2010

The Effects of Ecological Restoration on Soil Microbial Enzyme Activities and Leaf Litter Decomposition

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THE EFFECTS OF ECOLOGICAL RESTORATION ON SOIL MICROBIAL ENZYME ACTIVITIES AND LEAF LITTER DECOMPOSITION

A Thesis

Presented for the

Master of Science

Degree

The University of Mississippi

Anthony Rietl

December 2010

ABSTRACT

This study examined the immediate effects of ecological restoration on soil enzyme activities and decomposition. The activities of five enzymes: phosphatase, β-glucosidase, β-N-acetylglucosadminidase (NAGase), phenol oxidase, and lignin-peroxidase were measured in both soils and on decomposing *Quercus falcata* leaf litter in unburned, burned, and burned and thinned plots in a mesic forest in northern Mississippi. Rates of decomposition were also assessed for *Q. falcata* leaf litter at each plot. The restoration treatments decreased phosphatase activity in relation to an increase in soil organic matter after the fire, and increased NAGase activity in relation to a decrease in leaf litter after the fire. Activities of extracellular enzymes associated with decomposing *Q. falcata* leaf litter in litterbags showed no consistent patterns amongst treatments or between individual enzymes**.** Decomposition of *Q. falcata* leaf litter was slightly accelerated in the treatment plots; however, decay rates were not significantly different from each other. Decomposition was related to cumulative enzyme activity, with phenol oxidase and peroxidase having the highest apparent efficiencies in degrading this material. When the activity of all enzymes was combined, the microbial degradation of *Q. falcata* leaf litter was more efficient in the burned frequently and thinned, and burned and thinned plots than in the other treated plots. The combination of burning and thinning can further a restoration project by reducing the amount of litter through both a reduction in inputs and more efficient decomposition of litter material. This reduction in the litter layer could allow for increased solar penetration to

the soil, and could allow the shade-intolerant species that once dominated the understory to proliferate

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INTRODUCTION

Historically, fire has been an integral part of eastern North American forests as a natural disturbance that shapes community composition and ecosystem function. The earliest record of fossil charcoal dates to the late Devonian period, with extensive fossil charcoal deposits found in the early Carboniferous (Scott, 2000). Pollen and charcoal deposits in the plateaus of the Appalachian Mountains give evidence of the prevalence of fire in this ecosystem for four thousand years (Delcourt & Delcourt, 1997). Likewise, historic woodlands of northern Mississippi were neither mid-successional nor late-successional forests, but rather fire-dependent open oak and pine woodlands (Brewer, 2001). The importance of fire was not limited to pre-settlement North America, but exerted major effects all over the world. Several of the world's major biomes may owe their distributions and ecological properties to a natural fire regime, and after accounting for climate limitations, large areas of C_4 grasslands and savannahs have the potential to form forests if not for frequent fires (Bond et al., 2005). Without frequent fires, it is thought that closed forests would increase from 27% to 56% in these areas (Bond et al., 2005). Such has been the fate of Midwestern North American grasslands, where fire threatened human development and agriculture. Without fire, these ecosystems converted into closed canopy forest very quickly, as in the case of southwestern Wisconsin (Cottam, 1949)

As recurrent fires formed unique ecosystems, over time this disturbance created a contrast between fire-dependent and fire-sensitive plant communities that has arguably increased biodiversity in pre-settlement ecosystems (Delcourt & Delcourt, 1998). With European colonization and the demise of Native American populations, major changes to the landscape of North America occurred and organized fire suppression became common practice beginning in the early twentieth century (Harmon, 1982). European colonization not only increased fire suppression, but in many instances also increased fire frequency as forests were cut and burned (Nowacki & Abrams, 2008). The cumulative effects of fire suppression and European colonization (agriculture, logging, grazing) led to a drastically different landscape in oak-pine systems, where tree densities increased up to ten times that of historic levels (Nowacki & Abrams, 2008). Presently, fire-sensitive mesophytic trees invade forest gaps and create a dense canopy that doesn't allow for oak regeneration or herbaceous plant growth. This is further intensified by various positive-feedback loops, one example being when increased fire suppression leads to less oak regeneration, increasing both canopy closure and litter moisture, further suppressing fire. This "mesophication" (Nowacki & Abrams, 2008) effectively limits the amount of light reaching the forest floor, thereby selecting for shade-tolerant vegetation that outcompetes the shade-intolerant herbaceous understory of a fire maintained system, and lends a distinct advantage to larger organisms. Plants that have evolved adaptations to cope with fire, such as thick bark and fire-stimulated flowering, are no longer dominant in these systems and many can only thrive with moderate fire frequencies (Van Lear & Harlow, 2002).

The Society for Ecological Restoration defines ecological restoration as the process of renewing and maintaining ecosystem health (Higgs, 1997). This usually entails reintroducing fire into closed canopy forests that were historically fire maintained. Depending upon tree densities and the goal of the restoration project, thinning of trees is often coupled with a prescribed burn plan as a way of opening the canopy to allow more solar radiation to penetrate to the forest floor, and as a means of shifting the plant community to that of the historically shade-intolerant herbaceous understory (Delcourt & Delcourt, 1998). The use of prescribed fire for land-management purposes increased substantially in the fifteen years prior to 1999 (Neary et al., 1999). Fire-induced changes in aboveground vegetation, along with the absence of fire-produced compounds such as charcoal and the build up of moist fuels, likely led to changes in soil organic matter quality and quantity and nutrient availability, as well as changes in the soil microbiota and microbial activity (Giai & Boerner, 2007). However, the effects of restoration efforts on soil abiotic parameters and microbiota are underrepresented in ecological restoration literature relative to effects on plant communities, and there is a lack of data for interpreting how basic belowground processes mediated by microorganisms are affected by fire.

Soils lay the foundation upon which a terrestrial ecosystem sustains itself by supporting hydrologic and biogeochemical cycles and containing a multitude of living organisms from earthworms to bacteria. Aboveground structure and function is highly influenced by soil processes, and as a result, soil disturbance can have long-lasting consequences for the whole ecosystem (Perry et al., 1989). Fire can alter soils physically, chemically, and biologically (Certini, 2005). Soils can be radically changed under conditions of frequent or high severity fire,

which results in a significant loss of organic matter and nutrients, or can be left relatively unaltered with occasional low severity fires (Certini, 2005). Generally, prescribed fires are less severe and are used to reduce fuel loads, or to promote the germination and growth of desired herbaceous species for ecological restoration (Jackson et al., 1995b). Wildfires, on the other hand, are often more severe due to the abundance of dry fuels and often burn chaotically with areas of high and low intensity (Rab, 1996). Organic matter is combusted at temperatures between 200 and 460°C (Giovannini et al., 1988) with approximately 10 - 15% of the heat energy absorbed by the mineral soil (Debano, 1974). Black carbon, or charcoal, is produced at temperatures between 250 and 500°C (Baldock and Smernik, 2002) and can form a layer on the soil that is akin to activated charcoal and has the same adsorbing properties, binding large molecules such as phenolic and aromatic compounds (Zackrisson et al., 1996).

Given the changes that fire causes in physical parameters, fire can also have major influences on belowground ecological processes. Estimations of the concentration of charred materials from a chronosequence of 12 boreal forest soils where wildfire is a major disturbance factor are well within the range to exert important ecological effects (Zackrisson et al., 1996). Some of these effects include an increased rate of nitrogen uptake by certain plant species (Wardle et al., 1998) and significant increases in net and gross nitrification (DeLuca et al., 2006). Charcoal also has been shown to result in an increase in the decomposition rate of forest humus (Wardle et al., 2008). Charcoal itself may harbor a distinct microbial community compared to the surrounding humus, (Pietikainen et al., 2000), and the role of this community in soil processes are unknown. Charcoal can also enhance seedling germination (Keeley et al., 1985; Keeley and

Pizzorono, 1986), so that the effects of charcoal range from biogeochemical changes to potential changes in both microbial and plant community structure.

Fire can alter the structure (Pietikainen & Fritze, 1995), activity (Fritze et al., 1993), and function (Staddon et al., 1998) of microbial communities. Microbial biomass is usually reduced immediately following a fire, and in cases of high intensity fire the topsoil can be completely sterilized (Certini, 2005). It is in the upper few centimeters of mineral soil and organic horizon, where fire most impacts the soil. Indeed, heating does not often occur below 20 - 30 cm and temperatures at 5 cm rarely exceed 150ºC (Debano, 2000). The upper few centimeters of soil are also where microorganisms are most abundant (Neary et al., 1999), and fatal temperatures for most microorganisms occur below 100°C (Debano et al., 1998). Some groups of microorganisms are more sensitive to high temperatures than others, resulting in a change in community composition post-fire. The temperature at which there is nearly 100% mortality of fungi is between 60 – 80ºC (Dunn et al., 1985). Certain soil bacteria, however, can survive up to temperatures between 100 – 120ºC (Dunn and Debano, 1977; Hart et al., 2005). This has been confirmed by studies in which post fire community analysis showed the relative abundance of bacteria to fungi to have increased (Fuller et al., 1955; Bååth et al., 1995).

As well as the direct effects of fire on microbial communities, many abiotic factors related to microbial communities change post-fire, which could lead to long-term effects on both vegetation and microbial communities. These effects include increased solar incidence levels, changes in mineral soil microclimate, and chemical alteration of the soil (Hart et al., 2005).

Along with these abiotic changes, fire induces biotic changes that could indirectly affect microbial communities, the most important of which is a change in vegetation, which is typically the goal of restoration. Vegetation alters soil microbial communities by regulating the quantity and quality of resources in the soil, competition for nutrients, and by forming mutualisms, all of which can also be directly influenced by fire (Hart et al., 2005).

Soil microbial communities release and recycle nutrients through processes such as the decomposition of organic matter and the transformation of nitrogen into forms available for plants. Bacteria and fungi decompose soil organic matter through the release of extracellular enzymes that break down insoluble macromolecules into smaller soluble molecules and oxidize aromatic and phenolic compounds such as lignin and humic material (Skujins, 1976). Many abundant soil organic compounds are degraded enzymatically, such as cellulose, lignin, chitin, proteins, and lipids (Skujins, 1976; Burns, 1982). Assaying the activity of these extracellular enzymes can provide insights into the metabolic requirements of the existing soil microbial community and provide information on nutrient and substrate availability (Skujins, 1976). Extracellular enzymes in soil have been studied since the late nineteenth century (Skujins, 1976) and have been used to investigate soil health, quality, and the biological status of the soil (Dick, 1992). Enzyme assays measure activity of active microorganisms and activity from enzymes in the soil matrix that are stabilized on soil colloids (Burns, 1982). The enzymes that are no longer associated with living cells have been termed “abiontic” by Skujins (1976), and can make up a considerable portion of total enzyme activity in a soil sample (Busto & Perez-Mateos, 1995). Typically, the enzymes used to study soil microbial communities belong to classes of enzymes

directly related to the degradation of cellulose and lignin (Allison et al., 2007), largely hydrolases and oxidases. The hydrolase enzymes that have generally been the most assayed include phosphatase (Rogers, 1942; Skujins, 1967; Eivazi & Tabatabai, 1977), β-glucosidase (Hildebrand & Schroth, 1964; King, 1986; Eivazi & Tabatabai, 1988), and β-N-acetylglucosadminidase (NAGase; Kanazawa & Filip, 1986; Sinsabaugh & Linkins, 1990; Kang et al., 2005). The laccase phenol oxidase has also been extensively studied (Turner, 1974; Li, 1981; Filip & Preusse, 1985), as has another oxidative enzyme, lignin-peroxidase (Bartha & Bordeleau, 1969; Vaughan & Malcolm, 1979; Markkola et al., 1990).

Of the three hydrolases most examined in extracellular enzyme studies, phosphatase has been given considerable attention (Rogers, 1942; Tabatabai & Bremner, 1969; Skujins, 1976; Eivazi & Tabatabai, 1977). The enzyme is involved in mineralizing phosphorus by cleaving the ester bonds of a variety of organic phosphorus containing compounds and releasing inorganic phosphate (Tabatabai & Bremner, 1969; Eivazi & Tabatabai, 1977). The name phosphatase is actually a general term describing a broad group of enzymes that all catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Schmidt & Laskowski, 1961). Another hydrolase, β-glucosidase, is produced by a variety of organisms and is involved in cellulose degradation, acting as part of a consortium of enzymes that break down labile cellulose into glucose units small enough to be taken into cells (Eriksson & Wood, 1985; Esen, 1993). β-glucosidase catalyzes the final step in the biodegradation of cellulose; the step responsible for releasing glucose for use by microorganisms (Esen, 1993). NAGase is an essential enzyme in the pathway of chitin degradation and hydrolyzes the N-acetyl-β-D-glucosamine residues of

chitooligosaccharides (Bielka et al., 1984; Parham & Deng, 2000). Chitin is the second most abundant biopolymer on Earth (Stryer, 1988), and NAGase is produced by a wide variety of organisms including bacteria, fungi, plants, invertebrates (Trudel & Asselin, 1989) and even humans (Neufeld, 1989).

The oxidative enzymes phenol oxidase (laccase) and lignin-peroxidase differ biochemically from the hydrolytic enzymes and are thought to play an important role in incorporating phenolic compounds into humus components (Skujins, 1976). While hydrolase enzymes are involved in substrate specific hydrolysis, phenol oxidase and lignin-peroxidase are not substrate specific and can oxidize a variety of aromatic compounds (Skujins, 1976). These enzymes are important for their ability to degrade lignin (Ander & Eriksson, 1976), the most abundant aromatic polymer in litter (Criquet et al., 2000). Phenol oxidase is a copper containing oxireductase that catalyzes the oxidation of phenolic compounds (Duran and Esposito, 2000). Lignin-peroxidase is a hemoprotein containing iron (Duran & Esposito, 2000) and has been shown to mineralize a variety of recalcitrant compounds and to oxidize many polycyclic aromatic and phenolic compounds (Karam & Nicell, 1997). One of the most important functions of lignin-peroxidase is its role in the decomposition of wood via release by white-rot fungi (Niku-Paavola et al., 1988), where the enzyme acts upon highly recalcitrant compounds that other enzymes cannot break down.

The effects of fire on soil extracellular enzyme activity are potentially complex and conclusions vary in the literature. Studies have shown activities of β-glucosidase and

phosphatase to increase in a tall-grass prairie (Ajwa, 1999), decrease in oak-hickory forests (Eivazi & Bayan, 1996; Boerner & Brinkman, 2003), or not change in an oak-hickory forest (Boerner et al., 2000) after prescribed fire. In a study that examined the activity of phenol oxidase, phosphatase, and chitinase in eight North American forest ecosystems being restored by the use of prescribed fire and mechanical thinning, Boerner et al. (2008) found that when the data from the eight sites were pooled together, fire lowered phenol oxidase activity, but did not affect phosphatase or chitinase activity. When the effects on three plots in the eastern United States (a central Appalachian plateau in Ohio, a southern piedmont in South Carolina, and a site in the southern Appalachians of North Carolina) were pooled together, phosphatase activity was significantly lowered in plots that were both burned and thinned, but no effect was seen on either phenol oxidase or chitinase (Boerner et al., 2008). Gutknecht et al., (2010) measured enzyme activities of β-glucosidase, α-glucosidase, cellobiohydrolase, β-xylosidase, NAGase, and phosphatase for three years following a wildfire in Californian grassland. Wildfire decreased enzyme activity as a whole by 10 - 20% in the first year, 25 - 50% the following year, with the response completely disappearing in the third year when activities returned to pre-fire levels (Gutknecht et al., 2010). Rather than providing generalizations about extracellular enzyme activity that may be applicable to management efforts, the literature shows that soil enzyme responses to prescribed burning are variable and could be ecosystem specific, as well as varying between differing stages of a restoration project.

