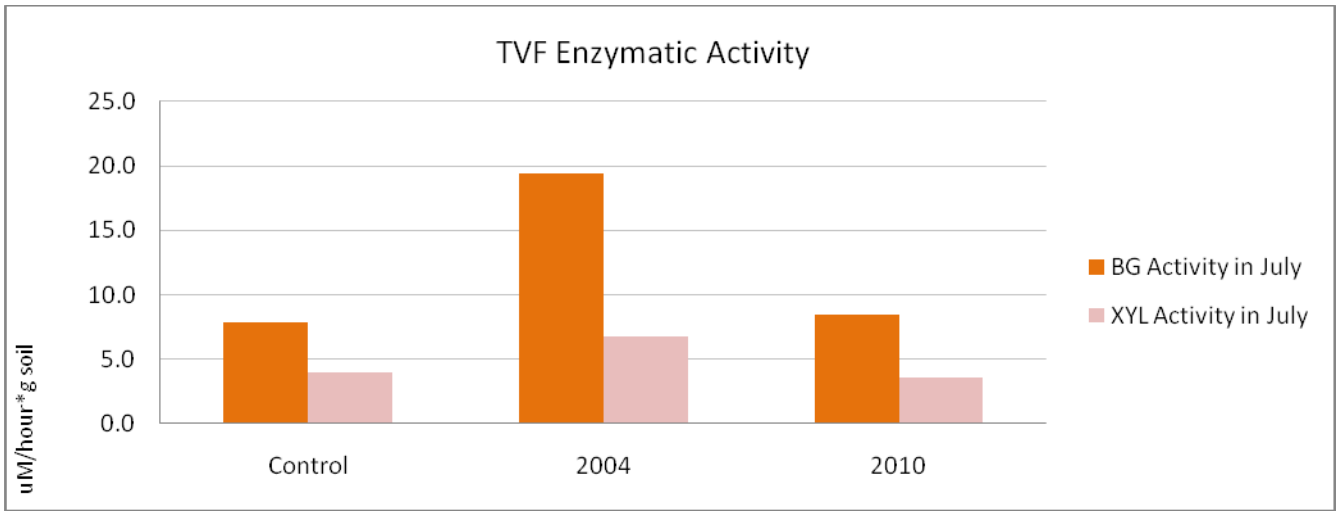


Figure 5: The enzymatic activity of BG and XYL in soils from July calculated in uM/hour*g soil. This graph shows the activity peaking in 2004.

