AN EVALUATION OF SOIL EXTRACELLULAR ENZYME ACTIVITY IN RESPONSE TO BURNING AS A FOREST RESTORATION TECHNIQUE

by
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ABSTRACT

William Steward: An Evaluation of Soil Extracellular Enzyme Activity in Response to Burning As a Forest Restoration Technique

Burning is a method of forest restoration with the goal of returning natural tree species to ecosystems. Burning has a wide range of effects on an ecosystem, including the alteration of important soil processes and characteristics. One of the key components of soil is the activity of extracellular enzymes, which can be used to provide insight into the nutritional requirements of soil microbes as well as nutrient availability and cycling. Because enzymes are important in the overall functioning of soil and can be used to assess soil health, there is great need to examine the effects of burning on soil enzyme activity. The aim of this study was to assess differences in the activity of five enzymes (phosphatase, β-glucosidase, NAGase, phenol oxidase, and peroxidase) in response to historical burning, as compared to an unburned plot. Soil samples were collected from three sites (unburned, prescribed burn, and wildfire) in an upland forest of north Mississippi and assayed for enzyme activity. NAGase and phenol oxidase activity was higher in the burned plot, while phosphatase and β-glucosidase activity was lower, and peroxidase was generally unaffected. While enzyme activity is subject to change following burning, how certain enzymes respond is yet to be determined. However, the enzymes assayed in this study proved to be sensitive to fire, suggesting that soil enzyme activity can be used as a measure of soil quality or health during the restoration process.

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Introduction

Enzymes are specialized biological molecules that are produced and used by all organisms. Enzymes are vital to the existence and survival of organisms as they catalyze essential metabolic processes by both speeding up the rate of these reactions and specifying the molecules to be involved. Likewise, enzymes play a significant role in the function of the soil environment and are a key part of maintaining soil characteristics such as its ecology, physical and chemical properties, fertility, and health (Das & Varma, 2011). One of the most important processes in soil is the decomposition of organic matter, which is directly dependent on the activity of various enzymes produced by microorganisms. Microorganisms are found abundantly throughout soil environments and use enzymes for their essential life processes (Caldwell, 2005), often releasing these enzymes extracellularly. Soil microbial enzymes stabilize its structure, decompose organic waste, form new organic matter, and are involved in the cycling of soil nutrients (Das & Varma, 2011). While all soils contain specific enzymes that regulate its processes, the level of enzymes and their activities in soil environments varies greatly with differing organic matter content, composition, and the activity of living organisms (Burns, 1982). Enzymes degrade organic compounds found in soil extracellularly, and the calculation of the activity of these enzymes can provide understanding of requirements of soil microbes, as well as insight into nutrient availability (Burns, 1982). While

microorganisms may be the main producers of soil enzymes, they can be produced and released from a wide range of organisms, including plants, animals, and microbes.

Some of the enzymes that are particularly important in soils include those related to carbon and nutrient mineralization, such as phosphatase, β-glucosidase, β-N-acetylglucosaminidase (NAGase), phenol oxidase, and peroxidase. Phosphatase plays a crucial role in the phosphorous cycle and can be a good indicator of overall soil fertility (Das & Varma, 2011). Phosphatase hydrolyzes compounds containing organic phosphorous, releasing inorganic phosphate. This inorganic phosphate, an essential nutrient for organisms, can then be taken up and used by plants and microorganisms (Piotrowska-Długosz & Wilczewski, 2014). β-glucosidase is another widely prevalent enzyme in soil and catalyzes the hydrolysis of various glucose polymers. This enzyme is involved in the hydrolysis of cellulose, one of the most prominent compounds found in decomposing plant matter, into glucose, an important source of carbon for microbial growth (Das & Varma, 2011). NAGase is involved in the breakdown of the long-chain polymer chitin, which is found in the cell walls of fungi. Chitin degradation provides significant sources of nitrogen and carbon to soil microorganisms and plants, and is important to the cycling of carbon and nitrogen in soil (Zeglin et. al, 2013).

Phenol oxidase and peroxidase differ from the previously mentioned enzymes in that they are categorized as oxidoreductases rather than hydrolases, and are involved in the breakdown of lignin, a component of the cell wall of plants and one of the most abundant organic polymers on Earth. The degradation of lignin contributes to soil pools of carbon and nitrogen and provides microbes with these essential nutrients. Phenol

oxidase and peroxidase can both play a role in the detoxification of the soil as well, as they can help alleviate the toxicity of metal ions and phenolic molecules. Peroxidase also is able to help mitigate the harmful effects of reactive oxygen species that can accumulate in the soil (Sinsabaugh, 2010).

All of the enzymes mentioned above are important in soil function, and can also be used to assess soil health in the context of restoration efforts, such as those accomplished through burning. Although fire can be looked at as a destructive force, it is an innate occurrence that contributes vitally to the natural functioning and composition of ecosystems (Scott et. al, 2000). A wide range of fire-adapted plant communities once covered a large portion of the United States, where periodic, naturally occurring fires helped establish ecosystems that contained fire-dependent plant species and contributed to increased biodiversity (Nowacki & Abrams, 2008). However, policies of fire suppression that were implemented in North America in the 1920's have greatly reduced fire occurrences in natural habitats, which has resulted in environmental consequences. Following fire suppression, shade-tolerant, fire-intolerant tree species began to invade forests and created dense canopies that inhibited oak regeneration and plant growth, as well as outcompeted the indigenous shade-intolerant understory, that were all maintained by a fire-dependent system (Rietl & Jackson, 2012). The normal shade-intolerant, fire-tolerant species have decreased substantially after dominating these forests for thousands of years. These changes have also likely altered soil properties, including levels of organic matter, nutrients, and microbial activity (Giai & Boerner, 2006). However, the harmful ecological impact of these changes has

encouraged a new approach that involves using fire as an instrument of forest restoration (Lafon et. al, 2007). Because soil enzymes are crucial to carbon and mineral cycling in soil, the impacts of fire on soil enzyme activity is also of interest.

A recent study on the effects of ecological restoration of a forest in northern Mississippi revealed an increase in NAGase activity and a decrease in phosphatase activity immediately following a prescribed fire, compared to an unburned control plot (Rietl & Jackson, 2012). Another similar study showed a reduced phosphatase activity and an increased NAGase activity following a prescribed fire in an oak-hickory forest, while β-glucosidase was found to be mostly unaffected and phenol oxidase activity highly variable (Boerner et. al, 2000). A later study in the same oak-hickory forest was done after burning annually and periodically over a period of five years. In that study, phosphatase and β-glucosidase activities decreased in the burned plots while phenol oxidase displayed an increased activity, and NAGase activity changed minimally (Boerner & Brinkman, 2003). Another study examined the effects of the frequency of fires and determined that frequencies of less than 2.5 fires per decade showed no relevant changes in soils of an upland oak forest, while fires occurring at a frequency of five or more per decade did cause significant changes in soil microbial function (Williams et. al, 2012). Thus, the effects of fire on soil enzyme activity may vary, although there is a trend towards a pattern of NAGase activity increasing in burned systems.

Given the variable outcome on enzyme activity when soil is impacted from forest restoration, there is still ample need to investigate the effects that fire has on soil enzyme activity. As part of a broader project examining the role of burning in the

structure and function of soil fungal communities (A. Rasmussen, personal communication), samples were taken from sites in the Tallahatchie Experimental Forest (TEF), a mixed upland forest with second growth oak stands in upland areas, within a division of the Holly Springs National Forest in Lafayette County, Mississippi. Sites used for soil collection include an unburned control plot, a prescribed burn plot, and a plot that had experienced a previous wildfire. Soil plug samples of different types were taken from each site and assessed for the activity of phosphatase, β-glucosidase, NAGase, phenol oxidase, and peroxidase in order to see how these enzymes were influenced by fire history.