Microbial extracellular enzyme activity is linked to organic matter decomposition (Sinsabaugh et al., 1991, 1994), suggesting that any changes in enzyme activity because of

prescribed fire could affect decomposition rates. Other ecosystem changes induced by fire (physical, chemical, and biological) could also affect decomposition rates as could changes to the quantity and quality of litter inputs. The historical fire frequency of an area has been shown to have indirect effects on litter characteristics with an increase in litter C:N ratio in frequently burned areas (Hernández & Hobbie, 2008). In that study, C:N ratio was the dominant predictor of decomposition rates of *Quercus ellipsoidalis* in a Minnesota oak savanna, with increased C:N leading to slower decomposition. The decline in leaf and litter nitrogen is thought to be a response to the reduced availability of soil nitrogen in frequently burned areas (Reich et al., 2001). Recurrent fires reduce nitrogen availability (Harden et al., 2002), rates of nitrogen mineralization (Reich et al., 2001; Dijkstra et al., 2006), and soil organic nitrogen, due to nitrogen's relatively low volatilization temperature (~200°C). These nutrient shifts may have a strong influence on litter decomposition rates (Melillo et al., 1982; McClaugherty et al., 1985; Taylor et al., 1989).

As is the case with soil extracellular enzyme activity, there is no general consensus as to how decomposition rates are affected by fire. Increases in the decomposition rate of wood in both burned and mechanically thinned and burned plots have been reported relative to a control (Gundale et al. 2005), as has a reduction in the decomposition rate of pine needles as a result of prescribed burning (Monleon & Cromack, 1996). Both Grigal & McColl (1977) and Hernández & Hobbie (2008) reported no differences in mass loss for both aspen and aster leaf, and pin oak litter between burned and unburned areas. However, if fire does indeed alter decomposition rates,

either directly or indirectly, this response could be significant at the ecosystem scale by altering carbon and nitrogen cycling (Hernández & Hobbie, 2008).

In this experiment I analyzed soil enzyme activities, leaf litter enzyme activities, and leaf litter decomposition rates in plots that have been burned, or burned and thinned as part of ongoing restoration efforts. While other studies have related decomposition to litter enzyme activity (Sinsabaugh, 1991; Sinsabaugh et al., 1993; Jackson et al., 1995a; Carreiro et al., 2000) and soil enzyme activity to litter enzyme activity (Saiya-Cork et al., 2002; Sinsabaugh et al., 2005), to my knowledge no study has examined all of these aspects in the context of restoration, or in fire impacted systems. By measuring these interacting variables in the context of a fire disturbance, it may be possible to determine what influence forest restoration practices have on belowground processes, in terms of both long and short term effects.

METHODS

Study Area and Sampling Plots

Sampling was conducted at Strawberry Plains Audubon Center, a 2500-acre sanctuary located in Holly Springs, Mississippi (Huffman, 2005), where experimental restoration plots were established by Dr. Steve Brewer (Department of Biology, University of Mississippi) in 2004. Two restoration sites (Sites 1 and 2) were established approximately 2 km apart. Both sites initially contained upland closed-canopy deciduous forest with little understory vegetation and were dominated by a mixture of oaks (*Quercus* spp.) and mesophytic species [e.g., *Liquidambar styraciflua* (sweetgum)] that invaded upland portions of the landscape following prolonged fire suppression in the 20th century (Surrette, Aquilani, & Brewer 2008). Site 1 consisted of three 70 x 75 m plots (one control, one burned frequently plus mechanically thinned of off-site tree species, and one burned infrequently plus mechanically thinned of off-site tree species), and Site 2 consisted of three 30 x 30 m plots (one control, one burned only, and one burned plus mechanically thinned of off-site tree species). Here, off-site species refer to those tree species that were historically were not common or abundant within upland portions of the landscape in north-central Mississippi (Surrette, Aquilani, & Brewer 2008). At Site 1, the experimental plot designated as burned infrequently was burned in March 2005 and April 2010 and the burned frequently plus thinned plot was burned in September 2004, March 2005, October 2006, July 2008, and April 2010. At Site 2, the burned, and burned and thinned plots were both burned in

July 2008 and April 2010. At each of the two sites, portions of the burned and mechanically thinned plots now have less canopy cover and an increased cover of grasses typically associated with open woodlands and savannas (e.g., *Schizachyrium scoparium*). Hence, these portions of the burned and thinned plots are approaching reference conditions with respect to canopy cover and understory plant species composition (Brewer and Menzel 2009).

Within each treatment plot a smaller 10 x 30 m soil-sampling plot was established in April 2009 specifically for this study. A smaller plot was established in order to have less spatial variability in measurements, and to reduce the chance of sampling towards the edge of the larger plot. Within each sampling plot, 15 sampling points were determined randomly by using randomly generated numbers as plotting points for a Cartesian coordinate system. Each point was marked with a flag, and was used for the duration of the experiment so that samples at different times were taken from the same locations within each plot. Litter depth was measured in late fall of 2009 with a standard ruler, and four measurements were taken at each sampling point and averaged. Measurements of litter depth were also taken after the April 1st fire to assess the immediate effects of fire. Canopy density was measured in late summer of 2009 with a densitometer, where four measurements were taken from each sampling point and averaged.

Soil Sampling and Processing

Soil samples for enzyme analysis were collected on a bi-monthly basis from April 2009 until August 2010. Additional sampling was also conducted within minutes following the fire of April 2010, as well as 4, 10, and 21 days following the fire. On each sampling date, one sample

was taken from each sampling point (15 samples from each 10 x 30 m plot) by filling a sterilized 50 ml centrifuge tube with soil from the top 5 cm. Samples were collected in the morning and returned to the laboratory by early afternoon. In the laboratory, soils were emptied into pre-weighed aluminum pans and mixed with a spatula. Six subsamples (five for enzyme assays plus one control; 0.2 - 0.4 g each) of each soil sample were transferred to microcentrifuge tubes, weighed, and stored at room temperature overnight until enzyme assays were conducted the next morning. A larger subsample (approximately 6 g) of soil was weighed, oven dried (75 ºC, 48 h) and reweighed to determine dry weight and % soil moisture. A subsample (approximately 4 g) of the oven dried soil was transferred to a pre-weighed crucible, weighed, and ashed (500 ºC, 2 h). The resulting ash was weighed and organic matter content for each soil sample determined as ash free dry weight.

Soil Enzyme Assays

For each soil sample, the activities of five enzymes were measured: phosphatase (EC 3.1.3.2), β-glucosidase (EC 3.2.1.21), NAGase (EC 3.2.1.52), phenol oxidase (EC 1.10.3.2 and 1.14.18.1), and lignin-peroxidase (EC 1.11.1.7). All assays included substrate and sample controls, as per Jackson et al. (2006). Enzyme assays were conducted by incubating the soil subsample in a microcentrifuge tube with 300 µL of either a p-nitrophenyl (pNP)-linked artificial substrate or L-3,4-dihydroxyphenylalanine (L-DOPA) at room temperature. Substrates for the hydrolytic enzymes were 5 mM pNP-phosphate, 5 mM pNP-β-glucopyranoside, and 2 mM pNP-β-N-acetylglucosaminide for phosphatase, β-glucosidase, and NAGase, respectively. 5 mM L-

3,4,-dihydroxyphenylalanine (L-DOPA) was the substrate for both phenol oxidase and lignin-peroxidase, the latter also received hydrogen peroxide to a final concentration of 0.015 %. For each enzyme, the substrate was dissolved in 50 mM acetate buffer (pH 5.0; 50 mL of 0.1 M acetic acid, 150 mL 0.1 M sodium acetate, 200 mL H_2O) and concentrations of substrate were high enough so that the reactions were not limiting. Incubation times for the hydrolytic enzymes were 0.5 – 1 h for phosphatase and β-glucosidase, and 3 – 4 h for NAGase. For the oxidative enzymes, incubation time was 1 – 2 h for both phenol oxidase and lignin-peroxidase. After incubation, the microcentrifuge tubes were centrifuged (4,500 xg, 5 min) and 100 µL of the supernatant transferred to a 96-well microplate containing 190 µL $H_2O$and 10 µL of NaOH per well (for assays using pNP-linked substrates), or 200 µL H_2O per well (for assays using L-DOPA). Absorbance was determined at 410 nm (pNP-linked substrates) or 460 nm (L-DOPA) using a microplate reader (Synergy HT, Biotek, Winooski, VT) and adjusted according to sample and substrate controls. Enzyme activity was calculated by dividing the adjusted absorbance by 14.71 (the absorbance of 1 µmol of pNP under these specific assay conditions) for the pNP-linked substrates, or 2.387 (the absorbance of 1 µmol of completely oxidized L-DOPA under these specific assay conditions) for the L-DOPA substrate (Jackson et al., 2006). All soil enzyme activities were expressed as µmol substrate consumed h^{-1} g^{-1} dry weight soil.

Leaf Litter Decomposition

Aboveground litter traps were placed under *Quercus falcata* (southern red oak) trees throughout control plots at both sites in September 2009. Leaves were collected independently at

each site, and were only collected from control plots to avoid complications from potential fire history effects on C:N content of plant material (Hernández & Hobbie, 2008). Leaves from *Q. falcata* were collected from the litter traps on a bi-weekly basis for six weeks, when an adequate amount of leaves had been collected. After each collection, leaves were taken to the laboratory and air-dried at room temperature until constant mass was obtained (7 d). Air dried leaves were weighed and placed in 20 x 24 cm litterbags made from 2 mm fiberglass screen and sealed with a 120 V Impulse Sealer (KF Industries, Taiwan). A subsample of the air dried leaves was weighed, oven dried (75 ºC, 48 h), and then reweighed to obtain a conversion factor between air dry weight and oven dry weight.

Litterbags were labeled and placed in the field on November 19th, 2009. Three groups of 20 bags each were placed in each plot for a total of 360 bags (3 groups x 20 bags x 3 plots x 2 sites). Three bags from each plot (one from each group of bags) were immediately collected to account for initial handling. Subsequent collections (three bags per plot) occurred after 7, 14, 28, 55, 98, 126, 143, 180, 207, and 277 days. After each collection, bags were returned to the laboratory and the contents weighed to determine field weight. A subsample (1 – 3 g) was removed for enzyme assays, and the remaining material was oven dried (75 ºC, 48 h) and reweighed to convert overall field weight into dry weight. On the day of the April 2010 fire, bags from all plots were taken up before the fire, placed in a plastic bag, and set to the side. Immediately following the fire, bags were re-staked in the same spot and bags were collected and processed from each plot to assess any disturbance effects of this moving action.

Leaf Litter Enzyme Assays

Leaf litter material was assayed for the activity of phosphatase, β-glucosidase, NAGase, phenol oxidase, and lignin-peroxidase. A known mass (1 – 3 g) of litter material was placed in a pre-weighed 50 mL centrifuge tube to which 20 mL of 50 mM acetate buffer (pH 5.0) was added. The mixture was homogenized using a Powergen 500 homogenizer (Fisher, Pittsburgh, PA) to form a slurry. For each enzyme assayed, four 150 µl replicates of each slurry sample were pipetted into a 96 deep-well block. 150 µl of the appropriate substrate solution (as described for soil enzyme assays) were added to each well, and reactions were incubated at room temperature for 1 - 4 h as described for soil enzyme assays. After incubation, the deep-well block was centrifuged (3,000 xg, 5 min), and 100 µL of the supernatant transferred to a 96 well microplate containing either 190 µL $H_2O$and 10 µL NaOH for pNP-linked substrates, or 200 µL H_2O for L-DOPA. Absorbance at 410 nm or 460 nm was then determined, as for soil enzyme assays. Enzyme activity was calculated as for soil assays, but using the mean of the four absorbance readings for each enzyme for each sample and expressed as µmol substrate consumed h^{-1} g^{-1} dry weight leaf material.

Statistical Analysis

Activities of soil enzymes were used to calculate change in enzyme activity before and after the fire event in treated and control plots. Change in enzyme activity was calculated by taking the mean of all enzyme activity measurements after the fire (April 2010 through August 2010) and subtracting the mean of all enzyme activity before the fire (April 2009 through March

2010) for each enzyme. This change in enzyme activity was used in a multi-source regression against mean canopy density and changes in other environmental variables (litter depth, soil organic matter content, and soil moisture). Significant changes in enzyme activity in response to those variables that differed significantly between control and treatment plots were taken as indirect evidence of significant treatment effects on enzyme activity. Change in litter depth was calculated by subtracting litter depth measurements taken after the fire from those taken before the fire. Change in soil organic matter content and soil moisture was calculated using the same method as change in soil enzyme activity. Univariate analysis was done using JMP version 5.1.2. Primer version 6 was used for a PERMANOVA analysis to examine any site effect.

Leaf litter decomposition rates at each plot were determined by generating a mass loss curve where percent original dry weight remaining is regressed against number of days in the field. For each mass-loss curve, the decomposition constant (k) was calculated as the slope and reported as % mass lost d^{-1} and differences in decomposition rates between treatments and sites analyzed via MANOVA using JMP version 5.1.2. Enzyme activity in the three litterbags sampled per plot per date was used to determine mean activity for each enzyme, and cumulative activity (the total amount of potential enzyme activity seen on leaf litter since the start of the study) was estimated by integrating mean activities over time. Apparent enzyme efficiencies were determined for each enzyme by regressing % mass remaining as a function of cumulative enzyme activity. The slope of the regression can be used as a measure of the apparent efficiency of that enzyme in degrading *Q. falcata* leaf litter (Sinsabaugh et al., 1994; Jackson et al., 1995a). Enzymes showing significant relationships between cumulative leaf litter enzyme activity and

mass loss were combined into an integrated index of microbial activity (Jackson et al., 1995a, Alvarez and Guerrero, 2000). Enzyme data were standardized to a 0 to 1 scale by dividing each activity measurement by the highest activity recorded for that enzyme. Mean standardized leaf litter enzyme activity was calculated for each date and plot and then integrated over time. Linear regressions of this cumulative standardized microbial leaf litter enzyme activity against % initial organic matter remaining were used to determine global apparent enzyme efficiencies for each plot at each site (Alvarez and Guerrero, 2000, Jackson et al., 1995a).

RESULTS

Site Differences in Environmental Variables

Canopy density at Site 1 was greater in the control plot, with the burned infrequently and thinned, and burned frequently and thinned plots having canopy densities approximately 10% lower than that of the control (Table 1). The same pattern was seen at Site 2; however, canopy density in each of the plots at this site was lower than those at Site 1 (Table 2). Litter depth before the prescribed burn at Site 1 was greater in the control plot than in treatment plots whereas at Site 2, litter depth was similar for both control and burned plots and lower in the burned and thinned plot (Tables 1 and 2). There were no significant differences in soil organic matter content prior to the burn between the plots at either site (Tables 1 and 2). At Site 1, soil moisture content was lower in the burned infrequently and thinned plot, whereas at Site 2 the burned only plot had the lowest soil moisture content (Tables 1 and 2).

Canopy density was unaffected by the fire, and showed the same trends after the burning as before the fire, being higher in the control plots at both sites. However, surface litter was entirely removed by the fire in the treated plots at both Site 1 and Site 2. At Site 1, the greatest reduction in litter was seen in the burned infrequently and thinned plot, whereas the greatest reduction at Site 2 occurred in the burned plot (Table 3). The change in litter depth after the fire was nearly the same for both the burned infrequently and thinned and burned only plots, as pre-fire litter depths in these plots were similar (Tables 1, 2, and 3). Percent soil organic matter at

Table 1. Canopy density (% canopy cover), litter depth (cm), soil organic matter (% dry weight), and soil moisture (% field weight) for control, burned infrequently and thinned (BI/T), and burned frequently and thinned (BF/T) plots at Site 1 before and after the prescribed burn. Values are expressed as means (SE) of 15 sampling points per plot.