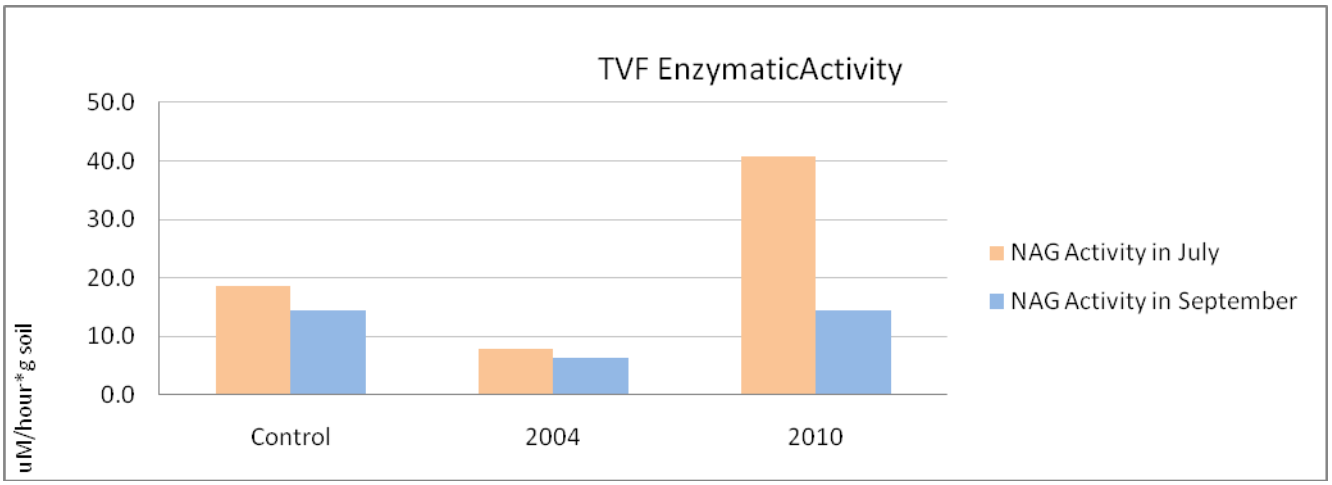


Figure 6: The enzymatic activity of NAG in soil from both seasons. This graph illustrates the peak in activity in soil exposed to wildfire in 2010.

In summary, among enzymes that degrade (hemi) cellulose (BG, CBH, and XYL), activity –as the ratio of enzymatic activity in soils exposed to wildfire to the activity in control soils- was higher in the summer months (Table 4). This effect was stronger in 2004 fire location than 2010 location. One notable observation was the high chitin related (NAG) activity in both seasons of the 2010 wildfire location. Among enzymes that degrade lignin, where POX activities increased in fire affected location, PO activities decreased (Table 5).

Table 4: The ratio of enzymatic activity of soils exposed to wildfire to compared control soils (uM/g soil* hour) in TVF for enzymes BG, CBH, NAG, and XYL.

Ratio of Enzymatic Activity of Soils Exposed to Wildfire to Control Soils
Measured in uM/g soil*hour

Soil Exposed to Wildfire in 2004		
	July	September
BG	2.5	0.1
CBH	0.4	0.2
NAG	0.4	0.4
XYL	1.7	0.3
Soil Exposed to Wildfire in 2010		
	July	September
BG	1.1	0.1
CBH	0.2	0.2
NAG	2.2	1.0
XYL	0.9	0.2

Table 5: The ratio of enzymatic activity of soils exposed to wildfire compared to control soils (relative absorbency/ g soil*hour) in TVF for enzymes PO and POX.

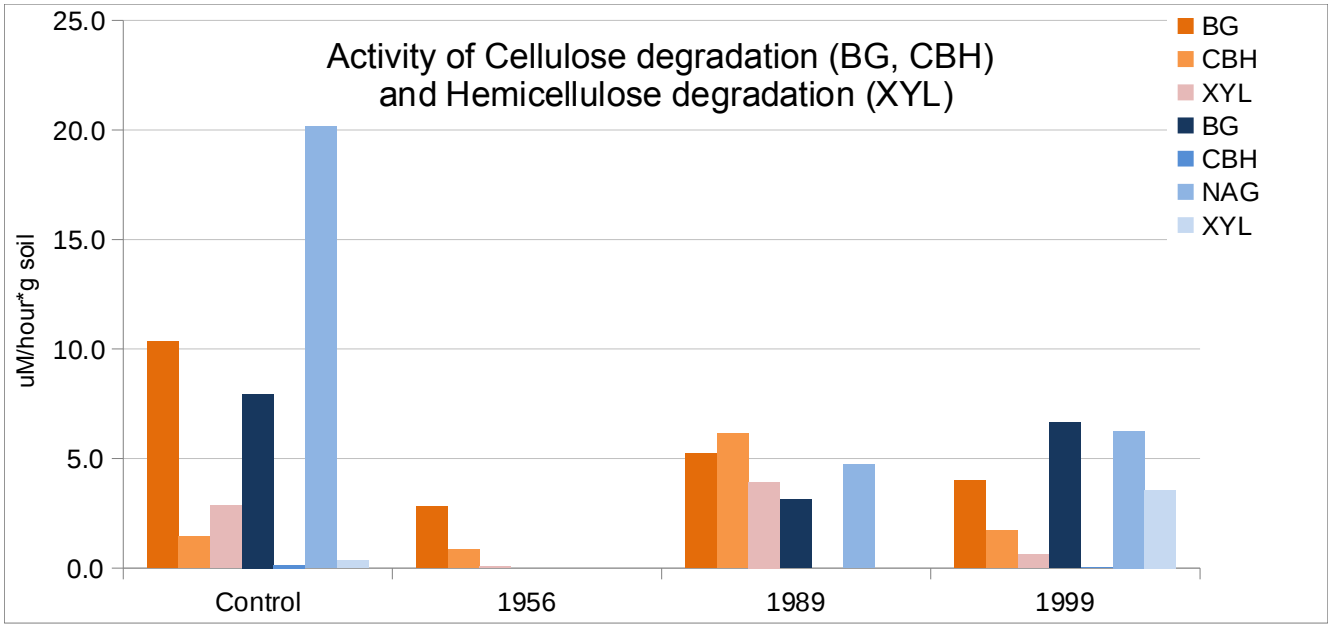
Ratio of Enzymatic Activity of Soils Exposed to Wildfire Compared to Control Soils Measured in relative absorbency/g soil*hour		
Soils Exposed to Wildfire in 2004		
	July	September
PO	0.5	0.3
POX	1.1	2.3
Soils Exposed to Wildfire in 2010		
	July	September
PO	0.1	0.2
POX	0.3	1.2