Methods

Sample Sites and Sample Collection

Research was conducted on soil samples collected from sites at the Tallahatchie Experimental Forest (TEF), part of the Holly Springs National Forest in Lafayette County, Mississippi. Samples were part of a larger project investigating fungal community structure and activity in response to forest restoration (A. Rasmussen, personal communication). The sites used in the enzyme assay were an untreated (unburned) control plot, a plot that was burned in 2005, 2010, and 2012, and an otherwise untreated plot that experienced a wildfire in July 2012. Potential enzyme activities in soil were tested on three experimental substrates collected from the same sites: unburned soil, prescribed burn soil, and wildfire soil. Four trees were selected in each of the unburned, prescribed burn, and wildfire sites at TEF. In spring 2013, two replicates of each substrate type were planted around each tree under the drip line, for a total of 72 substrate plugs (3 sites x 4 trees x 3 substrates x 2 replicates). Plugs were placed in a stratified random manner around each tree, with each substrate represented on the north half and on the south half of each tree. Each plug was 15 cm deep by 10 cm diameter. Soil for substrates was taken from holes dug for insertion of soil plugs, and all soil for each treatment was mixed and sieved to remove root tips and other large organic matter. Plugs were removed just before leaf drop in Fall 2013 in a series of successive weeks, taking samples from

one site each week. With their being six cores around each of the four trees, a total of 24 soil samples were extracted each week. Samples were removed and placed in a separate Ziploc bags marked with its respective soil plug number, and brought to the laboratory and immediately refrigerated while waiting for testing.

Sample Processing and Enzyme Analyses

A small amount (typically 0.1 g) of each soil plug sample was placed into six different colored microcentrifuge tubes and weighed. Each color tube represented samples that were to be tested for one of five enzymes, with the sixth being a control. Tubes were weighed prior to and after adding soil samples, and the mass of soil determined.

The activity of five microbial extracellular enzymes was determined: phosphatase, β-glucosidase, NAGase, phenol oxidase, and peroxidase. For the phosphatase, β-glucosidase, and NAGase enzyme assays, p-nitrophenyl (pNP) linked substrates were used, following the general procedures of Jackson et al. (2013). These substrates release pNP while undergoing enzymatic hydrolysis, which turns a bright yellow color when NaOH is added. The yellow color was then detected spectrophotometrically at 410nm. Enzymatic activity was calculated from this absorbance reading, as activity is directly related to the amount of pNP released, and the amount of pNP released gives a specific absorbance at this wavelength. Phenol oxidase and peroxidase enzyme assays required the use of L-3,4,-dihydroxyphenylanaline (L-DOPA) substrate. This substrate produced a red-brown compound from the oxidation of L-DOPA that could be read at 460nm. Again, absorbance is directly dependent on the amount of enzymatic activity, and then

used to calculate total activity. All assays were performed using 5mM substrate solutions, which were made on the day of testing by dissolving the appropriate substrate in 50mM (pH 5.0) acetate buffer.

Enzyme assays were performed directly in each microcentrifuge tube, totaling 24 tubes for every enzyme and the control. For the pNP linked assays, each tube received 300 µl of substrate solution. Phenol oxidase and peroxidase assays received 300 µl of L-DOPA solution, with the peroxidase assays receiving an additional 15 µl of 0.3% H_2O_2. The sixth group of tubes received 300 µl of the acetate buffer to serve as a substrate-free control. All of the tubes were vortexed to properly mix the solutions and soils and were incubated at room temperature for the appropriate time period required of each enzyme. Phosphatase assays were incubated for 0.5 h and β-glucosidase assays for 1 h. The NAGase, phenol oxidase, and peroxidase assays were each incubated for 2-3 h. Following incubation, tubes were centrifuged for 5 minutes at 5,000 g. 150 µl of the supernatant from each tube was then pipetted into microplate wells for absorbance readings. The pNP assay wells received an additional 140 µl of RO water and 10 µl of 1M NaOH, while an additional 150 µl of RO water was added to the L-DOPA assay wells to bring the total volume to 300 µl in each well. Microplates were read using a BioTek Synergy microplate spectrophotometer set to the appropriate wavelength. Final absorbance was calculated as follows:

Final Absorbance = Sample Absorbance – Control Absorbance.

Enzyme activity was then calculated as follows:

Enzyme Activity (μmoles/h/g sample) = Final Absorbance / (C x incubation time x g sample)

Because the peroxidase assay actually measures both phenol oxidase and peroxidase, final peroxidase activity was calculated from:

Peroxidase (μmoles/h/g sample) = [Final Absorbance / (C x incubation time x g sample)] – Phenol Oxidase Activity

The mass term in each equation was calculated from the dry weight of the samples, which was measured after allowing the tubes to dry in an oven for 24 hours at 70 °C. Incubation time was measured in hours. C represents the conversion factors for the assays, which was 21.69 for the pNP assays and 3.347 for L-DOPA assays.

The data was imported into Microsoft Excel spreadsheet for analysis. Mean (and standard error) enzyme activity was calculated for each enzyme with respect to both site (unburned, prescribed burn, or wildfire,) and the type of soil plug (unburned soil, prescribed burn soil, or wildfire soil.) Correlations between enzymes were calculated using Excel in a site-wise and plug-wise manner. Trends in enzyme activity were visualized in two ways: patterns between different sites, and patterns between different plug types.

Results

In general, phosphatase showed the highest overall amount of enzyme activity across all three sites and soil types tested. Phosphatase displayed the highest enzyme activity at the prescribed burn site, where its activity ranged from 1.2 to 2.4 µmoles/h/g sample in the different soils (Figure 1). Activity at the unburned and wildfire sites was lower, and generally equal. In regards to soil type, phosphatase showed the greatest overall activity in the unburned soil, with activity ranging from 1.3 to 2.4 µmoles/h/g sample across the different sites (Figure2). Lower phosphatase activity was seen in the prescribed burn soil compared to unburned soil, and the wildfire soil contained the least phosphatase activity of the three core types tested. The greatest phosphatase activity was just over 2.4 µmoles/h/g sample and was found in unburned soil cores at the prescribed burn site (Figure 1).

β-glucosidase exhibited lower overall enzymatic activity than phosphatase across all burn sites and different soil types, but was still one of the more active enzymes, with its activity being substantially higher than either phenol oxidase or peroxidase. β-glucosidase showed similar activity at the prescribed burn and wildfire sites, with slightly higher activity at the wildfire site (Figure 3). Higher β-glucosidase activity was detected in soils from both of these sites than at the unburned site, where it showed the least enzymatic activity and ranged from 0.1 to 0.15 µmoles/h/g sample (Figure 4). Concerning the different soil types, the greatest β-glucosidase activity was found in the