Before the prescribed burn

	Canopy density	*Litter depth*	*% Soil organic matter*	*% Soil moisture*
Control	97.5 (0.3)	5.4 (0.3)	14.1 (1.2)	73.9 (1.3)
BI/T	88.9 (1.3)	3.8 (0.3)	13.3 (0.7)	70.0 (0.5)
BF/T	89.1 (1.0)	3.2 (0.2)	13.5 (0.9)	72.0 (0.8)

After the prescribed burn

	Canopy density	*Litter depth*	*% Soil organic matter*	*% Soil moisture*
Control	97.5 (0.3)	5.4 (0.3)	12.4 (1.1)	73.0 (1.0)
BI/T	88.9 (1.3)	0 (0)	16.4 (2.6)	69.6 (0.9)
BF/T	89.1 (1.0)	0 (0)	11.6 (1.2)	72.3 (0.9)

Table 2. Canopy density (% canopy cover), litter depth (cm), soil organic matter (% dry weight), and soil moisture (% field weight) for control, burned, and burned and thinned plots at Site 2 before and after the prescribed burn. Values are expressed as means (SE) of 15 sampling points per plot.

Before the prescribed burn

	Canopy density	*Litter depth*	*% Soil organic matter*	*% Soil moisture*
Control	91.0 (1.0)	3.9 (0.4)	10.3 (0.9)	77.3 (1.1)
Burned	74.0 (2.8)	3.9 (0.6)	11.5 (0.7)	76.9 (0.8)
Burned and thinned	71.0 (4.8)	1.1 (0.3)	8.5 (1.9)	81.9 (1.1)

After the prescribed burn

	Canopy density	*Litter depth*	*% Soil organic matter*	*% Soil moisture*
Control	91.0 (1.0)	3.9 (0.4)	8.5 (0.8)	76.0 (1.4)
Burned	74.0 (2.8)	0 (0)	12.7 (0.9)	73.9 (1.4)
Burned and thinned	71.0 (4.8)	0 (0)	7.6 (1.1)	80.6 (1.1)

Table 3. Changes in Litter depth (cm), soil organic matter (% dry weight), and soil moisture (% field weight) after the prescribed burn for control, burned infrequently and thinned (BI/T), and burned frequently and thinned (BF/T) plots at Site 1 (A), and control, burned only, and burned and thinned plots at Site 2 (B). Values calculated by subtracting the mean of pre-fire measurements from the mean of post-fire measurements (SE).

A.

	% Soil Organic Matter	*% Soil Moisture*	*Litter Depth*
Control	-1.6 (0.5)	-0.9 (0.8)	0.0 (0)
BI/T	+3.1 (2.8)	-0.4 (0.8)	-3.8 (0.3)
BF/T	-1.9 (0.9)	+0.4 (0.8)	-3.2 (0.2)

B.

	% Soil Organic Matter	*% Soil Moisture*	*Litter Depth*
Control	-1.8 (0.5)	-1.3 (0.6)	0.0 (0)
Burned	+1.2 (0.7)	-3.0 (1.3)	-3.9 (0.6)
Burned and thinned	-0.9 (0.3)	-1.3 (0.5)	-1.1 (0.3)

Site 1 decreased in both the control and burned frequently and thinned plots, and increased in the burned infrequently and thinned plots after the prescribed fire (Table 3). At Site 2, a similar pattern was seen where both the control and burned and thinned plots decreased in soil organic matter after the fire, while the burned only plot increased (Table 3). Soil moisture decreased in all plots at both sites after the fire, except in the burned frequently and thinned plot at Site 1, where a slight increase was observed. Simple correlations between the changes in percent organic matter, percent moisture, and litter depth after the prescribed fire, along with canopy density showed that only the change in litter depth after the fire and canopy density were correlated ($r = 0.70$), and only at Site 1. Partial correlations, accounting for interactions between these variables showed the same pattern, a correlation between change in litter depth after the prescribed fire at Site 1 and canopy density ($r = 0.66$).

Post-fire changes in environmental variables (litter depth, soil organic matter content, and soil moisture) at all sample points were expressed as the mean of pre-fire measurements subtracted from the mean of post-fire measurements and analyzed by discriminant analysis along with canopy density. When sample points were distinguished by both site and plot treatment type, the two control plots were the most similar to each other based upon the environmental variables measured, with Site 1 having slightly higher canopy densities and percent organic matter (Fig. 1). At Site 1, the burned frequently and thinned plot was more similar to the burned infrequently and thinned plot than either of those plots was to treatments at Site 2 (Fig. 1). Permutational multivariate analysis of variance (PERMANOVA) detected a significant site effect ($p<0.05$; Appendix A), so data from the two sites was not pooled for any subsequent analyses. This separation was based upon a large increase in organic matter in the burned infrequently and thinned plot at Site 1, as compared to the small increase in soil organic matter in

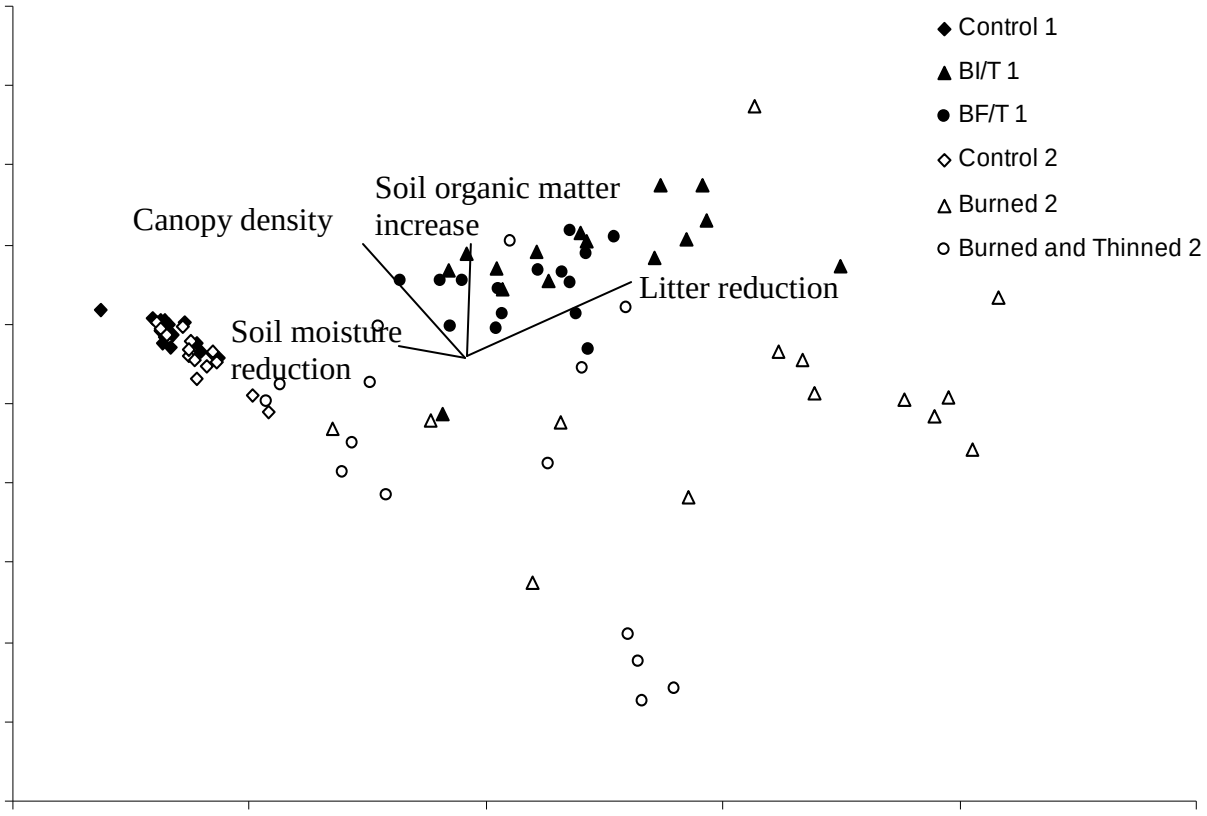

Figure 1. Discriminant analysis of treatments and site based upon mean changes in litter depth, soil moisture content, and soil organic matter content following the prescribed burn, along with canopy density. Site 1 points are shaded, Site 2 are open. Control plots are diamonds, burned infrequently and thinned (BI/T) and burned only plots are triangles, burned frequently and thinned (BF/T) and burned and thinned plots are circles. 87.8 % of variation is explained by axis 1.

the burned only plot at Site 2 after the prescribed burn (Fig. 1). Soil moisture and canopy density are the least important variables in distinguishing between treatment plots and controls, with percent soil organic matter and litter depth changes after the prescribed burn being most important (Fig. 1).

Soil Enzyme Activities

Soil enzyme activity was highest for phosphatase, which ranged between 1.8 and 62 µmol h^{-1} g^{-1} dry weight (Figs. 2 and 3). The highest observed phosphatase activity for all plots occurred in the control plot at Site 1 on the February 25th 2010 sampling (Fig. 2). The highest phosphatase activity at Site 2 occurred on the same sample date, but was detected in the burned plot, which generally showed the highest activity at that site, followed by the control and burned and thinned plots (Fig. 3). The lowest values recorded for phosphatase activity occurred on the June 13th 2010 sampling for all plots at both sites (Figs. 2 and 3).

The next highest values for soil enzyme activity was seen for the other hydrolytic enzymes ß-glucosidase, which ranged from 0.2 – 11 µmol h^{-1} g^{-1} dry weight (Figs. 4 and 5), and NAGase, for which activity varied from 0.05 – 7.1 µmol h^{-1} g^{-1} dry weight (Figs. 6 and 7). For all plots, the greatest amount of ß-glucosidase activity was recorded on the March 30th 2010 sampling date (Figs. 4 and 5), however activity declined on the next sample date (April 2nd 2010), which was the sampling that occurred immediately post-fire. ß-glucosidase activity resembled phosphatase activity in that at Site 1 the control plot showed the highest recorded activity, followed by the burned infrequently and thinned and burned frequently and thinned plots (Fig. 4), and in general the control plots at both sites showed the highest recorded activity, followed by the treatment plots (Figs. 4 and 5). The lowest values recorded for ß-glucosidase

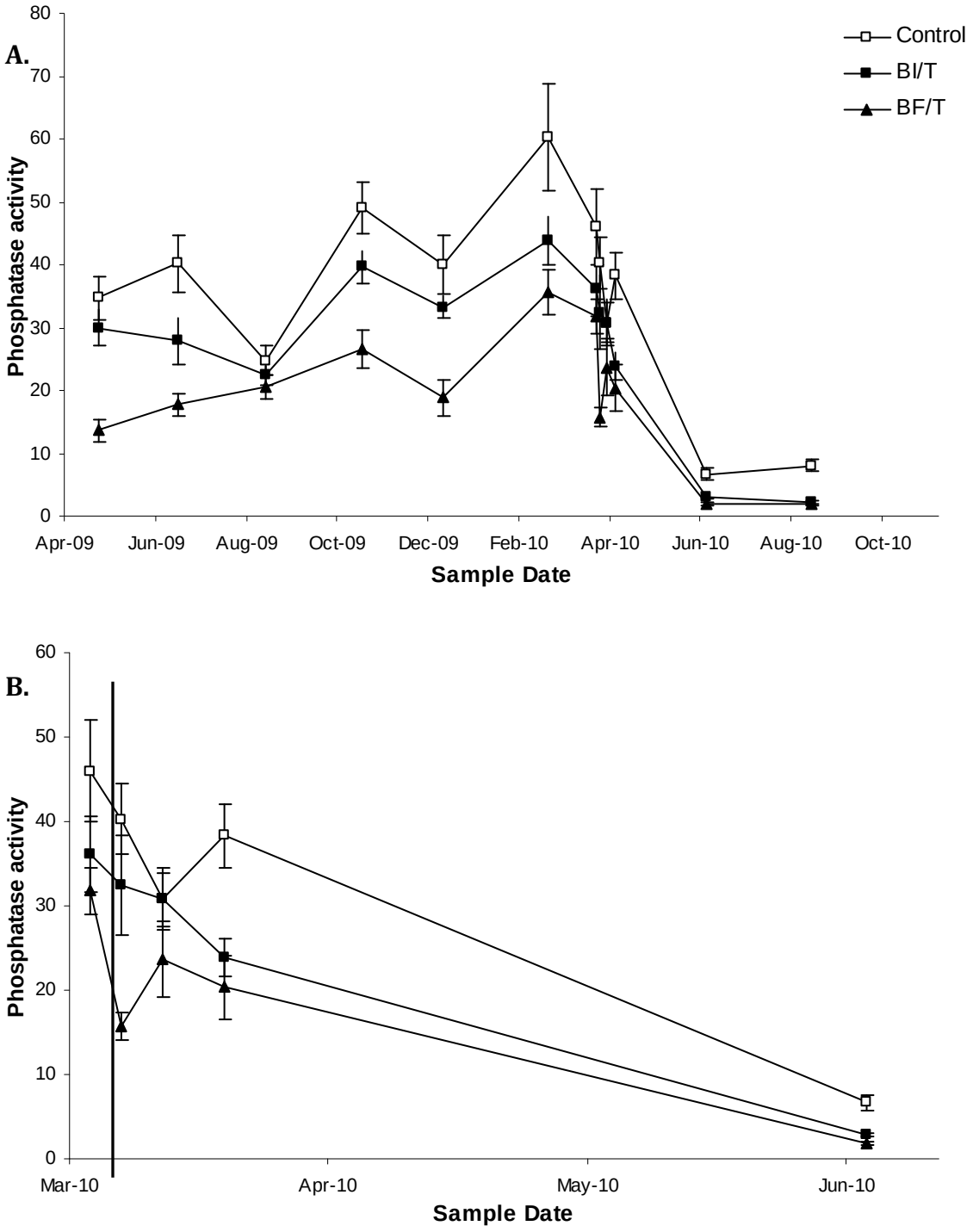

Figure 2. Soil phosphatase activity on each sampling date for control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned plots (BF/T; shaded triangles) at Site 1. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil phosphatase activity over the entire sampling period. B: soil phosphatase activity immediately preceding and following the prescribed burn (line).

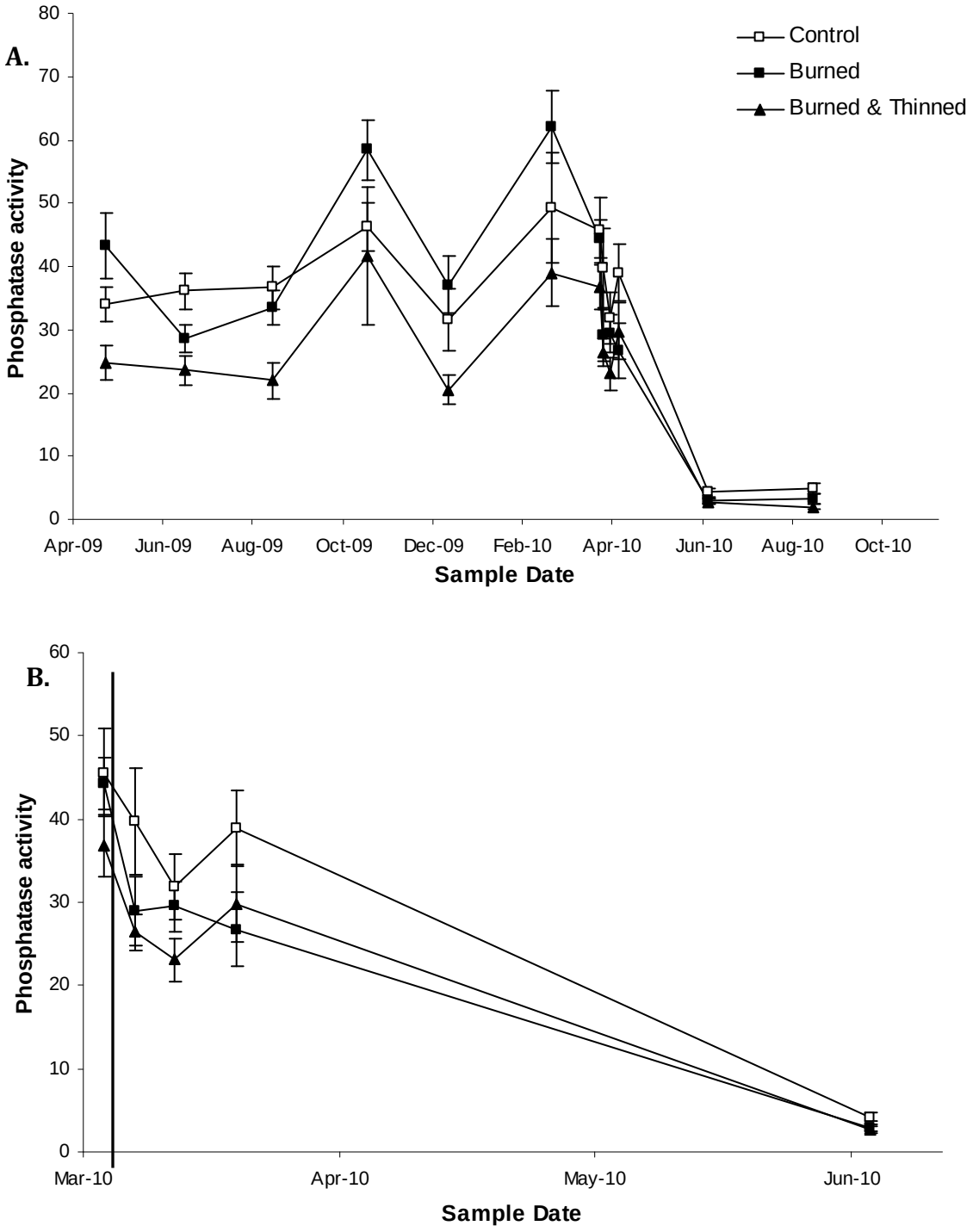

Figure 3. Soil phosphatase activity on each sampling date for control (open squares), burned (shaded squares), and burned and thinned plots (shaded triangles) at Site 2. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil phosphatase activity over the entire sampling period. B: soil phosphatase activity immediately preceding and following the prescribed burn (line).