Donnelly Flats (DF)

In DF soils, enzymes showed similar activity trends to the ones of TVF. The majority of the enzymes decrease in activity compared to the controls. The control had the highest amount of activity of BG (in July and September soil) and CBH (in September soil) compared to the corresponding enzymatic activity in wildfire soils (Figure 7a). Unlike TVF soils, the control had the highest amount of activity of NAG, responsible for chitin degradation, in September soil (Figure 7b). Overall, NAG activity in soil from both seasons exposed to wildfire in 1987 and 1999 was much lower than the control and soil exposed to wildfire in 1956.

In DF September soil, the control had the highest amount of activity of PO compared to corresponding activities in wildfire affected soils. The lowest amount of activity in soil from both seasons was in soil that had been exposed to wildfire in 1999 compared to all other samples. In July soil, the highest amount of activity of POX was in 1987 compared to all other samples while the lowest amount of activity was in both the control and soil exposed to wildfire in 1956. There was no clear change in the activity of POX in corresponding September soils.

(a)



(b)

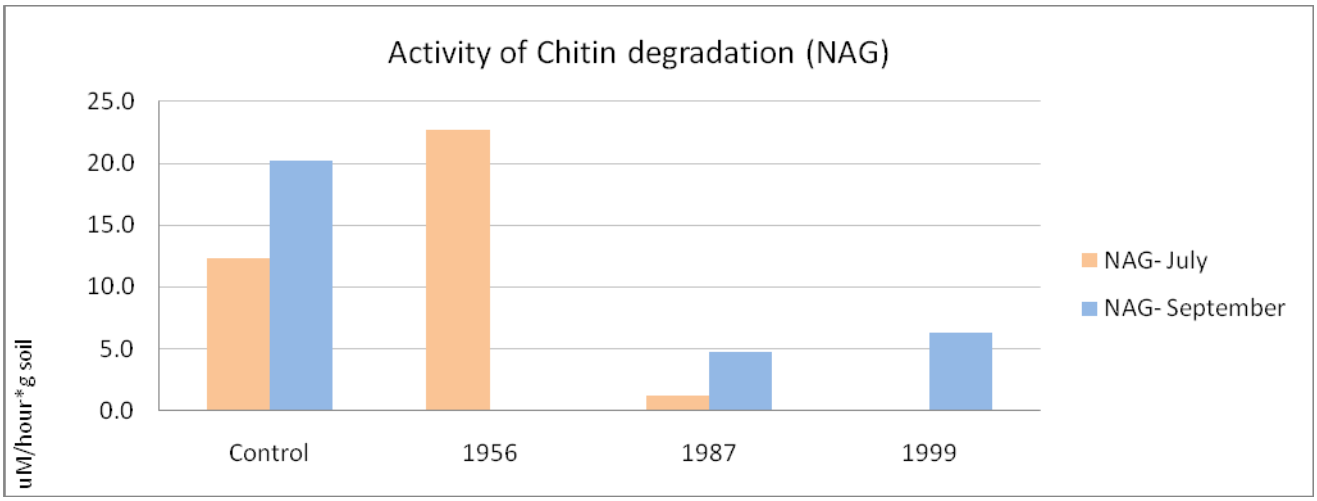
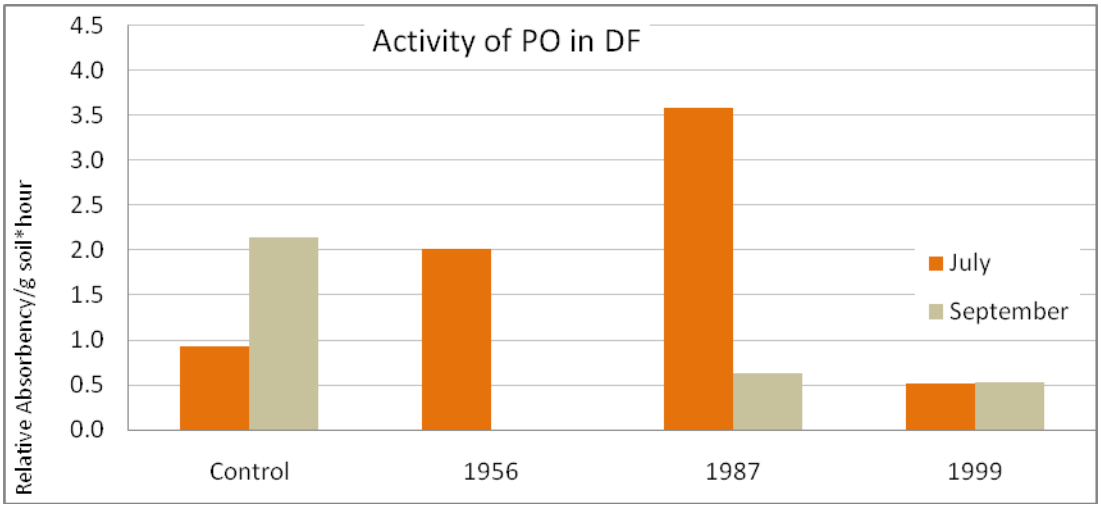