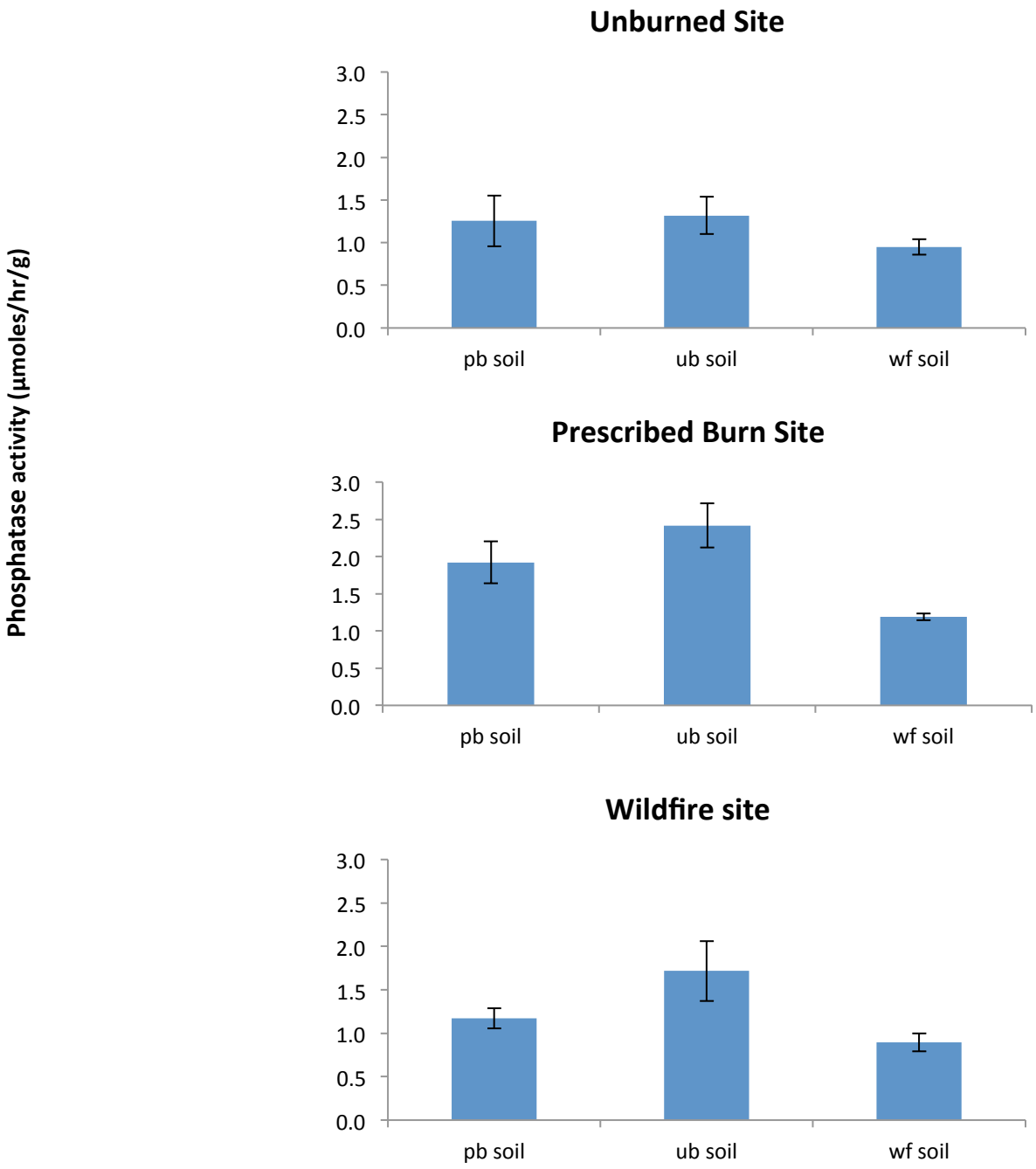

Figure 1. Phosphatase activity in prescribed (pb), unburned (ub), and wildfire (wf) soil cores taken from sites in Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples of each soil type at each of the three sites.

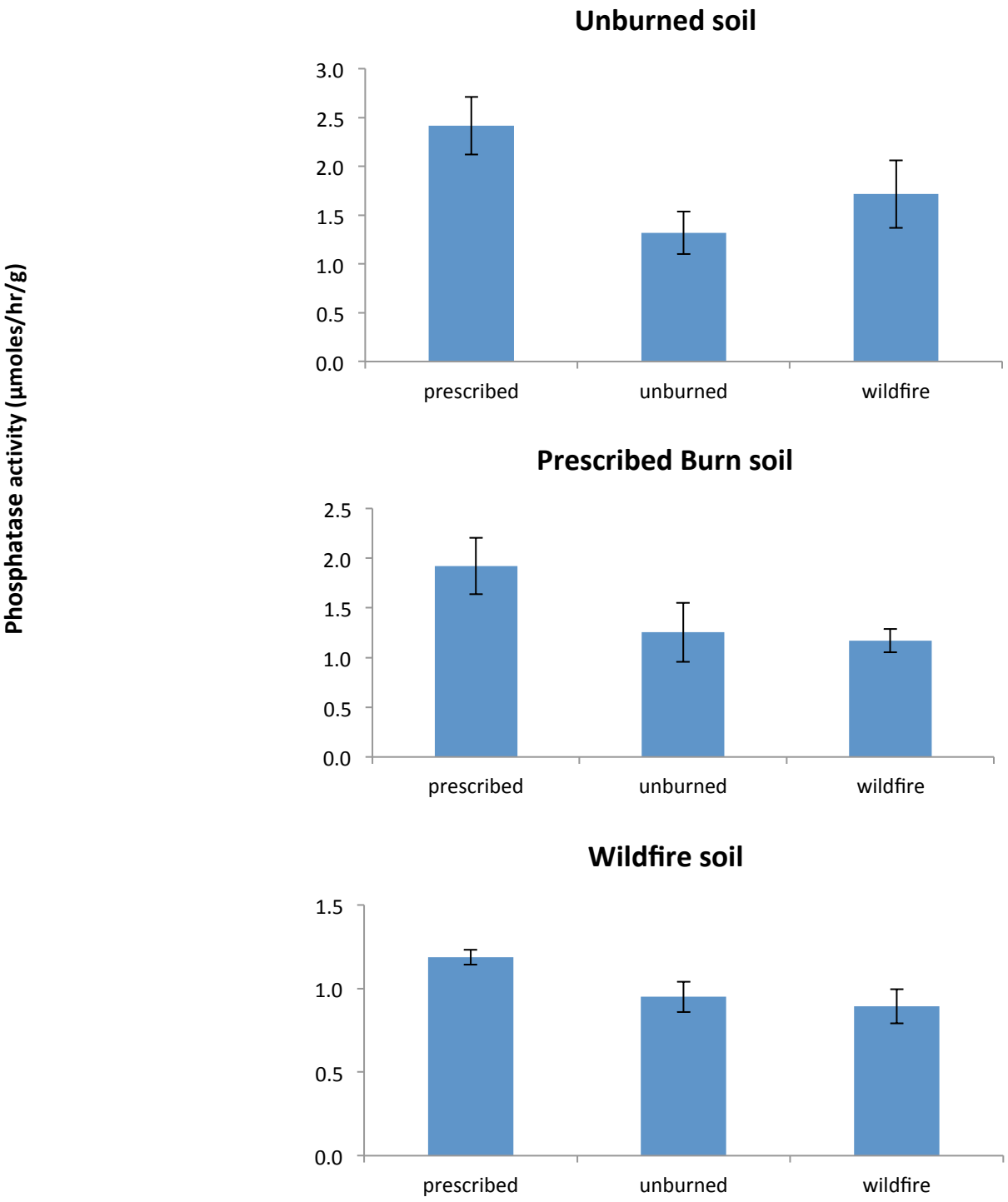

Figure 2. Phosphatase activity in each soil type at the different (prescribed, unburned, and wildfire) sites taken from Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples from each site of every soil.