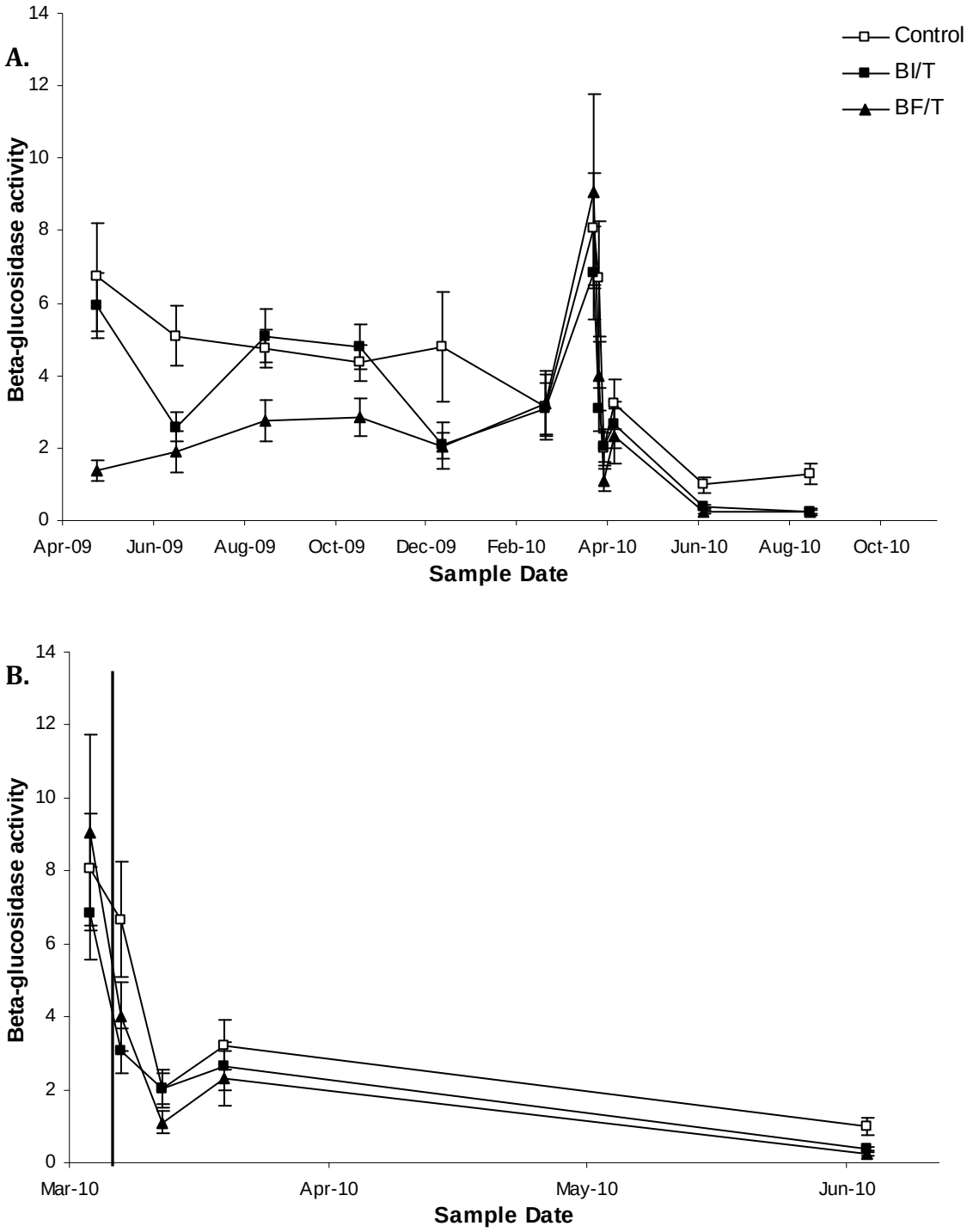

Figure 4. Soil β-glucosidase activity on each sampling date for control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned plots (BF/T; shaded triangles) at Site 1. Activity is reported in μmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil β-glucosidase activity over the entire sampling period. B: soil β-glucosidase activity immediately preceding and following the prescribed burn (line).

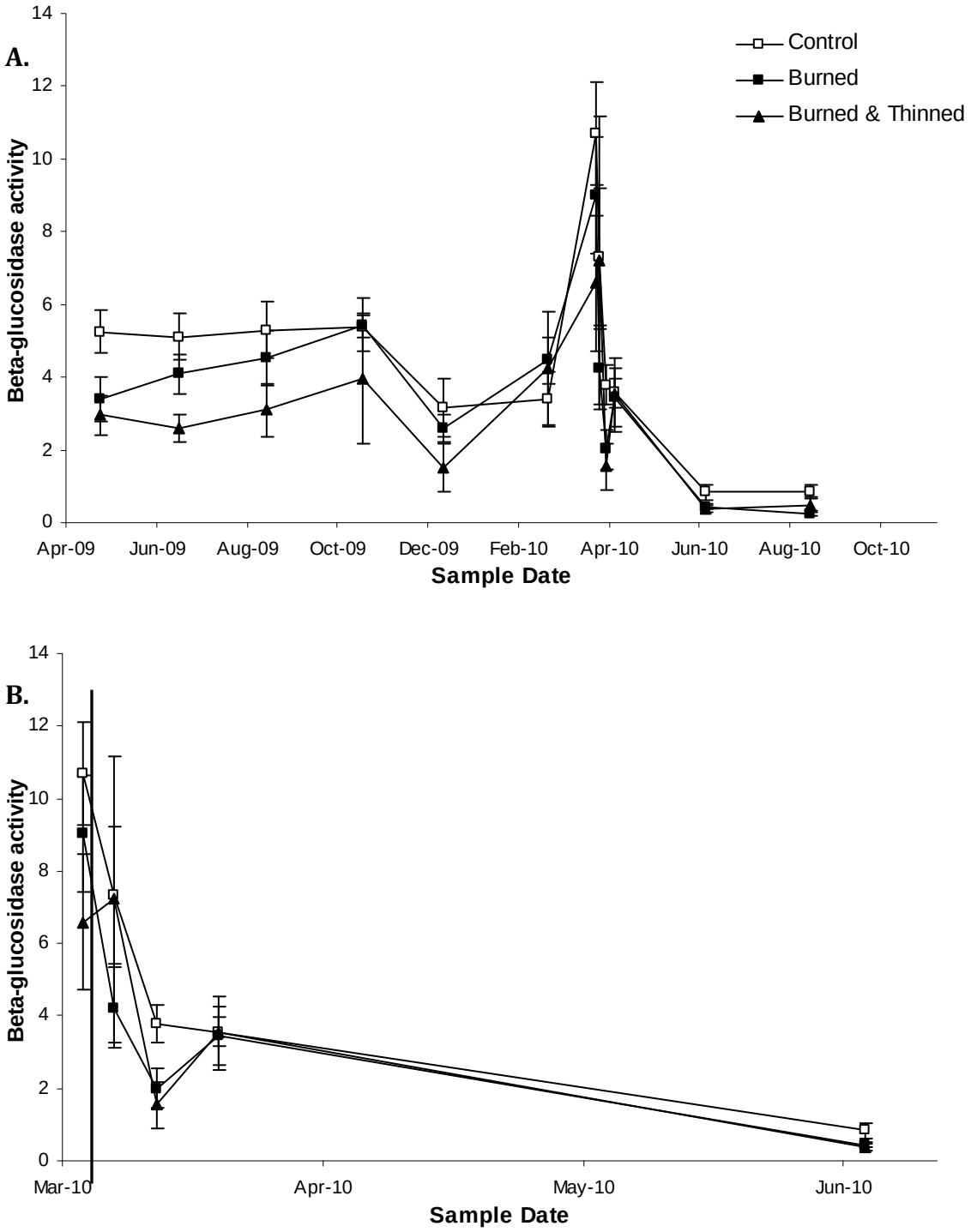

Figure 5. Soil β-glucosidase activity on each sampling date for control (open squares), burned (shaded squares), and burned and thinned plots (shaded triangles) at Site 2. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil β-glucosidase activity over the entire sampling period. B: soil β-glucosidase activity immediately preceding and following the prescribed burn (line).

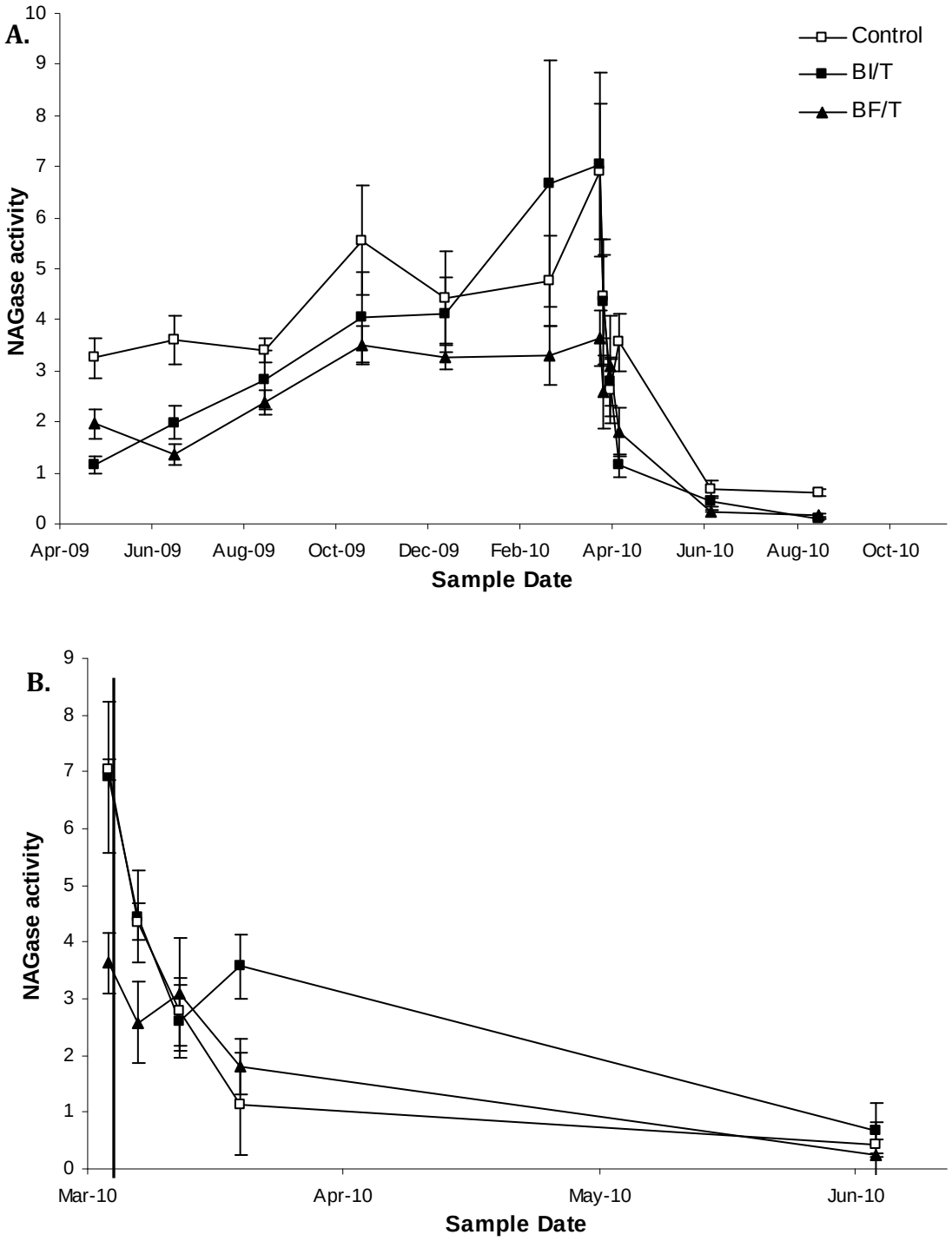

Figure 6. Soil NAGase activity on each sampling date for control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned plots (BF/T; shaded triangles) at Site 1. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil NAGase activity over the entire sampling period. B: soil NAGase activity immediately preceding and following the prescribed burn (line).