Figure 7: (a). The enzymatic activity of BG, CBH and XYL from soil in DF from both seasons. (b). The enzymatic activity of NAG from soil in DF from both seasons.

(a)



(b)

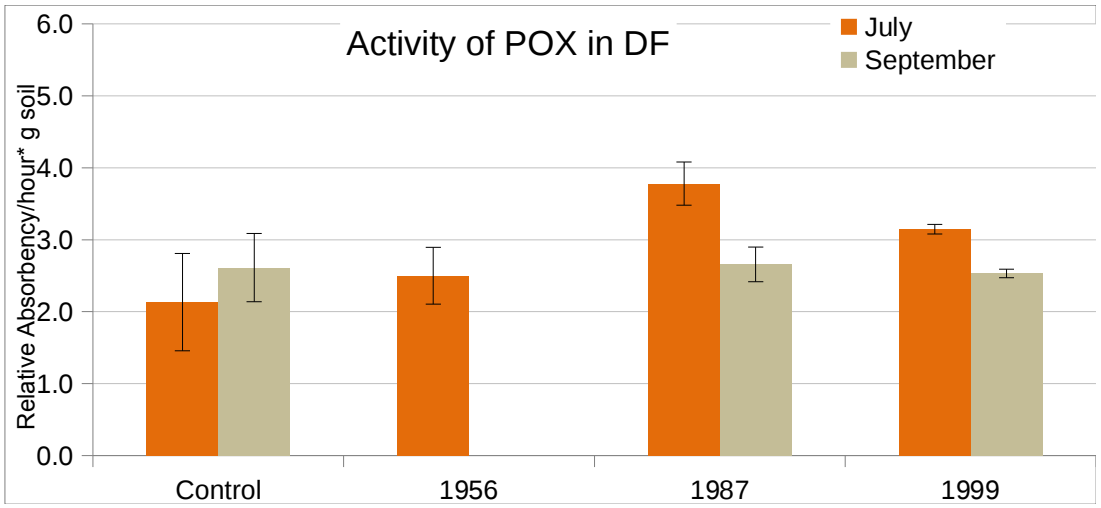


Figure 8: (a). The enzymatic activity of PO in soil from both seasons in DF. (b). The enzymatic activity of POX in soil from both seasons in DF. The activities in both of these graphs are calculated in relative absorbency/g soil* hour.