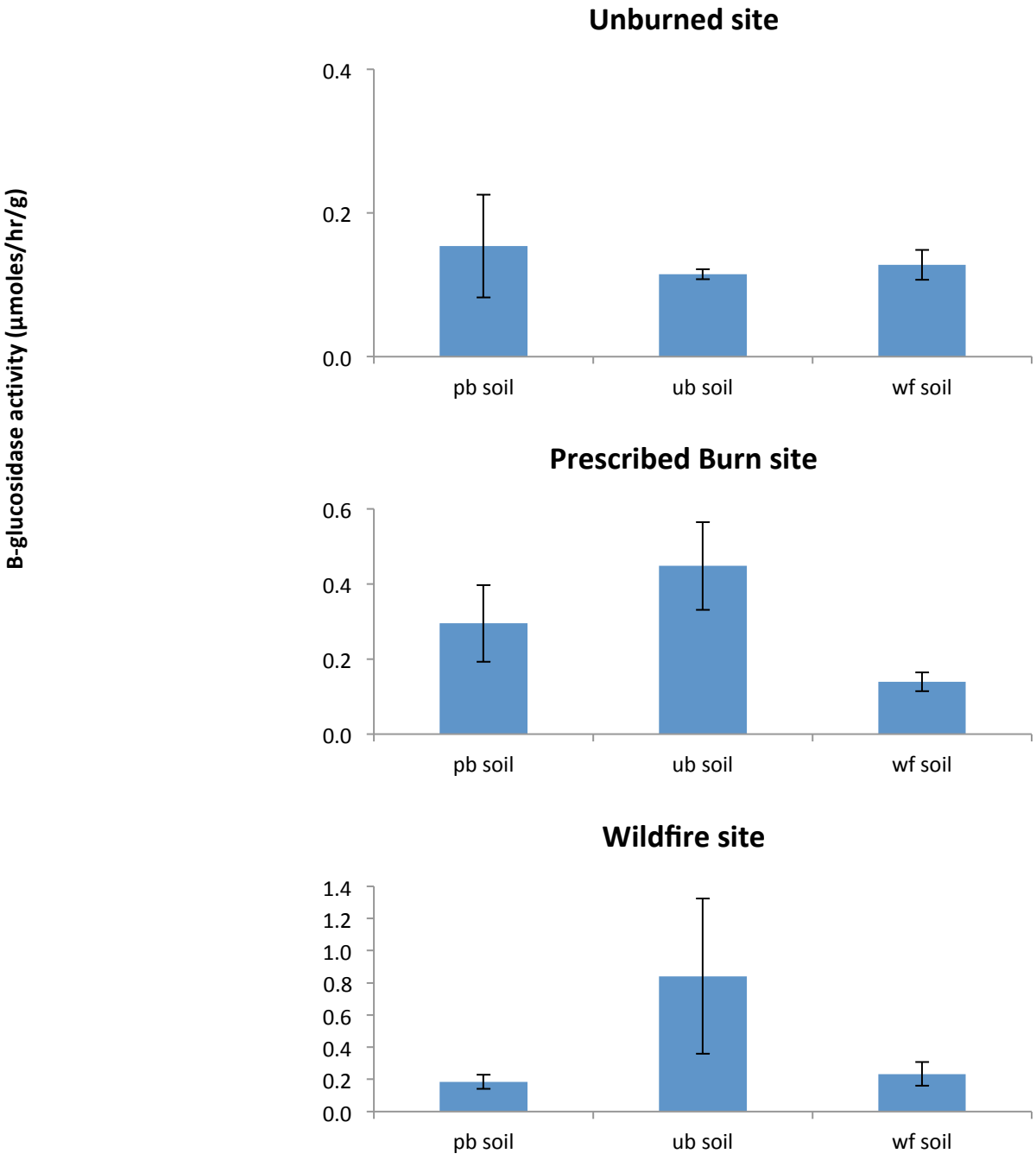

Figure 3. β-glucosidase activity in prescribed (pb), unburned (ub), and wildfire (wf) soil cores taken from sites in Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples of each soil type at each of the three sites.

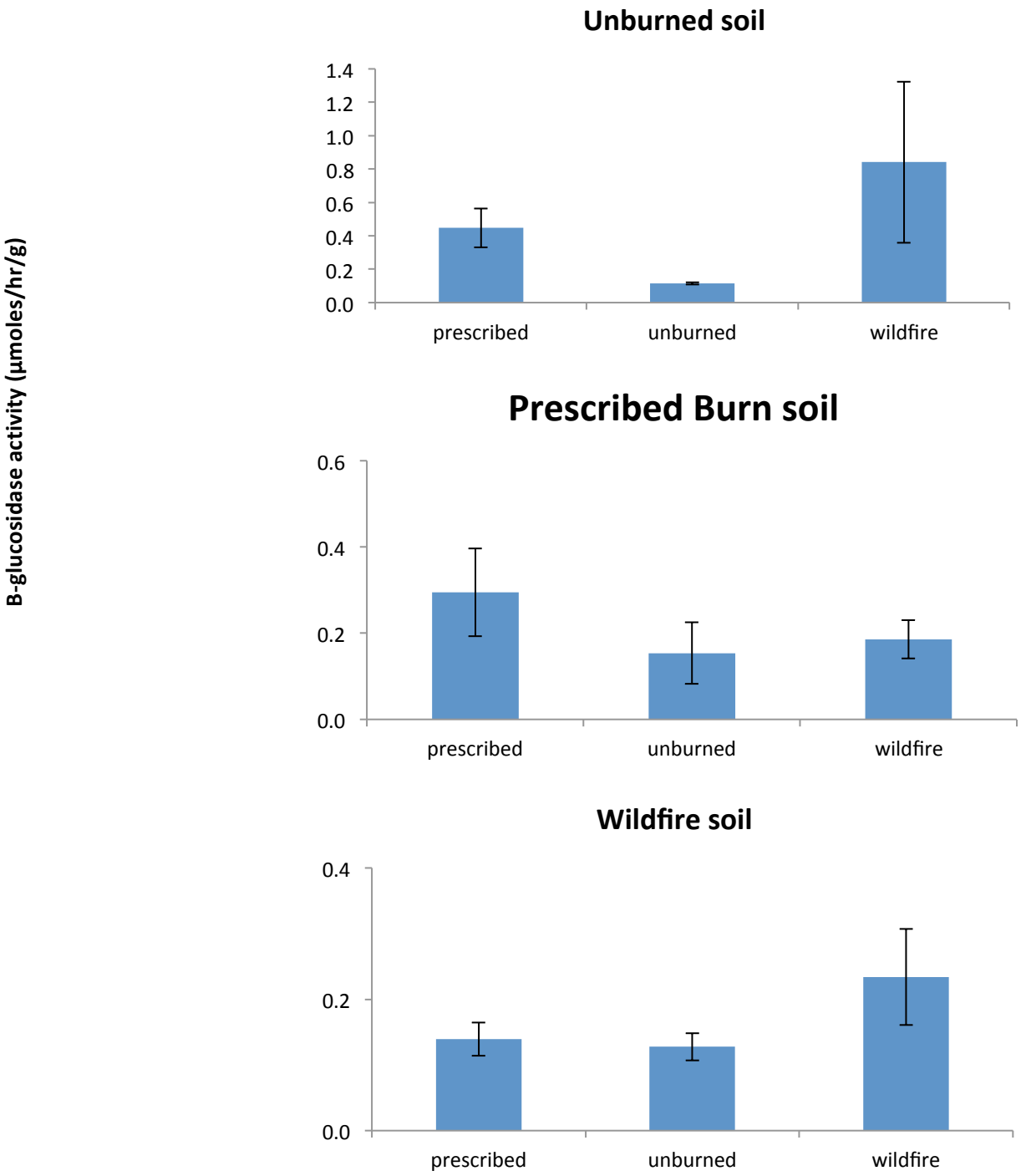

Figure 4. β-glucosidase activity in each soil type at the different (prescribed, unburned, and wildfire) sites taken from Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples from each site of every soil.

unburned soil, although activity fluctuated from 0.11 to 0.84 μmoles/h/g sample across the different sites (Figure 3). Lower β-glucosidase activity was found in the prescribed burn and wildfire soils. The greatest β-glucosidase activity was seen in unburned soil cores taken from the wildfire site (Figure 4).

NAGase displayed a similar level of enzymatic activity to β-glucosidase as it was less active than phosphatase but more active than either peroxidase or phenol oxidase. Regarding the different burn sites, the wildfire site resulted in the greatest amount of NAGase activity from 0.56 to 0.65 μmoles/h/g sample, while activity was progressively lower at the prescribed burn and unburned sites (Figure 5). NAGase proved to be more similar in its activity in different soil types than other enzymes, with the only noticeable difference being at the prescribed burn site where there was higher activity in the unburned soil than the other soils. NAGase activity was greatest in unburned soil at the wildfire site with a high of 0.65 μmoles/h/g sample (Figure 6).