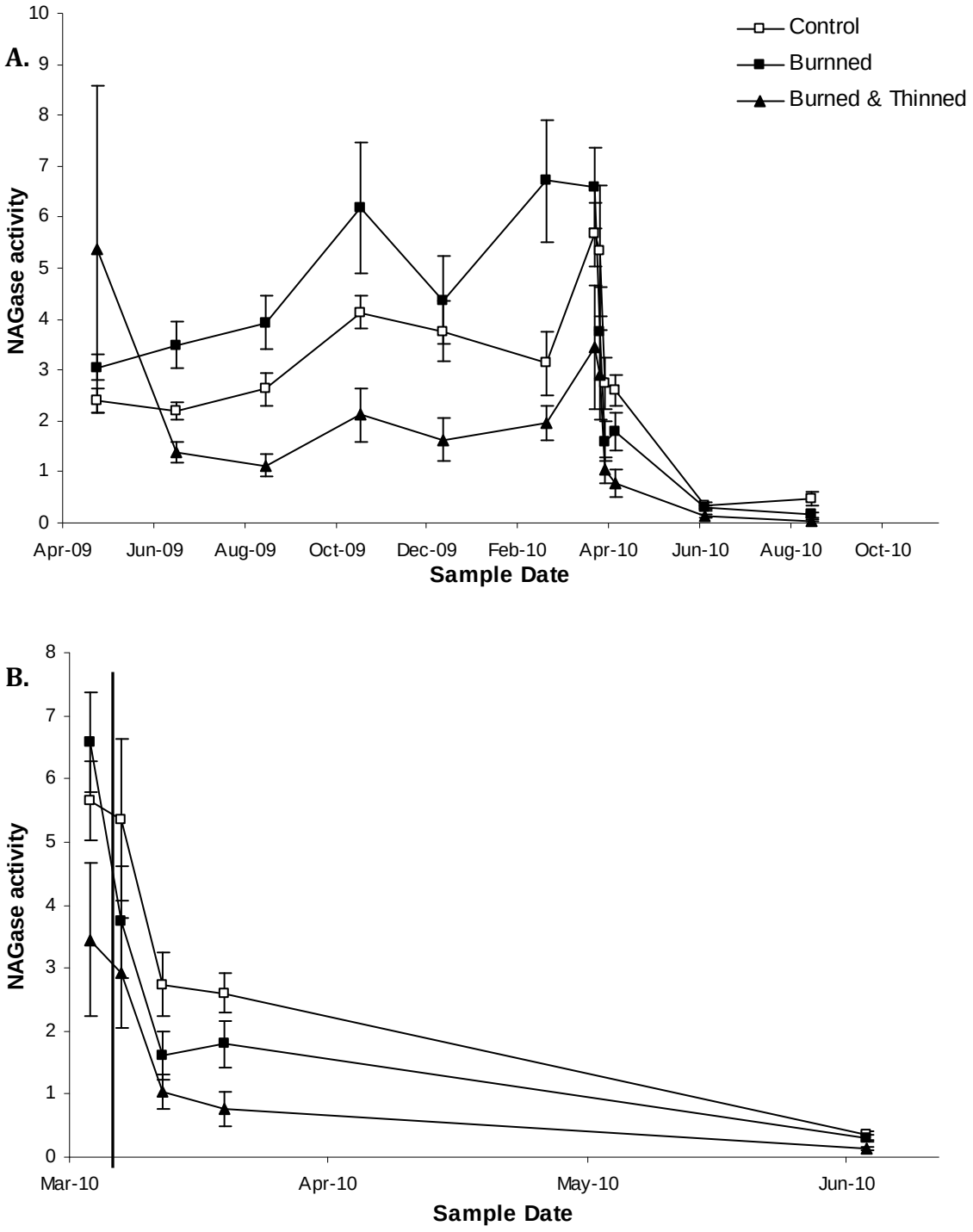

Figure 7. Soil NAGase activity on each sampling date for control (open squares), burned (shaded squares), and burned and thinned plots (shaded triangles) at Site 2. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil NAGase activity over the entire sampling period. B: soil NAGase activity immediately preceding and following the prescribed burn (line).

activity occurred on the June 13th 2010 sampling at both sites and for all plots (Figs. 4 and 5). Overall, the temporal patterns for β-glucosidase were similar to those for phosphatase, and ß-glucosidase activity in all plots (both treatments and controls) increased during the second spring of sampling, and decreased after the fire, (Figs. 4 and 5).

The highest observed values for NAGase activity occurred on the March 30th sampling at Site 1 and the February 25th 2010 sampling at Site 2 (Figs. 6 and 7). In each case, the highest NAGase activity was observed in the treated plots. As with the other hydrolytic enzymes, the lowest values recorded for NAGase activity occurred on the June 13th 2010 sampling at both sites and for all plots (Figs. 6 and 7). Overall temporal trends in NAGase activity at Site 1 suggested an increase in activity in all plots up to the March 30th 2010 sampling date, after which all NAGase activity dropped on the post-fire sampling dates (Fig. 6). Site 2 showed the same general pattern in soil NAGase activity; however there was more temporal variation at this site (Fig. 7). NAGase resembled phosphatase and ß-glucosidase at Site 1, in that the control plot typically showed the highest activity, followed by the burned infrequently and thinned and burned frequently and thinned plots, but differed in that activity at the burned infrequently and thinned plot surpassed that in the control plot on the February 25th 2010 sampling date (Fig. 6). At Site 2, the burned plot exhibited higher NAGase activity for most of the study period (Fig. 7). Activity of the oxidative enzymes was more erratic: phenol oxidase activity ranged from 0 - 2.1 $\mu mol\ h^{-1}\ g^{-1}$ dry weight, and was undetectable for 42 assays (Figs. 8 and 9). Lignin-peroxidase activity could always be detected, and ranged from 0.25 – 16 $\mu mol\ h^{-1}\ g^{-1}$ dry weight (Figs. 10 and 11). At Site 1, the highest phenol oxidase activity was observed in the burned frequently and thinned plot on March 30th 2010, while the burned infrequently and thinned plot showed the highest activity on April 24th 2009, and the control plot showed the highest activity on February

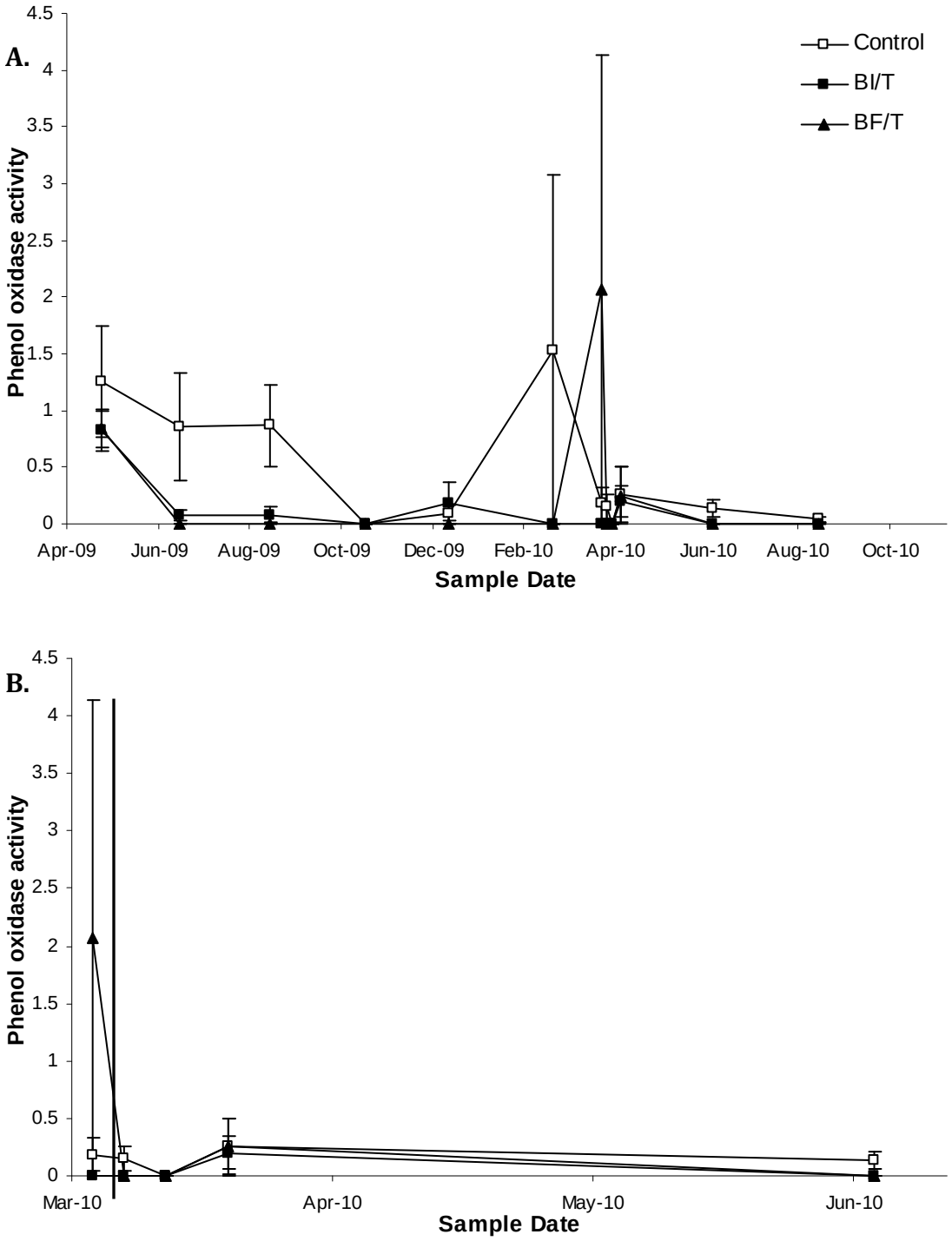

Figure 8. Soil phenol oxidase activity on each sampling date for control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned plots (BF/T; shaded triangles) at Site 1. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil NAGase activity over the entire sampling period. B: soil NAGase activity immediately preceding and following the prescribed burn (line).

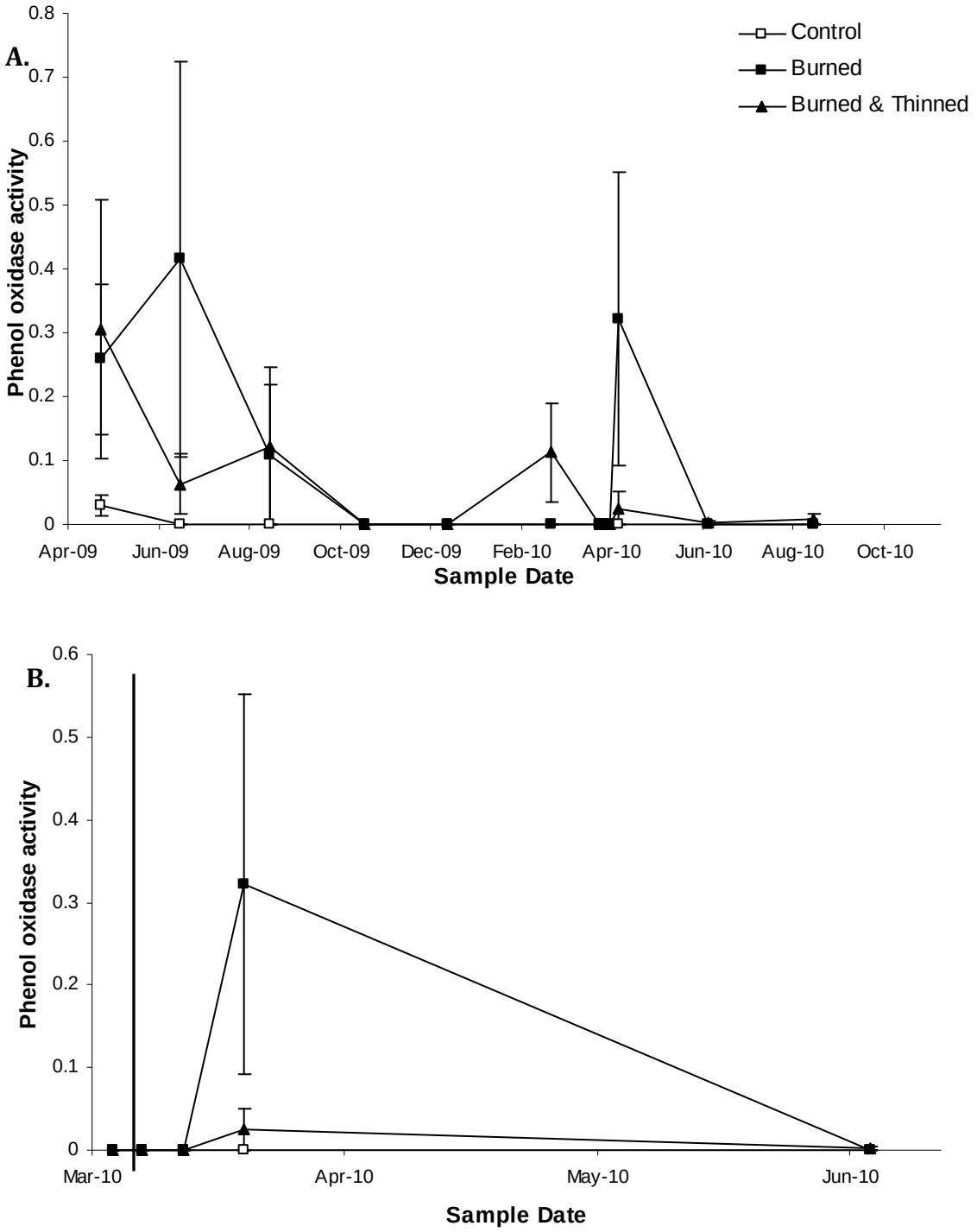

Figure 9. Soil phenol oxidase activity on each sampling date for control (open squares), burned (shaded squares), and burned and thinned plots (shaded triangles) at Site 2. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil phenol oxidase activity over the entire sampling period. B: soil phenol oxidase activity immediately preceding and following the prescribed burn (line).

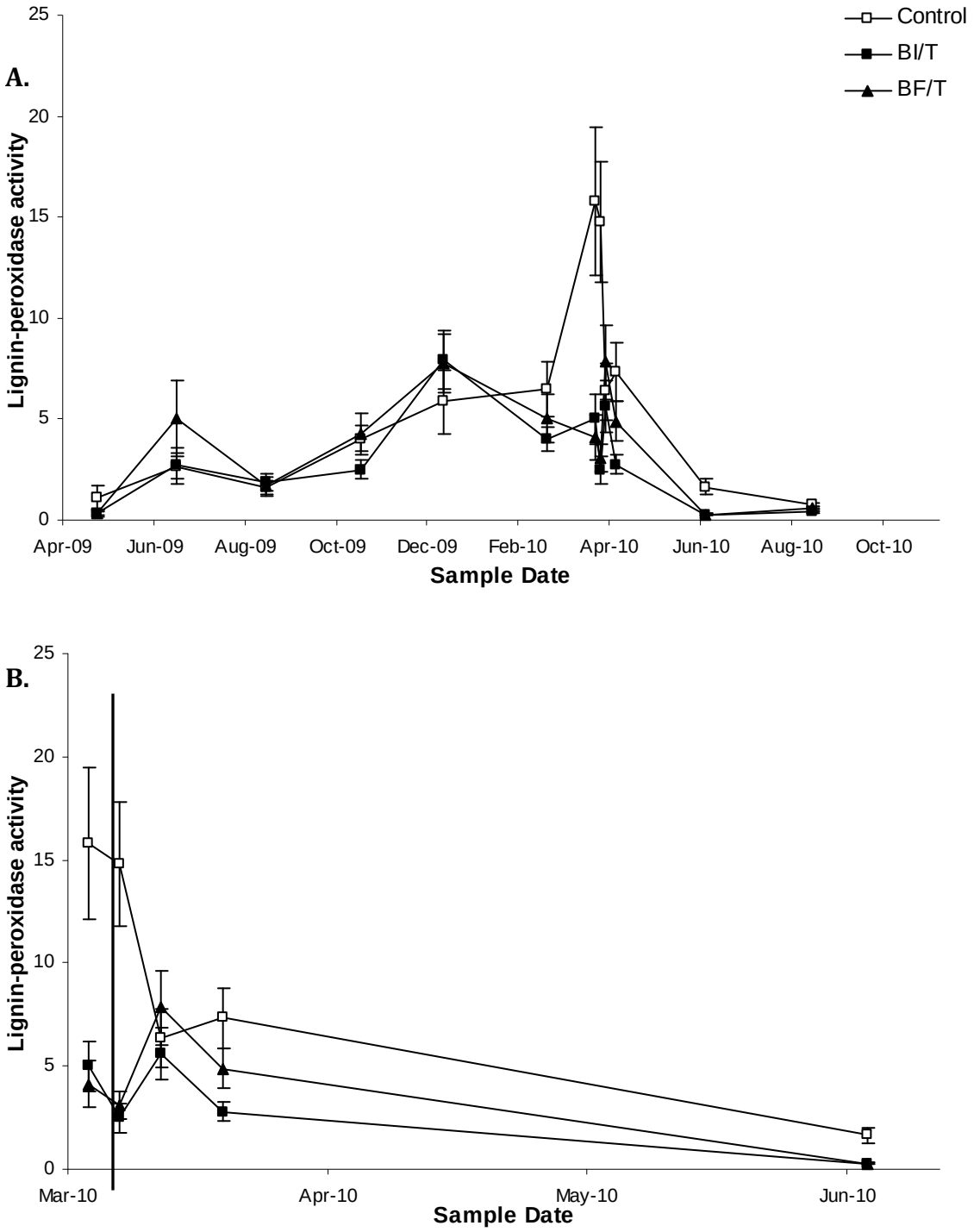

Figure 10. Soil lignin-peroxidase activity on each sampling date for control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned plots (BF/T; shaded triangles) at Site 1. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil lignin-peroxidase activity over the entire sampling period. B: soil lignin-peroxidase activity immediately preceding and following the prescribed burn (line).