Briefly, for the enzymes involved in (hemi)cellulose (BG, CBH and XYL) degradation no clear trend observed between different sampling seasons (Table 6a).Among the enzymes that degrade lignin (PO and POX), the ratio of PO activity in wildfire affected soil to activity in control soil decreases sequentially as the wildfire event becomes more recent.

Comparisons of the ratio of enzymatic activity in wildfire affected soil to activity in control soil between sites are complicated by the time difference in exposure to wildfire and the difference in location. Lower ratios in the DF site compared to the TVF site are not observed as expected.

(a)

Ratio of Enzymatic Activity of Soils Exposed to Wildfire Compared to Control Soils
Measured in uM/g soil*hour

Soil exposed to wildfire in 1956		
	July	September
BG	0.3	
CBH	0.6	
NAG	1.9	
XYL	0.0	
Soil exposed to wildfire in 1987		
	July	September
BG	0.5	0.4
CBH	4.2	0.0
NAG	0.1	0.2
XYL	1.4	0.0
Soil exposed to wildfire in 1999		
	July	September
BG	0.4	0.8
CBH	1.2	0.1
NAG	0.0	0.3
XYL	0.2	10.6

(b)

Ratio of Enzymatic Activity of Soils Exposed to Wildfire Compared to Control Soils
Measured in relative absorbency/g soil *hour

Soils Exposed to Wildfire in 1956		
	July	September
PO	2.2	
POX	1.2	
Soils Exposed to Wildfire in 1987		
	July	September
PO	3.9	0.3
POX	1.8	1.0
Soils Exposed to Wildfire in 1999		
	July	September
PO	0.6	0.2
POX	1.5	1.0

Table 6: (a). The ratio of the enzymatic activity of BG, CBH, NAG, and XYL in soils exposed to wildfire in DF during the years 1956, 1987, and 1999 compared to control soils (uM/g soil*hour). (b). The ratio of enzymatic activity of PO and POX in soils exposed to wildfire in DF during the years 1956, 1987 and 1999 compared to control soils (relative absorbency/g soil*hour).

DISCUSSION

Generally, our data supports our hypothesis that the sites exposed to wildfire would have a lower enzymatic activity for the enzymes involved in plant material degradation. In most cases, the activity of the enzymes in the soils collected in July were higher than that of corresponding soils collected in September, suggesting that increase in temperature might play a part in the amount of activity of the enzymes studied.

Cellulases

For the enzymes that degrade cellulose (BG and CBH) and hemicellulose (XYL), the control had the most activity in both TVF and DF sites. Additionally, for the BG activity in September, XYL activity in July, and the CBH activity in both season in TVF, each sample from the 2010 wildfire event had a lower activity than the sample from the 2004 wildfire event. This suggests that wildfires are suppressing the activity of these enzymes in the soil, and that soils from more recent wildfire events have more of a reduction in enzymatic activity. This also suggests that it might take an extended period of time for the activity of these enzymes to recover to pre-fire activity amounts. In DF, for example, the activity of these cellulases are much lower in soil exposed to fire in 1956 than in the control soils, indicating that full recovery might take longer than 54 years. An exception to this trend was the activity of BG and XYL in July soil from TVF. While the activity in the July soil from a 2004 wildfire is higher for these enzymes than the activity in the control, there is still an observed decrease in the activity of the soil from the 2010 wildfire event. At this point it is tempting to speculate that fire events effect every location differently where activity of the enzymes detected are linked to type and metabolic capabilities of the microorganism that survive the event. Additionally, amount of sites and samples analyzed in this study is not sufficient enough to significantly detect site dependent differences.