Along with peroxidase, phenol oxidase showed lower overall levels of enzymatic activity than the other enzymes. With respect to the different sites, phenol oxidase tended to have the highest amount of activity at the prescribed burn site at (0.01 to 0.14 μmoles/h/g sample) and was less active at the unburned and wildfire sites (Figure7). The enzyme also showed the greatest activity in the prescribed burn soil, ranging from 0.02 to 0.14 μmoles/h/g sample, depending on the particular site (Figure 8). Phenol oxidase activity was lower in the unburned soils and even lower in the wildfire soil. The highest phenol oxidase activity detected was 0.14 μmoles/h/g sample in prescribed burn soil at the prescribed burn site (Figure 8). Peroxidase also displayed

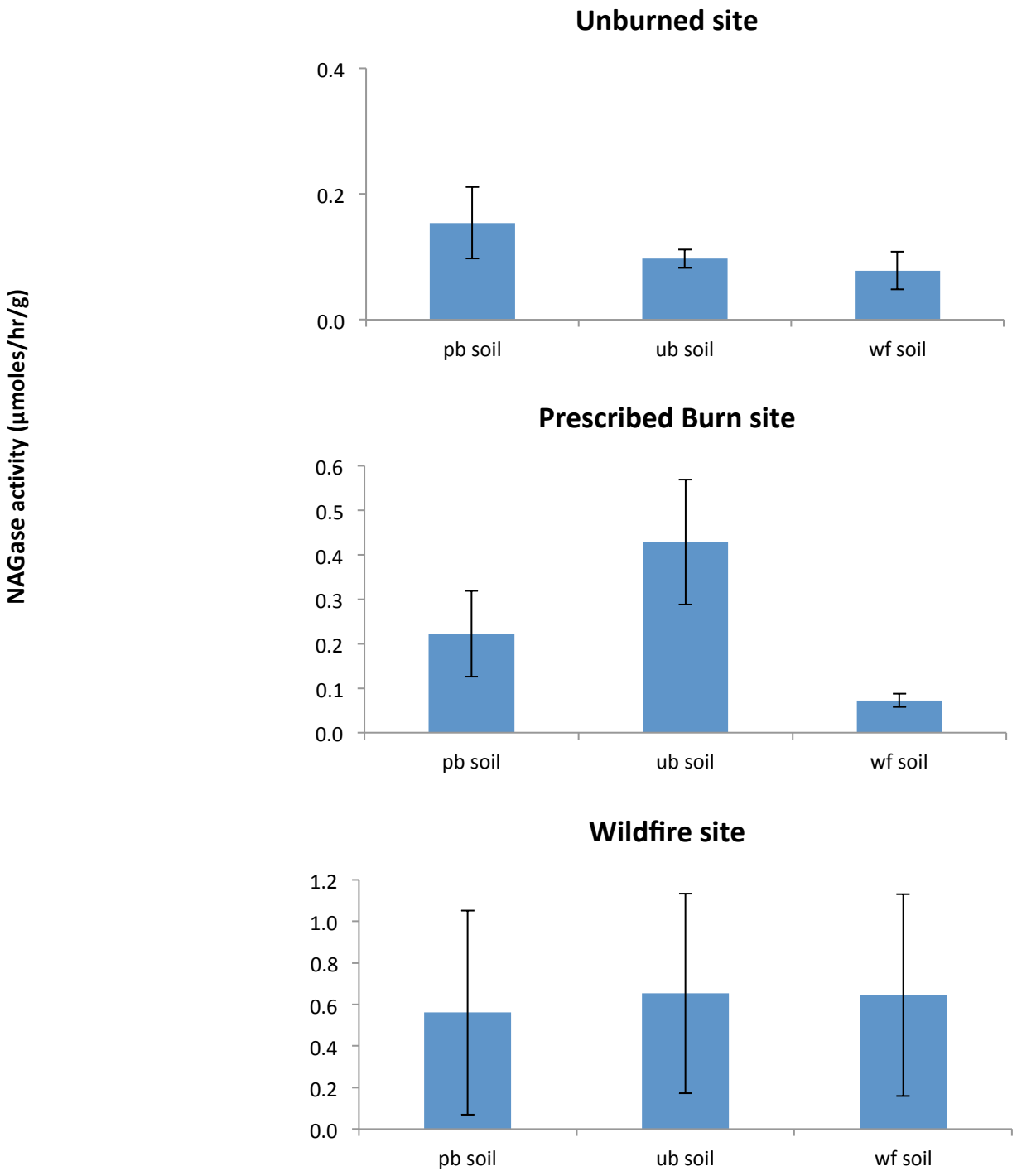

Figure 5. NAGase activity in prescribed (pb), unburned (ub), and wildfire (wf) soil cores taken from sites in Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples of each soil type at each of the three sites.

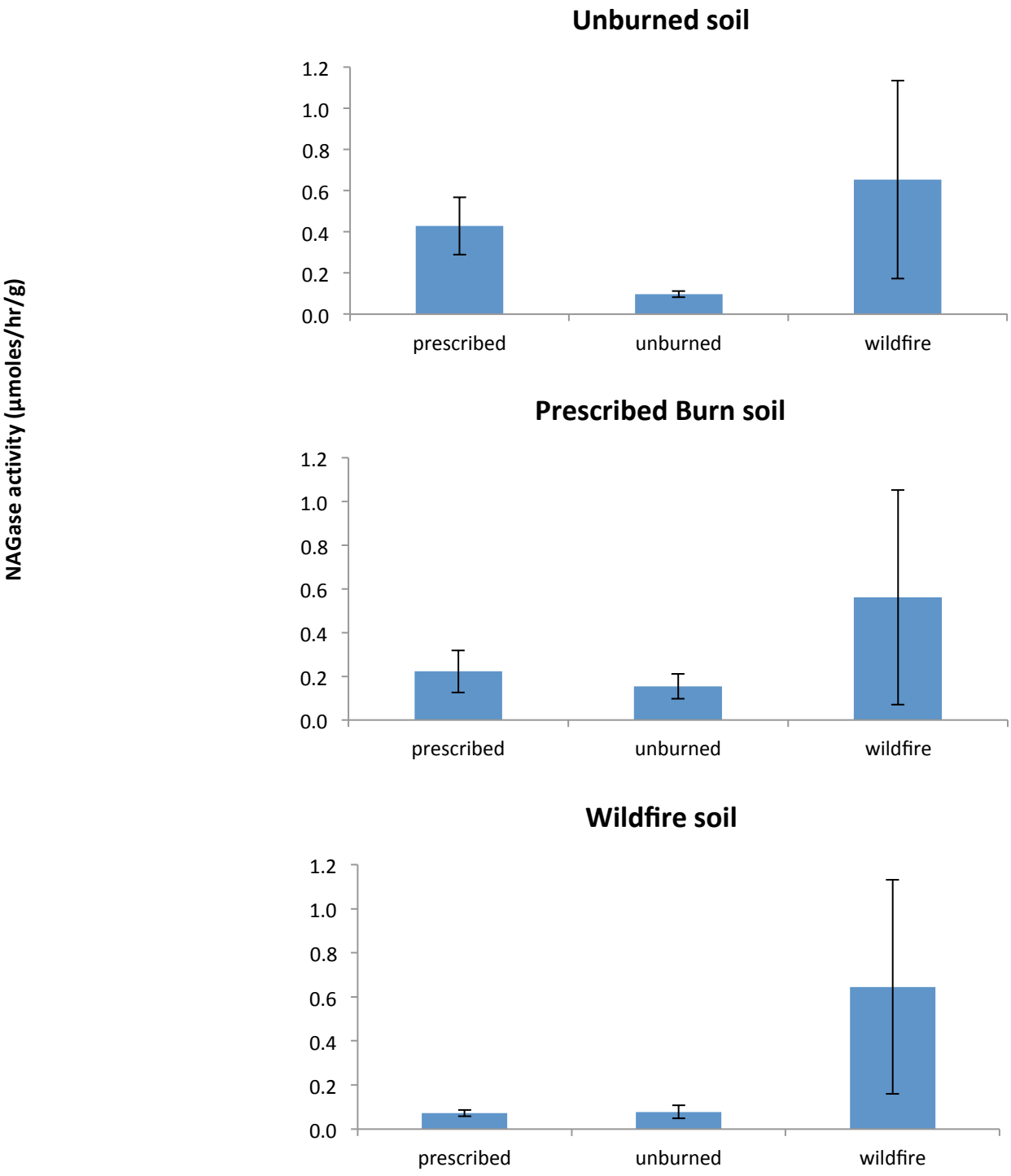

Figure 6. NAGase activity in each soil type at the different (prescribed, unburned, and wildfire) sites taken from Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples from each site of every soil.