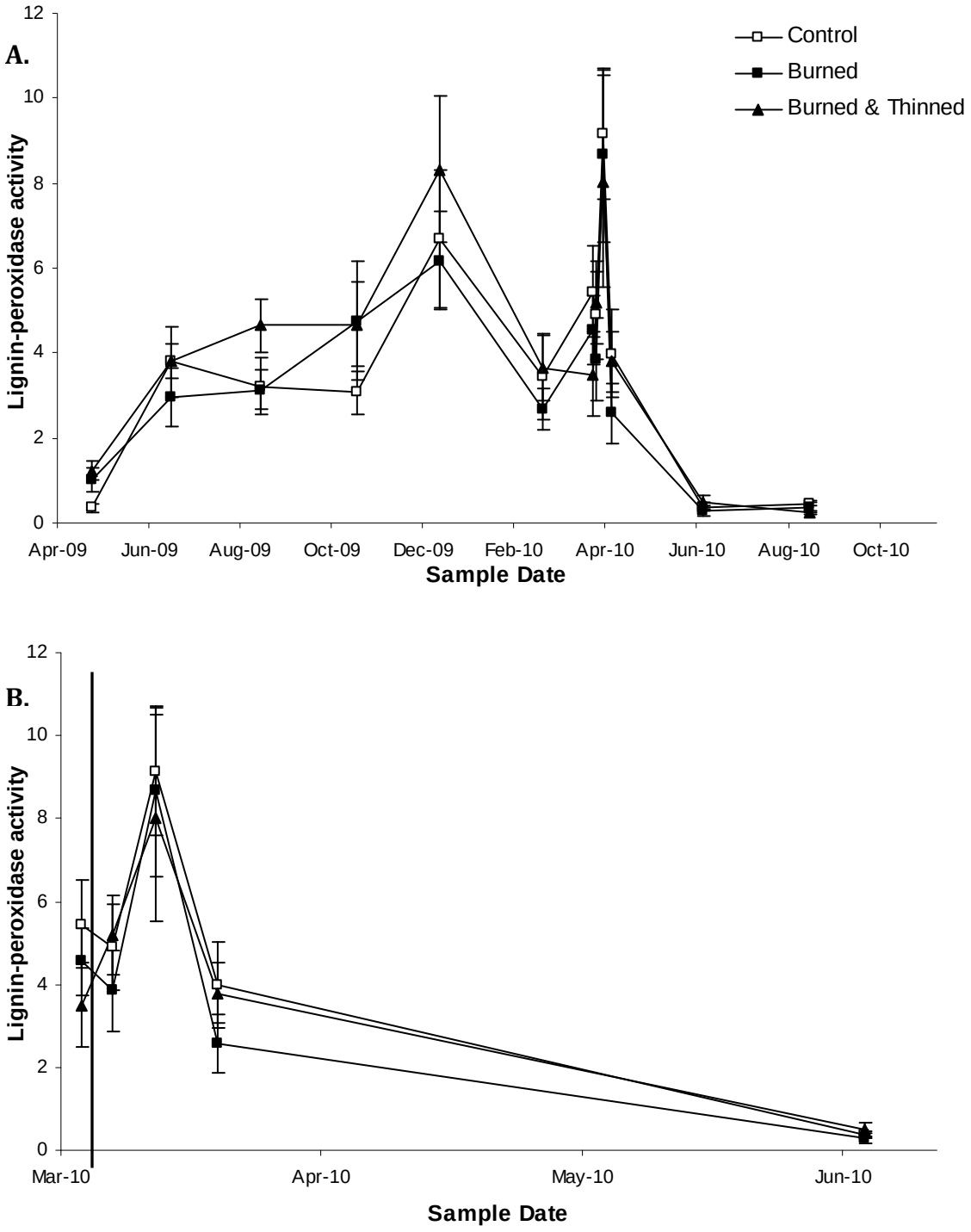

Figure 11. Soil lignin-peroxidase activity on each sampling date for control (open squares), burned (shaded squares), and burned and thinned plots (shaded triangles) at Site 2. Activity is reported in µmol h^{-1} g^{-1} dry weight soil and is the mean (+/- SE) of 15 sample points per plot. A: soil lignin-peroxidase activity over the entire sampling period. B: soil lignin-peroxidase activity immediately preceding and following the prescribed burn (line).

25th 2010 (Fig. 8). Soil phenol oxidase activity at Site 2 showed even more temporal variability than at Site 1 particularly in the burned plot, where the highest activity was recorded on June 17th 2009 (Fig. 9). Overall, phenol oxidase activity in the various plots at both sites was typically low for the duration of the experiment, and did not exhibit any clear temporal patterns (Figs. 8 and 9).

Lignin-peroxidase activity was also variable but showed somewhat more defined temporal patterns than phenol oxidase (Figs. 10 and 11). At Site 1, the control plot showed the highest observed activity on March 30th 2010 (Fig. 10), and the control plot at Site 2 showed the highest observed activity a week later on April 6th 2010 (Fig.11). Lignin-peroxidase activity in the Site 1 control plot generally increased up to the March 30th 2010 sampling date, after which point activities declined (Fig. 10). However, the burned infrequently and thinned and burned frequently and thinned plots at Site 1 did not exhibit this pattern as clearly (Fig. 10). All of the plots at Site 2 exhibited an increase in lignin-peroxidase activity over the first eight months of the study that was similar to that of the control plot at Site 1; however activity decreased after December 16th 2009 and then increased again on the April 6th 2010 sampling date (Fig. 11). Overall, lignin-peroxidase activities were generally similar for all plots, and the lowest observed activity for all plots at either site was recorded on the last two sampling dates (Figs. 10 and 11).

Discriminant analysis revealed how treatments were separated based upon the change in environmental variables after the prescribed burn, and which variables were the most important in separating the treatments. The change in each environmental variable was then used in a multi-source regression with the change in the activity of each enzyme and site, regardless of treatment. Treatments themselves were not directly used in the multi-source regression because of concerns over pseudo-replication and differences between sites. Thus, use of discriminant analysis detected indirect relationships between soil enzyme activity and treatments via

environmental variables that were affected by burning and burning and thinning treatments. When the data from both sites were analyzed together, changes in the activity of ß-glucosidase, phosphatase, and NAGase after the prescribed burn (calculated as mean post-fire activity subtracted from mean pre-fire activity) showed a significant ($p<0.05$) negative relationship with the change in soil moisture (Appendix B). However, changes in soil moisture were not related to the treatment type (Fig. 1), so this relationship is not attributed to the effects of burning. The change in phosphatase activity showed a significant relationship with site ($p<0.05$) but also showed a significant ($p<0.05$) negative relationship with the change in soil organic matter content. This relationship was attributed to an effect of burning, as soil organic matter is responding to a site by treatment interaction (Fig. 1). Together these two results suggest that fire increased soil organic matter, thereby reducing the activity of phosphatase. NAGase activity showed a highly significant ($p<0.01$) relationship with the change in soil moisture, but soil moisture content was not related to treatments (Fig. 1). Therefore, this relationship was not attributed to the effects of burning or thinning. Post-fire changes in phenol oxidase and lignin-peroxidase showed no relationship to either changes in environmental variables or site.

Because of the inherent differences amongst sites (as revealed by PERMANOVA, discriminant analysis, vegetation, and site history), change in enzyme activity (calculated as mean post-fire activity subtracted from mean pre-fire activity) was also analyzed individually for each site. At Site 1 (Appendix C), ß-glucosidase was the only enzyme that showed a significant ($p<0.05$) negative relationship between its change in activity after the fire and any of the changes in environmental variables; this was found for soil moisture, which not related to the treatments (Fig. 1). More soil enzymes showed changes in activities that were related to changes in environmental variables after the prescribed burn at Site 2 (Appendix D) than Site 1. The change

in phosphatase activity showed significant ($p<0.05$) negative relationships with both change in soil organic matter content and change in soil moisture. Soil moisture was not related to the treatment plots (Fig. 1), yet soil organic matter was, therefore the reduction in phosphatase activity was attributed to an effect of burning. Change in NAGase activity showed a very highly significant ($p< 0.001$) negative relationship to the change in litter depth, as well as a weaker relationship to change in soil moisture ($p<0.05$). Litter depth was related to treatment plots (Fig. 1), where the reduction in litter depth was greater in the burned infrequently and thinned and burned only plots; therefore the change in NAGase activity can be attributed to the treatment affects on litter depth where a large reduction in litter depth corresponded to only a slight reduction in NAGase activity. Post-fire changes in phenol oxidase activity also showed significant negative relationships to changes in environmental variables at Site 2, where changes in soil moisture and litter depth were related to phenol oxidase activity ($p<0.05$). As with NAGase activity, the change in phenol oxidase activity can be attributed to the treatments affects on litter depth. Lignin-peroxidase shows no significant relationships with measured environmental variables at either site.

Leaf Litter Decomposition

After 277 days in the field, 77% of the mass remained of *Q. falcata* leaves placed in litterbags in the control plot at Site 1 (Fig. 12). The burned infrequently and thinned and burned frequently and thinned plots, however, had less mass remaining, with 73% and 71% remaining at the end of the study, respectively (Fig. 12). Similar amounts of *Q. falcata* leaves remained at the end of the study at Site 2, 78% of the mass remained in the control plot, compared to 73% in the

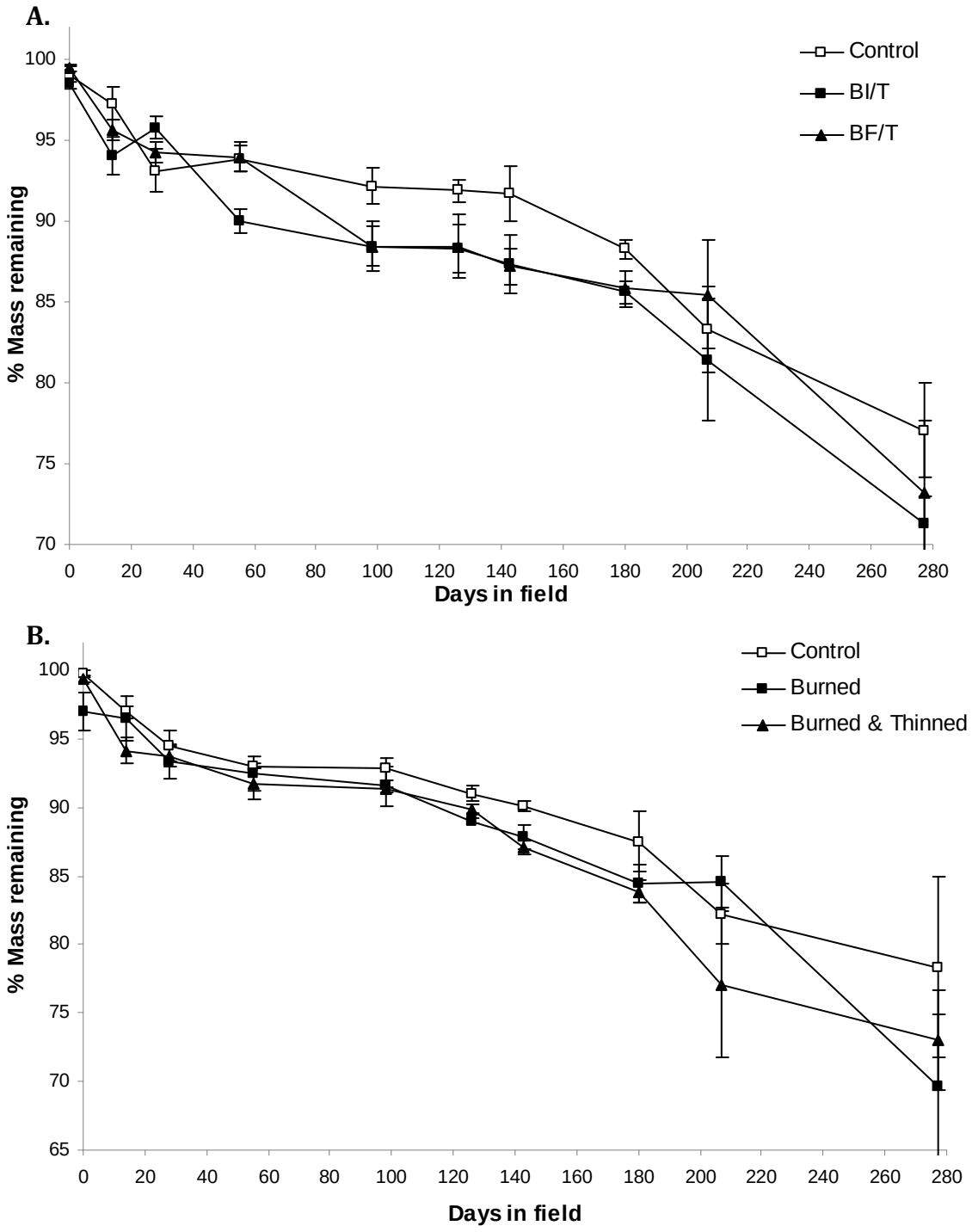

Figure 12. Mean (+/- SE) % initial dry mass remaining of *Q. falcata* leaves in litterbags in control (shaded squares), burned infrequently and thinned (BI/T; open squares), and burned frequently and thinned (BF/T; shaded triangle) plots at Site 1 (A) and control (shaded squares), burned (open squares), and burned and thinned (shaded triangle) plots at Site 2 (B). Day 0 = 11/19/2009. Treatment plots burned on day 133 = 4/1/2010.

burned plot and 70% in the burned and thinned plot (Fig. 12). A mass loss curve was generated using linear, exponential, and second order polynomial regression for each plot at each site (Table 4). R-squared values for each model were similar for each plot, with the second order polynomial model generally having only a slightly higher r-squared value. As the difference between the second order polynomial and linear models was negligible, the simpler linear model was used to calculate decay rates. The highest decomposition rate at Site 1 was seen in the burned infrequently and thinned plot, where leaves lost 0.082% of their original dry mass per day. The slowest decomposition at this site occurred for leaves in the control plot, with 0.068% of mass lost per day. At Site 2 the burned and thinned, and burned plots showed similar decomposition rates (0.086% and 0.082% dry mass lost per day, respectively) while, as with Site 1, the decay rate for litterbags in the control plot at Site 2 was lower (0.069 % dry mass lost per day; Table 4). However, these decay rates were not significantly different from each other ($p>0.05$) so there was no definite effect of treatments on decomposition rate.

Enzyme Activities on Decomposing Leaf Litter

Activities of extracellular enzymes associated with decomposing *Q. falcata* leaf litter in litterbags showed no consistent patterns amongst treatments or between individual enzymes (Figs. 13-17). ß-glucosidase showed the highest activity on leaf litter, and ranged between 14.5 and 138 µmol h^{-1} g^{-1} dry weight (Fig. 13). Litter at both sites showed a similar temporal pattern where ß-glucosidase activity was initially high, then decreased up until 55 days in the field when it leveled off to a consistent lower activity level for the duration of the study (Fig. 13). At Site 1, the highest ß-glucosidase activity was seen on leaf litter in the burned infrequently and thinned plot after 14 days in the field followed by litter in the control and burned frequently and thinned

Table 4. Slopes, R^2 values, and decomposition constant (k) for linear, exponential, and polynomial curves for control, burned infrequently and thinned (BI/T), and burned frequently and thinned (BF/T) plots at Site 1 and control, burned, and burned and thinned plots at Site 2.