Oxidases

In lignin degradation, the PO activity in both seasons in TVF and in September soil in DF showed highest amounts of activity in the control and a similar decreasing trend of activity from

earlier wildfire events to more recent wildfire events. This data supports the study by Waldrop and Harden [10] and our hypothesis. An exception to this trend was observed in the activity of PO in July soil of DF; however, it is important to note that this data does not completely conflict with our hypothesis. The lowest PO activity was still found in the sample from the most recent wildfire event--soil exposed to wildfire in 1999. In DF, the activity of POX in the July soil showed no real change between the control and the soil exposed to wildfire in 1956 and a decrease in activity from the soil exposed to wildfire in 1987 to the soil exposed to wildfire in 1999. This suggests that the activity of POX might be able to recover from wildfire after around 54 years of regrowth. The control does not contain the highest activity of POX for any of the samples in TVF or in DF, indicating that there might be some over expression of this enzyme as the soil recovers from a wildfire. Also, it is possible that fluctuations of POX activity in these locations vary by season. Though lignin degradation is usually associated with white-rot and brown rot fungus, the activity of PO and POX does not seem to correlate with high fungal/bacteria ratios in this soil (Table 2, 3). Nevertheless, recent publications have suggested that actinomycetes, α- proteobacteria and γ-proteobacteria may be responsible in large part for bacterial lignin degradation in the soil [10].

Chitinase

Measurements of NAG activity in TVF would suggest that wildfire is stimulating the activity, however, this observation conflicts with the measured activity of NAG in DF. NAG activity appears to be highly sensitive to location and an overall trend cannot be discerned from this data.

Future Work

As this study functions as a broad survey of the activity of enzymes important in key decomposition pathways in various wildfire affected and unaffected sites, it is difficult to identify what are the exact causes of these observed changes in activity. Future work would include not only repeated trails of the experiments performed in this study to insure more confidence in the results, but also trials that target elements of post-fire soil (such as BC-incubated soil,) to determine what is responsible for

these changes.

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APPENDIX

1. Appendix A: Protocol for Fluorometric Assays
2. Appendix B: Protocol for Colorimetric Assays

APPENDIX A:

Enzyme Activity Assay: beta- 1,4 glucosidase, beta-cellobiohydrolase, beta-xylosidase, and N-acetylglucosamidine

Goal: Measure the activity of enzymes by testing for amount of fluorescence over time when in presence of a MUB (4-methylumbelliferone) bound substrate. This, and other fluorometric enzyme assays, exploit the emission of 460nm light from fluorophore, a compound released when MUB-contain substrates are cleaved by their respective enzyme. This emission occurs when the molecule is excited by 365nm light.

The day before the experiment

1. Enzyme reaction buffer consists of sodium acetate (NaAc) and EDTA in ultra-pure Milli Q water. This buffer is required to keep the pH of the samples between 5 and 5.5 to mimic environmental conditions. Dissolve NaAc and EDTA, bring pH to approximately 5.0, adjust the volume and filter the finished buffer through 0.2ü filter.

Enzyme reaction Buffer	50 mM NaAc, 25 mM EDTA @ pH~5.0		
	NaAc	EDTA (0.5M)	Glacial Acetic acid
500 mL	2.05 g	25 mL	4.5 mL
1L	4.10 g	50 mL	10 mL

2. Create stock solutions of substrate by mixing 8.8mg MUB (1 mM), 1.69mg BG-MUB (0.1 mM), 2.50mg CBH-MUB (0.1 mM), 1.54mg XYL-MUB (0.1 mM) and 1.90mg NAG-MUB (0.1 mM) into 50mL of the above buffer solution.

in mg	MUB (1 mM)	BG	CBH	XYL	NAG
50 mL	8.81	1.69	2.50	1.54	1.90
100 mL	17.62	3.38	5.00	3.08	3.79
250 mL	44.04	8.46	12.51	7.71	9.48

3. Shake the substrate solutions in an incubator at 30°C for at least an hour to dissolve them. Then they can be kept for a week if they are covered from light and refrigerated at 4°C.

4. Weigh 2 g of each frozen soil solution into a container. If you don't have 2 g of sample to work with 1 g may be used with half the amount of buffer. Slightly crush the soil to avoid large clumps.

5. Make dilutions of the stock MUB substrate so that you have a stock of around 10 mL of MUB at 100, 75, 50, 37.5, 25, 12.5, 5, and 2.5 uM.

The experiment:

1. Add 200 uL of each diluted MUB solution to columns 3, 4, 5, 9, 10, and 11 of a Corning

Incorporated 96-well, flat bottom, non-treated, non-sterile assay plate. This will serve as the standard for the calibration curve.