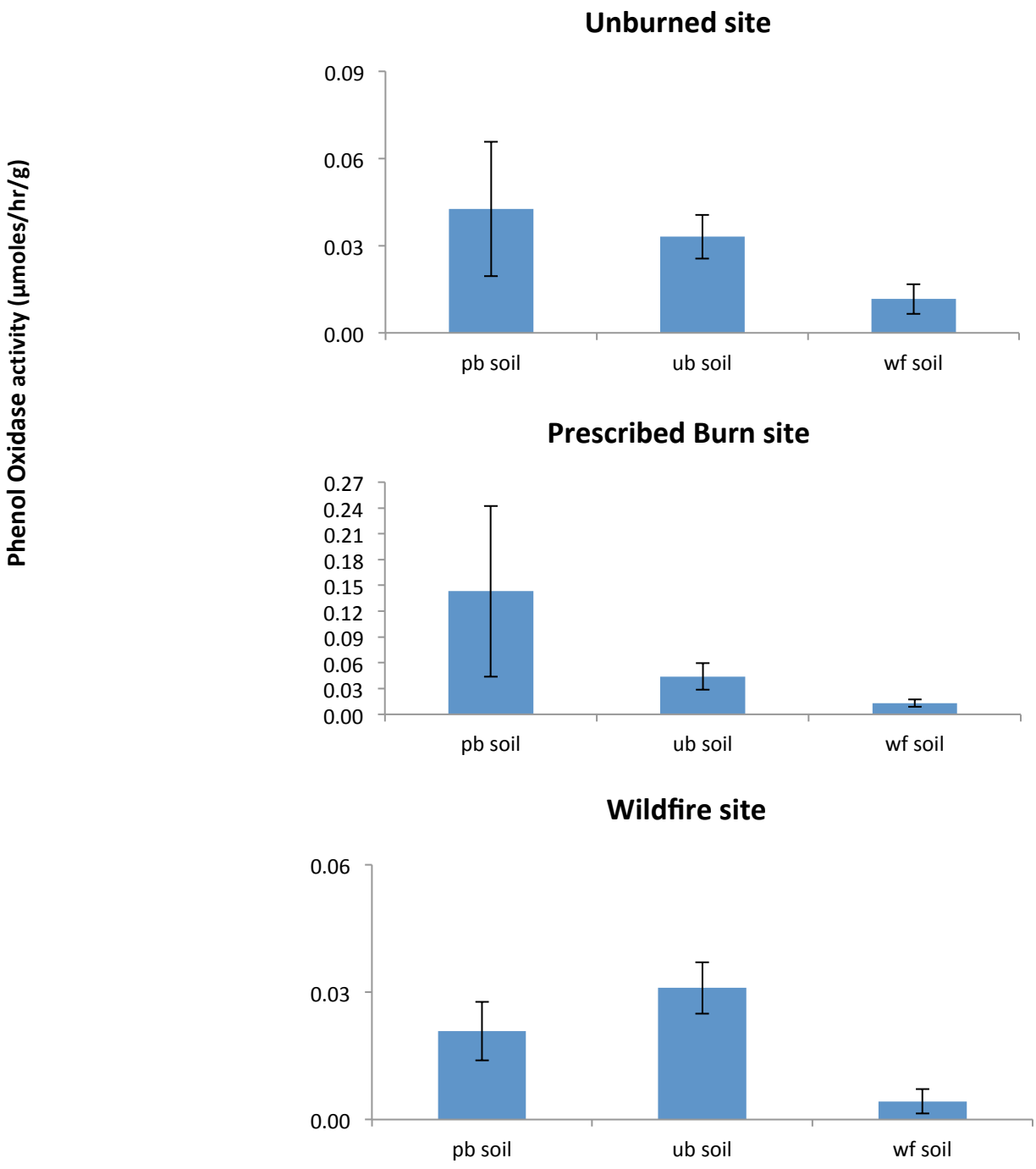

Figure 7. Phenol oxidase activity in prescribed (pb), unburned (ub), and wildfire (wf) soil cores taken from sites in Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples of each soil type at each of the three sites.

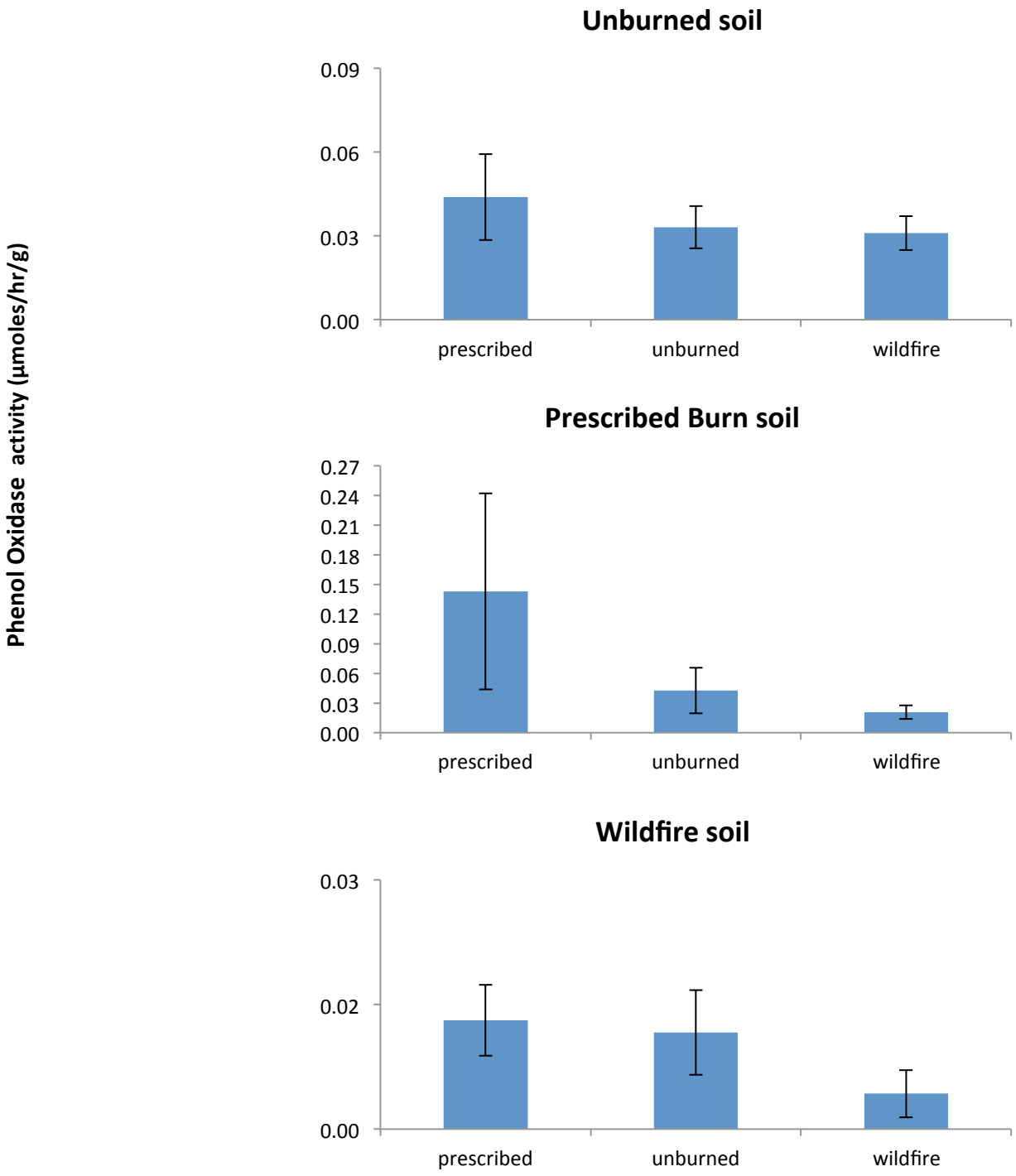

Figure 8. Phenol oxidase activity in each soil type at the different (prescribed, unburned, and wildfire) sites taken from Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples from each site of every soil.

low levels of activity across all of the sites and soil types tested. Peroxidase showed the highest activity at the unburned site, with activity levels between 0.08 and 0.24 μmoles/h/g sample, while it was least active at the prescribed burn site (activity of 0.05 to 0.07 μmoles/h/g sample) (Figure 9). It was also least active in the prescribed burn soil cores (regardless of site), where it ranged from .05 to .08 μmoles/h/g sample (Figure 10). Highest peroxidase activity (0.24 μmoles/h/g sample) was detected in wildfire soil at the unburned site.