Site 1

		Slope	R^2	*k*
Control				
	Linear	-0.0677	0.901	0.068
	Exponential	-0.0008	0.890	
	Polynomial	-0.0238	0.940	
BI/T				
	Linear	-0.0816	0.924	0.082
	Exponential	-0.0010	0.908	
	Polynomial	-0.0442	0.944	
BF/T				
	Linear	-0.0773	0.923	0.077
	Exponential	-0.0009	0.908	
	Polynomial	-0.0514	0.934	

Site 2

		Slope	R^2	*k*
Control				
	Linear	-0.0694	0.945	0.069
	Exponential	-0.0008	0.940	
	Polynomial	-0.0491	0.954	
Burned				
	Linear	-0.0828	0.894	0.083
	Exponential	-0.0010	0.862	
	Polynomial	-0.0125	0.961	
Burned and thinned				
	Linear	-0.0857	0.933	0.086
	Exponential	-0.0010	0.925	
	Polynomial	-0.0533	0.947	

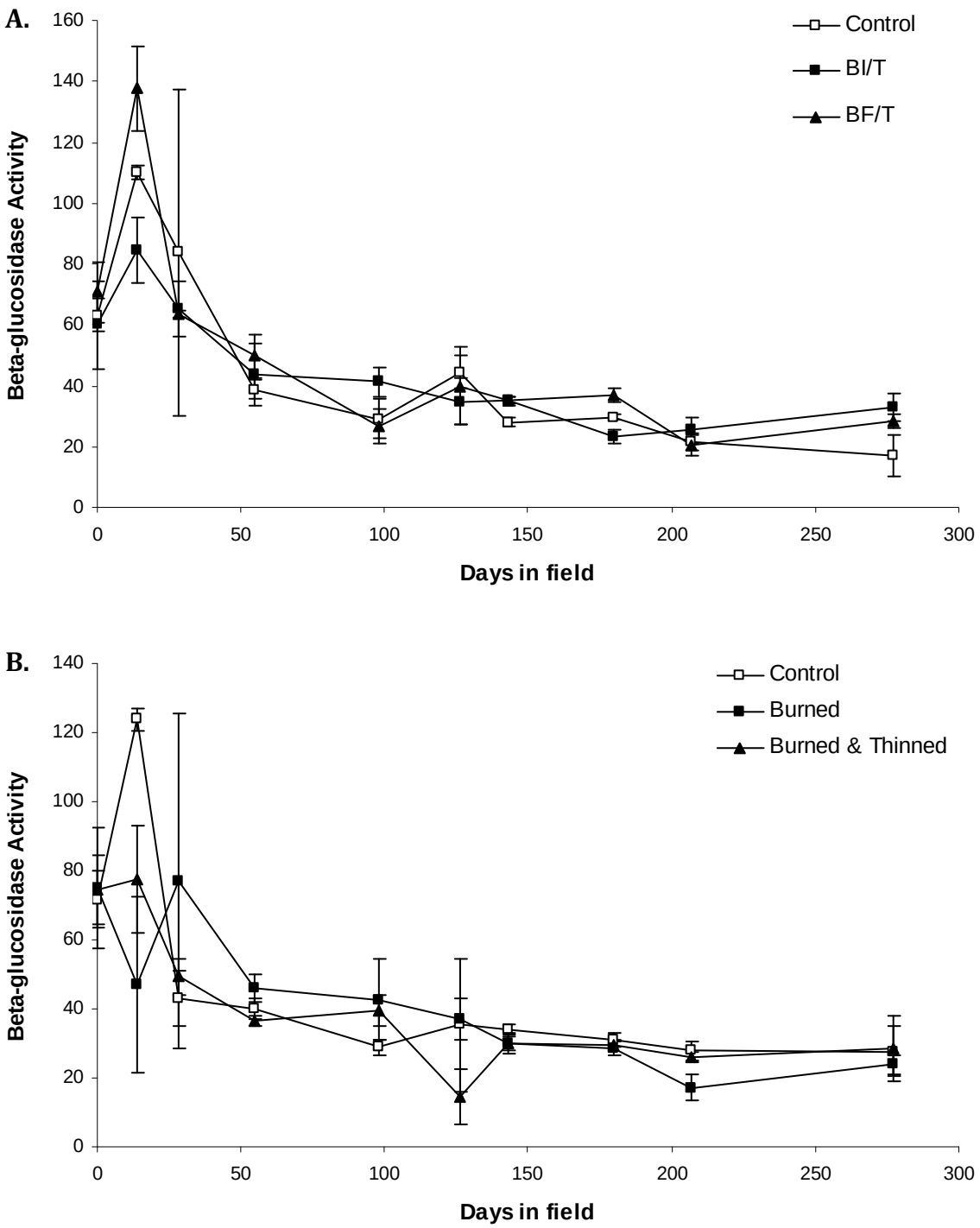

Figure 13. β-glucosidase activity associated with decomposing *Q. falcata* leaves in litterbags in control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned (BF/T; shaded triangles) plots at Site 1 (A) and control, burned, and burned and thinned plots at Site 2 (B). Activity is reported as μmol h^{-1} g^{-1} dry leaf weight and is the mean (+/- SE) of three litterbags per date. Day 0 = 11/19/2009. Treatment plots burned on day 133 = 4/1/2010.

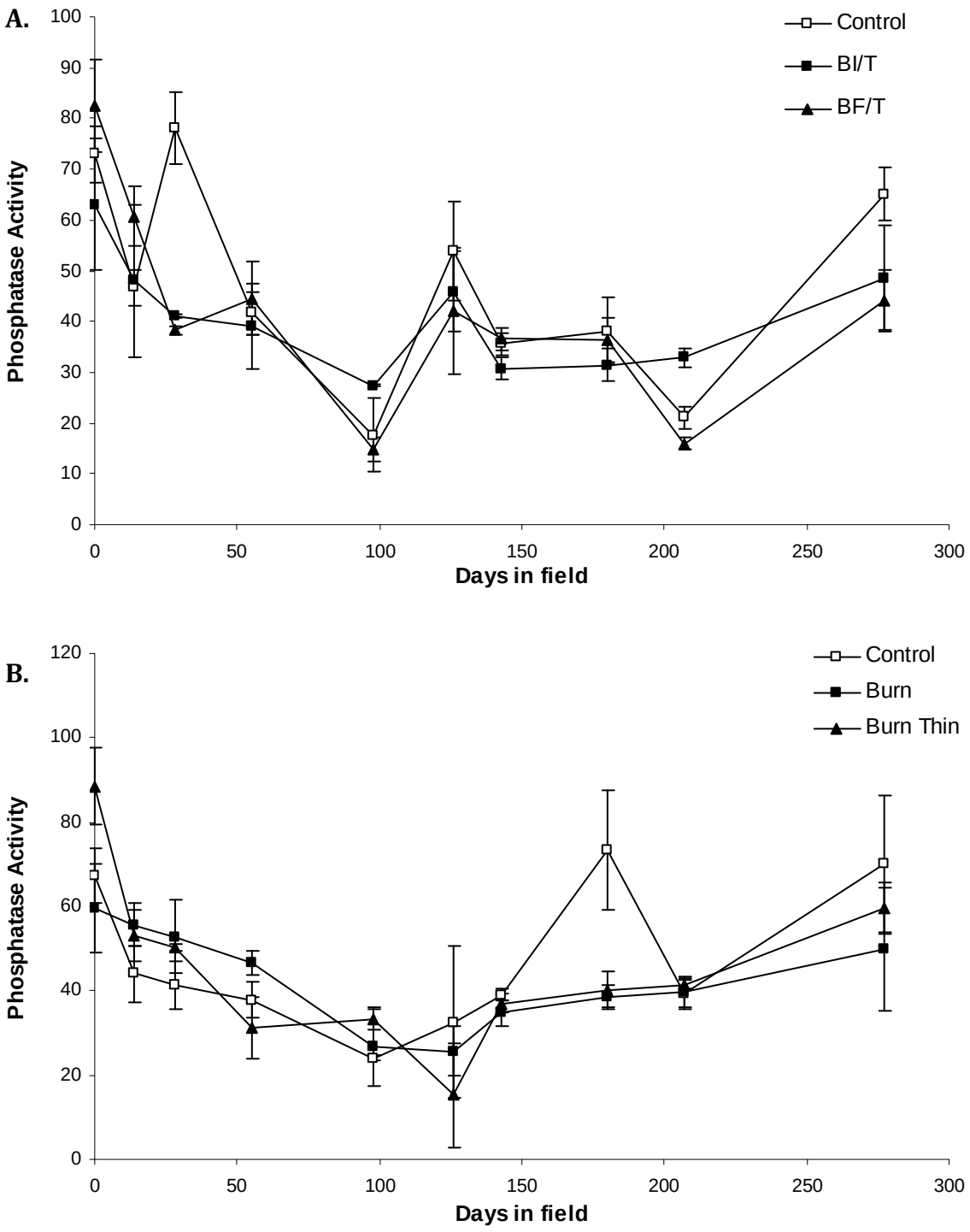

Figure 14. Phosphatase activity associated with decomposing *Q. falcata* leaves in litterbags in control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned (BF/T; shaded triangles) plots at Site 1 (A) and control, burned, and burned and thinned plots at Site 2 (B). Activity is reported as µmol h^{-1} g^{-1} dry leaf weight and is the mean (+/- SE) of three litterbags per date. Day 0 = 11/19/2009. Treatment plots burned on day 133 = 4/1/2010.

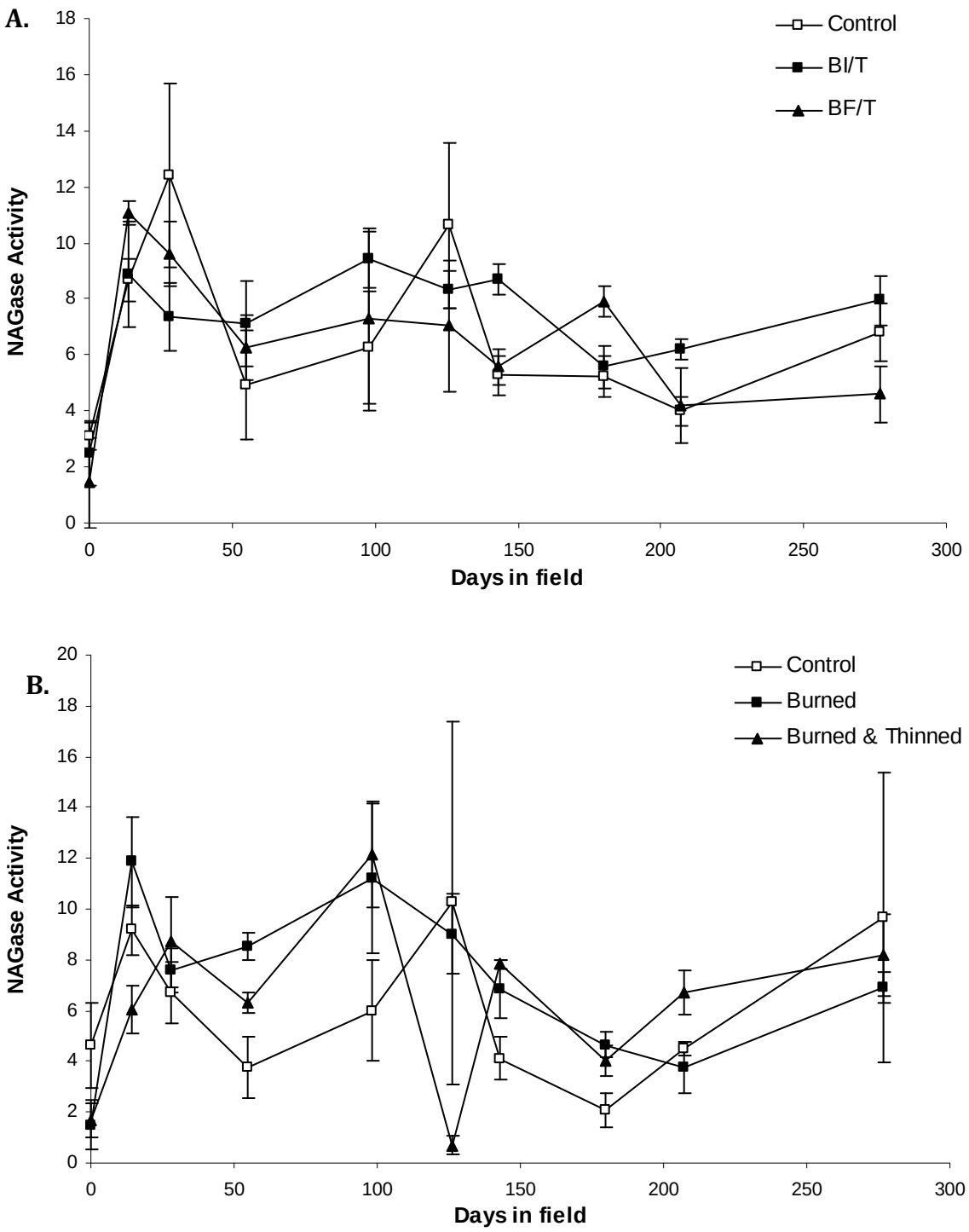

Figure 15. NAGase activity associated with decomposing *Q. falcata* leaves in litterbags in control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned (BF/T; shaded triangles) plots at Site 1 (A) and control, burned, and burned and thinned plots at Site 2 (B). Activity is reported as µmol h^{-1} g^{-1} dry leaf weight and is the mean (+/- SE) of three litterbags per date. Day 0 = 11/19/2009. Treatment plots burned on day 133 = 4/1/2010.

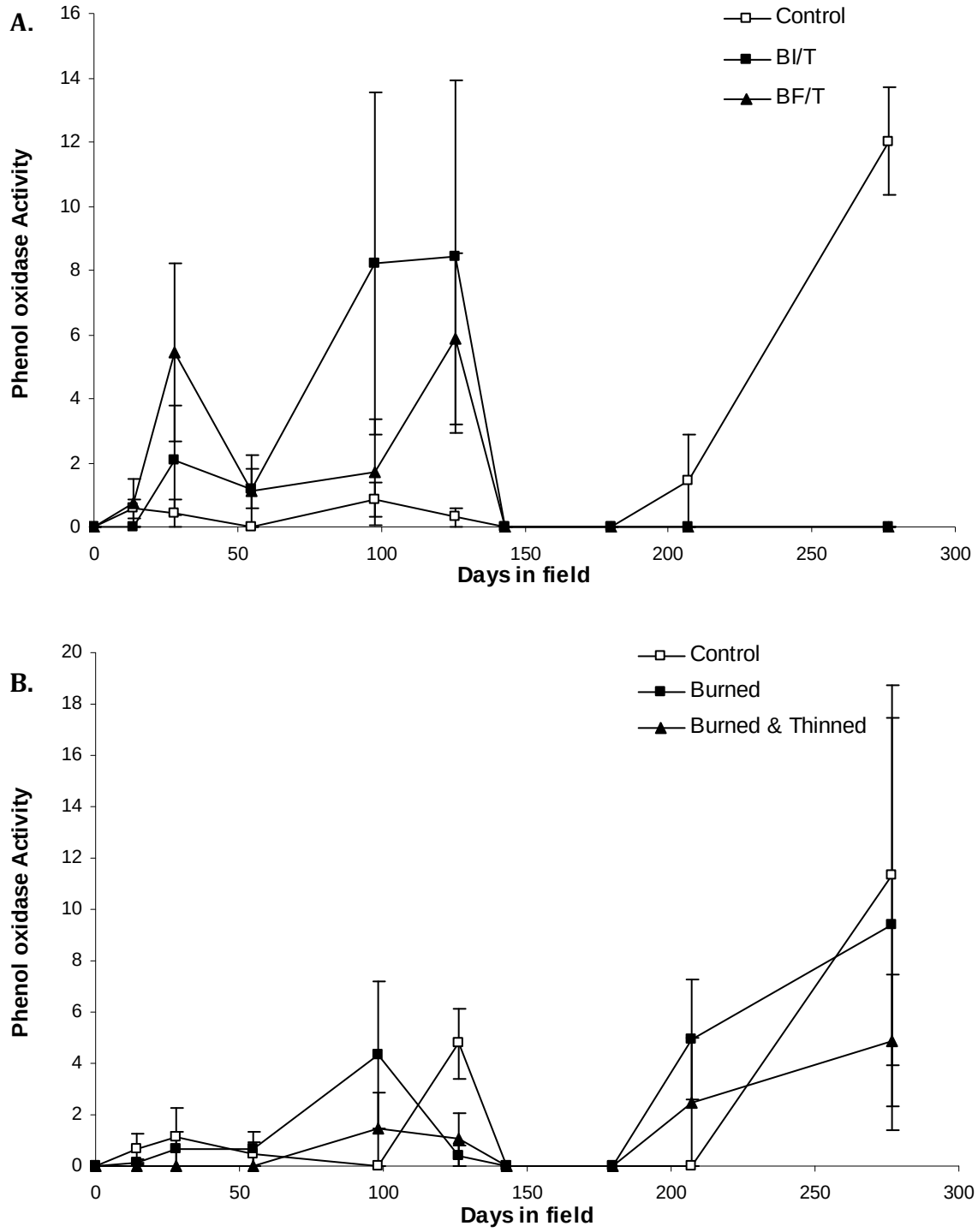

Figure 16. Phenol oxidase activity associated with decomposing *Q. falcata* leaves in litterbags in control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned (BF/T; shaded triangles) plots at Site 1 (A) and control, burned, and burned and thinned plots at Site 2 (B). Activity is reported as µmol h^{-1} g^{-1} dry leaf weight and is the mean (+/- SE) of three litterbags per date. Day 0 = 11/19/2009. Treatment plots burned on day 133 = 4/1/2010.