2. Add 200 uL of substrate into columns 1, 2, 7, and 8. Every plate will have the same substrate and therefore will test activity for only one enzyme per plate. This particular experiment tests four enzymes and requires four different plates.

3. Mix each 2 g soil sample with 40 mL of sodium acetate buffer using a stir bar. (Alternatively, 1 g of soil with 20 mL of sodium acetate buffer.) Two samples may be assayed per plate. Allow 8 minutes for the sample to thoroughly mix.

4. Pipette 50 ul of the first soil solution into columns 1-6 and 50 uL of the second soil solution into columns 7-12. Mix soil slurry with the pipette before adding to the plate and add to soil to each plate consecutively to prevent large time differences between sample additions.

5. Centrifuge the wells for at 3000 rpm for 1.5 minutes.

6. Measure fluorescence of each well with a fluorometric spectrometer that excites the samples with 365 nm light and repeat readings after 15 minutes for the first 1.5 hours and then at the 2 hour mark and the 3 hour mark. After each reading it is necessary to mix each well with a pipette to insure homogeneity and then centrifuge before each reading to reduce interference from large particles. Between readings it is good practice to keep the samples covered from light.

APPENDIX B:

Protocol for Colorimetric Assay: Phenol Oxidase (PO) and Peroxidase (POX)

Goal: Measure the activity of enzymes by testing for amount of color change over time when in presence of L-3,4-dihydroxyphenylalanine (L-DOPA).

The day before the experiment:
1. Enzyme reaction buffer consists of sodium acetate (NaAc) and EDTA in ultra-pure Milli Q water. This buffer is required to keep the pH of the samples between 5 and 5.5 to mimic environmental conditions. Dissolve NaAc and EDTA, bring pH to approximately 5.0, adjust the volume and filter the finished buffer through 0.2ü filter.

Enzyme reaction Buffer	50 mM NaAc, 25 mM EDTA @ pH~5.0		
	NaAc	EDTA (0.5M)	Glacial Acetic acid
500 mL	2.05 g	25 mL	4.5 mL
1L	4.10 g	50 mL	10 mL

2. Create stock solutions of 25 mM L-DOPA by mixing 0.49 g in 100 mL of sodium acetate buffer. Mix this solution with a stir bar for at least 3 hours or until all the L-DOPA is mixed. Keep this solution covered from light using aluminum foil and a cap to cover the glass beaker.

3. Weigh 2 g of each frozen soil solution into a container. Slightly crush the soil to avoid large clumps.

The experiment:
1. Add 750 uL of L-DOPA substrate to all wells in the Corning Incorporated 2 mL, 96 well, square V-bottom, certified Rnase/DNase free, sterile, polypropylene assay block except for wells 3A-D, 6A-D, 9A-D, and 12A-D

2. Add 750 uL of buffer to wells 3A-D,G,H 6A-D,G, H, 9A-D,G, H, 12A-D, G, H.

3. Add 15 uL of 30% hydrogen peroxide to columns 7-12.

4. Combine 2 g of soil with 50 mL of sodium acetate buffer. Mix with a stir bar for 8 minutes.

5. Add 750 uL of soil slurry to all wells except 3G, H, 6G, H, 9G, H, and 12G, H. Record time of addition. Two soil samples may be assayed per plate. Add the first sample to columns 1-6 (note exception) and the second sample to columns 7-12 (note exception).

6. Add 50 uL of 50 mg/uL tyrosinase to wells 3E-H, 6E-H, 9E-H, and 12E-H.

7. Centrifuge assay block for 1.5 minutes at 3000 rpm.

8. Transfer 100 uL of the upper phase to a Corning Incorporated 96 well, flat bottom, non-treated, sterile, polystyrene assay plate with low evaporation lid.

9. Measure color change with a colorimetric spectrometer at 480 nm every 15 minutes for the first 1.5

hours and then at the 2 hour point and 3 hour point. After each reading it is necessary to mix each well in the assay block with a pipette to insure homogeneity and then centrifuge before transferring 100 uL to a new assay plate. Between readings it is good practice to keep the reaction covered from light.