Across the entire data set, a positive correlation (R = 0.57) was found between β-glucosidase and NAGase as well as a weaker correlation (R = 0.43) between phosphatase and β-glucosidase. Site-specific correlations were also seen within the different burn sites. Phosphatase and β-glucosidase were positively correlated at each site, with R values ranging from 0.46 at the wildfire site to 0.81 at the prescribed burn site. The prescribed burn site actually showed strong correlations between all three of the hydrolytic enzymes tested (phosphatase, β-glucosidase, and NAGase; correlations between 0.81 and 0.86). The only other strong (R>0.50) correlations that were site-specific were between phosphatase and β-glucosidase at the unburned site (R = 0.67), and between β-glucosidase and NAGase (R = 0.54) at the wildfire site. When each soil type was examined individually, activities of β-glucosidase and NAGase were positively correlated in the wildfire (R = 0.59) and unburned soil (R =0.91), and phosphatase and β-glucosidase were correlated in the prescribed burn soil samples (R = 0.79).

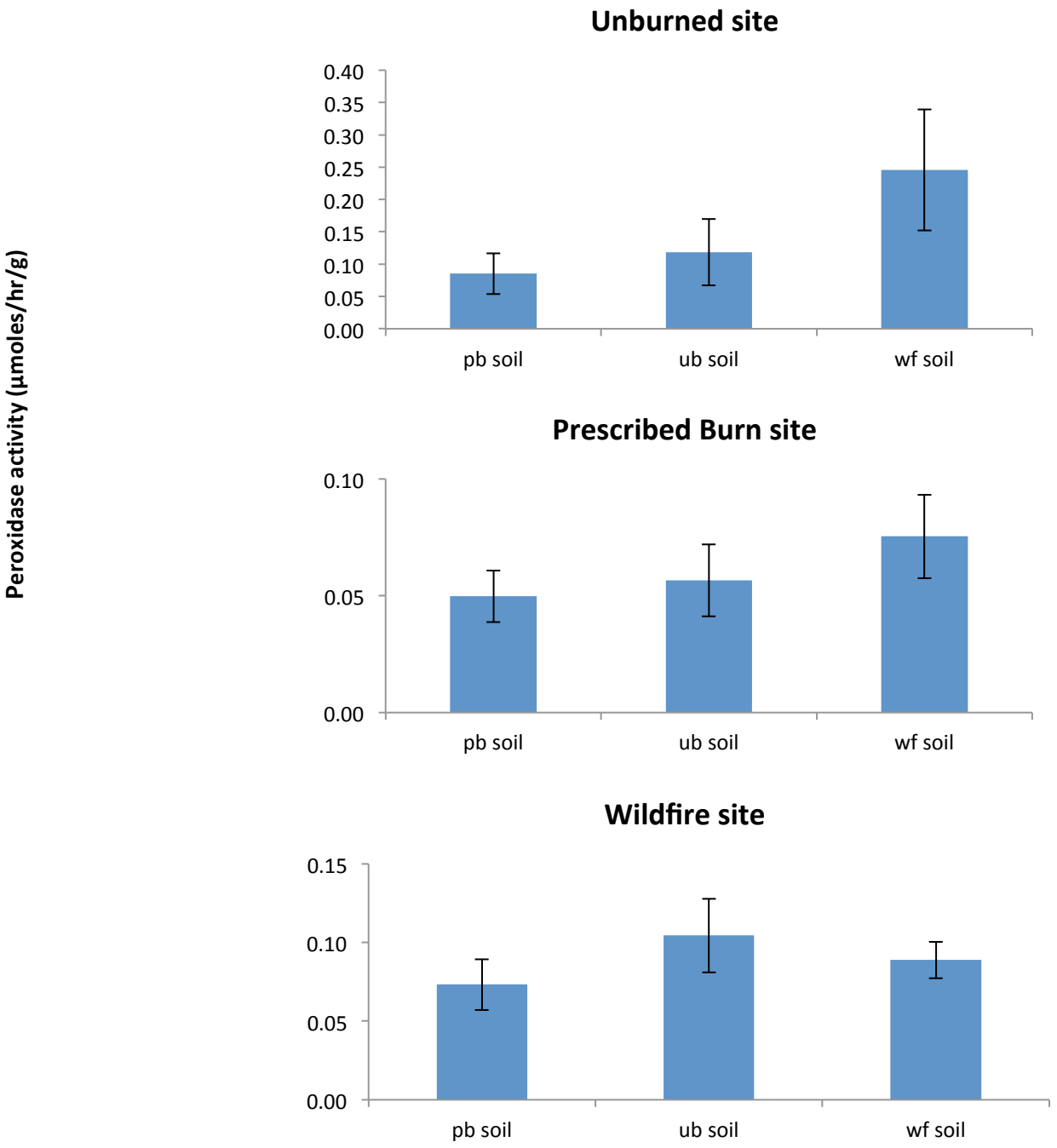

Figure 9. Peroxidase activity in prescribed (pb), unburned (ub), and wildfire (wf) soil cores taken from sites in Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples of each soil type at each of the three sites.

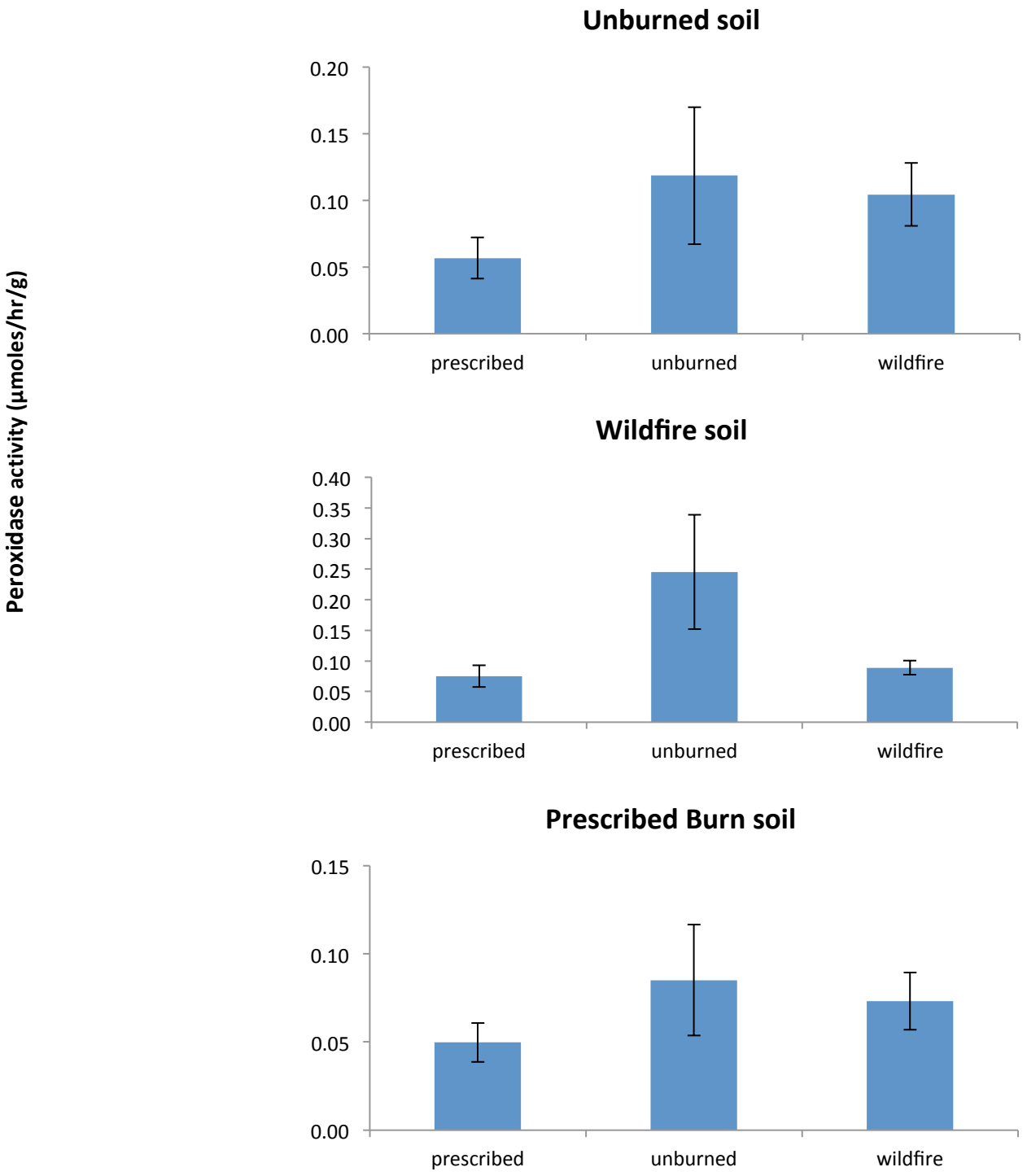

Figure 10. Peroxidase activity in each soil type at the different (prescribed, unburned, and wildfire) sites taken from Holly Springs National Forest, MS. Values represent mean (+/- SE) activity of 8 samples from each site of every soil.

Discussion

Microbial extracellular enzymes play a substantial role in the function of soil environments and are important in maintaining soil characteristics. One of the fundamental soil processes in which these enzymes are involved in is the decomposition of organic matter. Enzyme activity in soil can be used to assess soil health or quality in the context of restoration efforts, such as those accomplished through burning. While the amount of soil enzyme activity is clearly subject to change following burning, how activity differs between certain enzymes has yet to be determined. The results of this study show that the effect of burning on enzyme activity levels is widely variable and depends on the particular enzyme, soil type, and the site from which the soil was taken from. However, every enzyme assayed in this study was sensitive to fire disturbance in some way, showing either increased or decreased activity compared to an unburned site, suggesting that the activity of soil enzymes could be used as an indicator of soil quality or health during the restoration process.

Phosphatase showed greater activity at the prescribed burn site compared to the other sites, but had the greatest overall activity in unburned soil plugs across all sites. Phosphatase activity has been shown to decrease following burning (Boerner et. al, 2000;Rietl & Jackson, 2012) suggesting that, in this study, soil plug composition contributed more to the level of enzyme activity than the site in which the plug was placed. One of the main reasons for the depletion in phosphatase activity following fire

can be attributed to an increase in phosphorous availability, which can be liberated from soil organic matter following burning. With readily available phosphorous in the soil, there is less need for microorganisms to expend energy in producing phosphatases that free phosphorous for utilization (Morteza et. al, 2013).

β-glucosidase activity was found to increase at both burn sites compared to the unburned site, but as with phosphatase its overall activity was greatest in the unburned soil plugs across the different sites. β-glucosidase activity has been shown to decrease following burning (Boerner & Brinkman, 2003) or to be mostly unaffected (Boerner et. al, 2000), again suggesting that the soil type might have a greater influence on its activity than the specific burn site. Decreases in β-glucosidase activity can indicate a reduction in the quality of soil organic matter (Boerner & Brinkman, 2003), which can arise from burning. This change in soil organic matter can affect the availability of substrates (Rietl & Jackson, 2012), which could be the cause of the decreased levels of β-glucosidase activity.

NAGase showed the highest activity at the wildfire site and higher overall activity at both burn sites compared to the unburned site. However, it failed to display any noticeable differences in activity between the different soils used at each of the sites. Greater NAGase activity in areas subject to burning is consistent with other studies that have reported an increase in NAGase activity following fire (Boerner et. al, 2000;Rietl & Jackson, 2012). Initially, burning can result in decreased soil nitrogen because nitrogen is easily volatilized by high temperatures (Rietl & Jackson, 2012) and released from the soil as gas. Thus, NAGase activity may increase in burned areas as a response of the soil

microbial community to acquire more nitrogen from organic sources. Furthermore, NAGase is involved in the breakdown of chitin, a key component of the cell walls of fungi, and fire has been shown to stimulate fungal growth and turnover (Boerner et. al, 2000). This increase in fungi growth can then lead to an increase in the availability of chitin as a substrate and result in greater NAGase activity.

Phenol oxidase exhibited the highest amount of activity at the prescribed burn site and also showed the greatest amount of activity in the prescribed burn soil. However, it showed the least amount of activity in wildfire soil. An increase in the level of phenol oxidase activity following burning has been reported by other studies (Boerner & Brinkman, 2003), and is a good indication that the quality of organic matter in the soil has changed significantly at that site. Prescribed burning can lead to a reduction in available labile carbon compounds, thus resulting in increased activity of phenol oxidase as the soil microbial community switches to more recalcitrant substrates (Boerner & Brinkman, 2003). However, it is worth nothing that the unburned soil showed higher phenol oxidase activity than the wildfire soil. This could be an indication that there were substantial differences (e.g. temperature, duration) between the wildfire and prescribed burn at these sites, and that the frequency of fire may play as important a role in changing the activity of soil enzymes rather than the burn itself.

Peroxidase did not appear to show an increase in activity in areas subject to fire, as its activity level was highest at the unburned site and lowest at the prescribed burn site. The prescribed burn soil plugs were also the soil types that showed lowest peroxidase activity. Compared to the other enzymes, there is not a great amount of

published literature on the effects of burning on peroxidase activity, and the information that is present is widely varying or inconclusive. Peroxidase is involved in similar soil processes to phenol oxidase, but there was no agreement or similarity between the results of these two enzymes and no correlation was found from the data. Further experimentation is necessary to provide more conclusive evidence on how peroxidase activity is affected by fire.

The differences in enzyme activity between soil types and treatment areas observed in this study proved to be highly variable both in themselves and compared to other studies. Some of the aspects of this study that contributed to this variability are worth noting. One reason for the variability could be the length of time that elapsed from the extraction of the soil samples to when they were assayed for enzyme activity. Time constraints did not allow for immediate testing of the soil samples, and they were typically refrigerated for a period of 1-2 days before running assays, which could have affected enzyme activities. Another important component of other studies has been the frequency at which sites were subjected to burns, and the time elapsed from burns to when testing was done. Comparing studies that have tested enzyme activity in soils following a varying number of burns, over a wide range of years, and at different lengths of time since burning is likely to show a lot of variability amongst those comparisons. A final aspect that was unique to this study was that different types of transplanted soil were tested at each site, rather than the soil indigenous to that site. While this allows some comparison of site versus soil history, further testing on actual field soil is probably necessary to more accurately determine the fire frequency that provides the

most beneficial and lasting effect on ecosystems. For prescribed burns to become a more widely used and accepted form of restoration, validation of its use over a lengthy period of time is needed (Boerner et. al, 2004).

In order to fully understand an ecosystem and its functions, the underlying biological and biochemical processes must be taken into account. One of the most important components of ecosystems is the soil, which has its own ecology, physical and chemical properties, biological processes, and overall health. Microbial enzymes play an important role in the functioning of the soil environment, and are involved in key processes such as the decomposition of organic matter. Any sort of management or restoration process used in maintaining forest ecosystems should take into account the effects on soil processes and soil enzyme activity, although these are not often considered. Ultimately, soil enzyme activity could potentially be used as a management tool to assess the impacts of restoration on these systems.

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