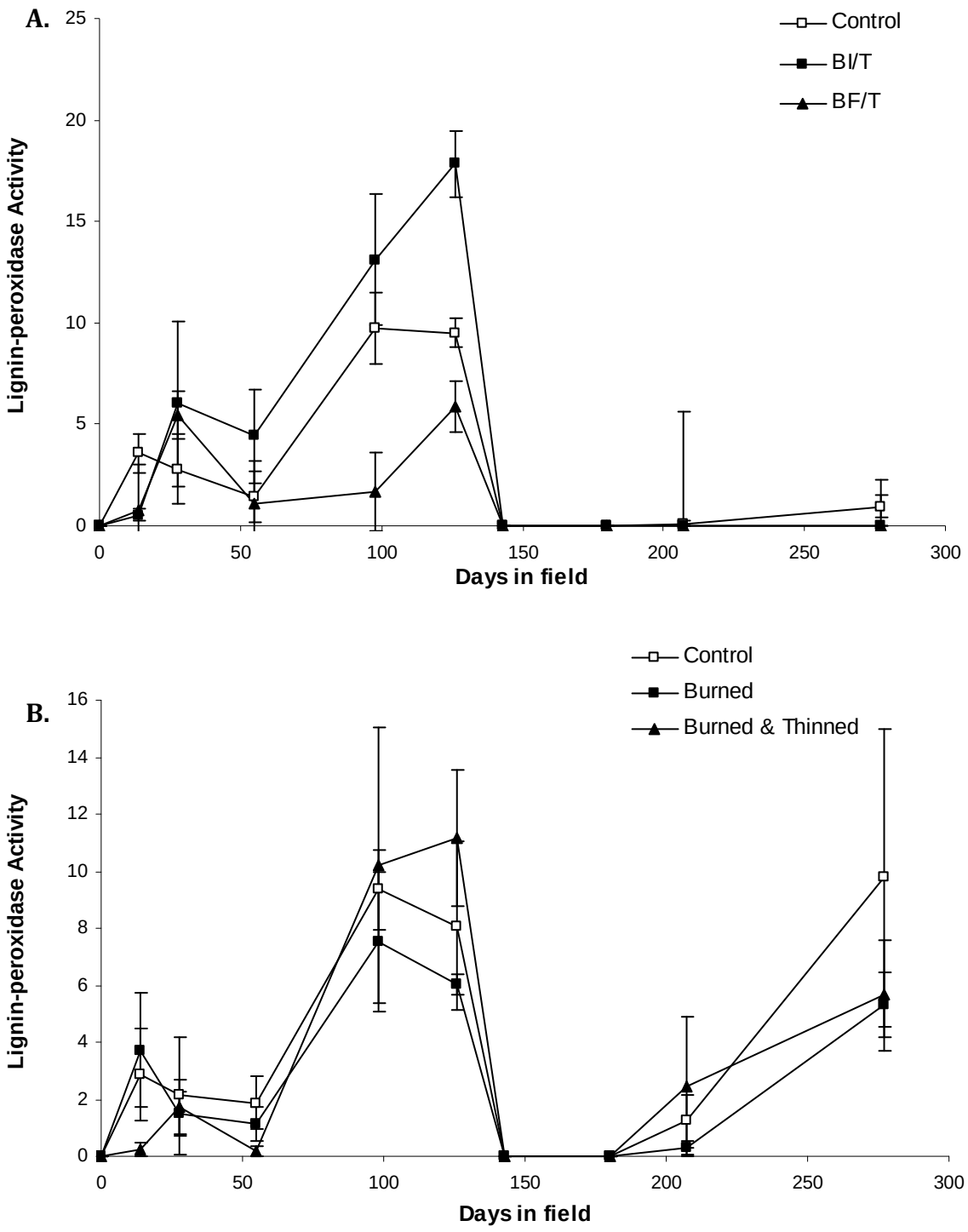

Figure 17. Lignin-peroxidase activity associated with decomposing *Q. falcata* leaves in litterbags in control (open squares), burned infrequently and thinned (BI/T; shaded squares), and burned frequently and thinned (BF/T; shaded triangles) plots at Site 1 (A) and control, burned, and burned and thinned plots at Site 2 (B). Activity is reported as µmol h^{-1} g^{-1} dry leaf weight and is the mean (+/- SE) of three litterbags per date. Day 0 = 11/19/2009. Treatment plots burned on day 133 = 4/1/2010.

plots on this date, respectively. The highest observed ß-glucosidase activity in litterbags at Site 2 occurred in the control plot, also after 14 days in the field (Fig. 13).

Phosphatase activity on leaf litter was also high, ranging from 14.7 to 89 µmol h^{-1} g^{-1} dry weight, and observed litterbag phosphatase activity was more variable than that of ß-glucosidase (Fig. 14). For both sites, leaf litter phosphatase activities were initially high, decreased over the first half of the study (approx. between 50 and 200 days in the field), and then increased again by the last collection after 277 days (Fig. 14). Phosphatase activity on leaves decaying at Site 1 was highest in the control plot after 28 days in the field and lowest in the burned frequently and thinned plot after 98 days in the field (Fig. 14). At Site 2, leaf litter phosphatase activity was highest in the control plot after 180 days in the field and lowest in the burned and thinned plot after 126 days in the field (Fig. 14).

The activity of NAGase on leaf litter ranged between 0.7 and 12.4 µmol h^{-1} g^{-1} dry weight and was more variable throughout the experiment than the other hydrolytic enzymes (Fig. 15). NAGase activity on decaying leaves varied over the sampling period, but generally exhibited mid-range levels of activity compared to the other enzymes. At Site 1, the highest NAGase activity occurred on leaves decaying in the control plot after 28 days in the field, with the lowest recorded activity occurring at the initial sampling of the burned frequently and thinned plot (Fig. 15). At Site 2, the highest observed NAGase activity was in litterbags in the burned and thinned plot after 98 days in the field, and the lowest recorded activity occurred in bags in the same plot on the next collection date (Fig. 15).

As with the soil enzymes, phenol oxidase activity on *Q. falcata* leaves showed the most erratic patterns of any leaf litter enzyme and had the most recorded litterbag collections with zero activity (30 collections). Lignin-peroxidase activity was also often low on the decaying leaves

and could not be recorded in 22 collections (Figs. 16 and 17). The highest recorded phenol oxidase activity on leaf litter was found after 277 days in the field in the control plot at Site 1; however this was the only point during the experiment at which observed activities from the control plot surpassed that of the other treatment plots (Fig. 16). The final litterbag collection also had the highest recorded phenol oxidase activity at Site 2, but for all plots rather than just the control (Fig. 16).

Lignin-peroxidase activity on decaying leaf litter was less variable than that of phenol oxidase (Fig. 17). The highest lignin-peroxidase activity recorded in the litterbags at Site 1 was in the burned infrequently and thinned plot after 126 days in the field (Fig. 17). Other plots at this site showed a similar pattern, and all plots showed no recorded activity on the next two collections (after 143 and 180 days; Fig. 17). Litterbags in both treatment plots continued to show zero activity levels for the duration of the experiment, however activity was observed in the control plot on the last collection date (277 days in the field; Fig. 17). At Site 2, a similar pattern is seen early on in the experiment, where highest observed litterbag lignin-peroxidase activity was recorded for the burned and thinned plots after 126 days (Fig. 17). Bags in the other plots also showed this spike in activity after both 98 and 126 days in the field, but had zero lignin-peroxidase activity for the next two collections (after 143 and 180 days; Fig. 17). However, in contrast to Site 1, all litterbags in all plots showed activity on the last two collections, with the control plot having its highest recorded leaf litter lignin-peroxidase activity on the final collection date (Fig. 17).

Linear regressions of % dry leaf mass remaining as a function of cumulative leaf litter enzyme activity showed significant negative ($p<0.05$) relationships for all enzymes in all plots (Table 5). Based on r-squared values, cumulative leaf litter phosphatase activity was the most

Table 5. Statistics from linear regressions of % dry leaf mass remaining as a function of cumulative leaf litter enzyme activity for *Q. falcata* leaves decomposing in litterbags in control, burned infrequently and thinned (BI/T), and burned frequently and thinned (BF/T) plots at Site 1 and control, burned and burned and thinned plots at Site 2.

Site 1

Treatment	*Enzyme*	*Slope*	R^2	*p-value*
Control	β-glucosidase	-0.002	0.760	0.002
	Phosphatase	-0.002	0.870	<0.001
	NAGase	-0.010	0.821	0.001
	Phenol oxidase	-0.031	0.714	0.004
	Lignin-peroxidase	-0.014	0.523	0.028
BI/T	β-glucosidase	-0.002	0.839	0.001
	Phosphatase	-0.002	0.916	<0.001
	NAGase	-0.010	0.880	<0.001
	Phenol oxidase	-0.021	0.546	0.023
	Lignin-peroxidase	-0.011	0.554	0.021
BF/T	β-glucosidase	-0.002	0.836	0.001
	Phosphatase	-0.002	0.891	<0.001
	NAGase	-0.011	0.856	<0.001
	Phenol oxidase	-0.037	0.598	0.014
	Lignin-peroxidase	-0.023	0.855	<0.001

Site 2

Treatment	*Enzyme*	*Slope*	R^2	*p-value*
Control	β-glucosidase	-0.001	0.907	<0.001
	Phosphatase	-0.002	0.972	<0.001
	NAGase	-0.012	0.909	<0.001
	Phenol oxidase	-0.032	0.776	0.002
	Lignin-peroxidase	-0.015	0.811	0.001
Burned	β-glucosidase	-0.002	0.748	0.003
	Phosphatase	-0.002	0.900	<0.001
	NAGase	-0.011	0.784	0.001
	Phenol oxidase	-0.033	0.956	<0.001
	Lignin-peroxidase	-0.027	0.744	0.003
Burned and thinned	β-glucosidase	-0.003	0.863	<0.001
	Phosphatase	-0.002	0.932	<0.001
	NAGase	-0.012	0.876	<0.001
	Phenol oxidase	-0.057	0.767	0.002
	Lignin-peroxidase	-0.019	0.781	0.002

correlated with mass loss of litter at Site 1, followed by activities of NAGase and then β-glucosidase (Table 5). The pattern at Site 2 was similar, where phosphatase activity on decaying leaves was also the most correlated to loss of litter; although cumulative NAGase and β-glucosidase activities predicted mass loss rates equally well (Table 5). Phenol oxidase activity on decaying leaves showed the least amount of correlation with mass loss of litter in all plots at both sites, followed by lignin-peroxidase, although these regressions were still significant (Table 5). The slopes of regressions of mass loss as a function of cumulative enzyme activity can be used as measures of the apparent efficiency of each enzyme at degrading *Q. falcata* leaf litter. In the control plots, the highest slopes were seen for phenol oxidase, with the next highest slope being for lignin-peroxidase and NAGase (Table 5). For the treatment plots, the same pattern was observed (phenol oxidase being more efficient than lignin-peroxidase which was more efficient than NAGase; Table 5) although the efficiency of phenol oxidase at Site 1 was much higher than at Site 2 (Table 5). Regressions between mass remaining and cumulative β-glucosidase and phosphatase activity had the lowest slopes in all plots, and within each plot the slopes for the two enzymes were generally similar (Table 5). For litterbags at Site 1, slopes relating mass remaining to activities of phenol oxidase and lignin-peroxidase (the two most efficient enzymes) were greatest in the burned frequently and thinned plot, followed by the control plot, and then the burned infrequently and thinned plot (Table 5). This pattern was not the same for litterbags at Site 2; the slope of phenol oxidase was greatest in the burned and thinned plot, followed by the burned and then control plot (Table 5), while the slope for lignin-peroxidase was greatest in the burned plot, followed by the burned and thinned and then control plots (Table 5).

Since all leaf litter enzyme activities showed significant ($p<0.05$) relationships for linear regressions of % mass remaining as a function of cumulative leaf litter enzyme activity, all

enzymes were combined into an integrated index of microbial activity (Table 6). Regardless of site or plot, all relationships between cumulative overall enzyme activity and mass loss were very highly significant ($p<0.001$; Table 6). At both sites, overall microbial activity was more efficient (higher slopes) at degrading *Q. falcata* leaf litter in the burned only and burned infrequently and thinned plots than in the other plots (Table 6). However, at Site 1, the efficiency was lower in the burned infrequently and thinned plot compared to the control, while at Site 2 the burned plot showed greater efficiency than the control (Table 6).

Table 6. Statistics from linear regressions of % dry leaf mass remaining as a function of cumulative standardized microbial activity in control, burned infrequently and thinned (BI/T), and burned frequently and thinned (BF/T) plots at Site 1 and control, burned and burned and thinned plots at Site 2.

Site 1

	Slope	R^2	*p-value*
Control	-0.178	0.814	0.001
BI/T	-0.149	0.815	0.001
BF/T	-0.188	0.837	0.001

Site 2

	Slope	R^2	*p-value*
Control	-0.168	0.926	<0.001
Burned	-0.176	0.856	<0.001
Burned and thinned	-0.204	0.894	<0.001

DISCUSSION

The overall aim of this study was to investigate changes in soil enzyme activity and litter decomposition rates in areas affected by fire. Fire has been an important natural disturbance in the history of eastern North American forests. More recently, prescribed burning has been introduced into fire suppressed closed canopy deciduous forests as a means of ecologically restoring historical plant communities and increasing overall biodiversity. Prescribed burning, coupled with thinning of the canopy aims to allow for greater solar penetration to the forest floor and a clearing of the understory to promote the germination of shade-intolerant herbaceous species. Indeed, much of the literature surrounding ecological restoration is focused on shifting plant communities to desired historical precedents; comparatively little attention has been paid to how restoration practices affect belowground processes.

When soils are burned, many physical changes occur that can affect microbially mediated processes. One of these changes is that fire tends to reduce soil moisture as well as soil water retention capabilities (DeBano, 2000). In this study, once correlations between measured environmental variables were accounted for by discriminant analysis, changes in soil moisture did not distinguish treatment plots. Fire is typically thought to reduce the amount of organic matter in the soil as well (Gonzalez-Perez et al., 2004). In the current study, however, fire appeared to interact with fire history and thinning to increase soil organic matter content in some plots. In areas with high pre-fire litter amounts (i.e. infrequently burned or unthinned areas), the

fires in 2010 were associated with either no decline in organic matter content (Site 2) or even an increase in soil organic matter content (Site 1). Changes in soil organic matter levels could affect belowground processes such as decomposition, microbial enzyme production and activity. Prescribed burning also has a large effect on the litter layer covering a closed canopy deciduous forest floor. In this study, the litter layer was completely combusted in burned plots, leaving behind only a blanket of ash, and the magnitude of litter reduction was greatest in areas that had not been thinned (burned only at Site 2), or that had not been burned in over six years (burned infrequently and thinned at Site 1). This likely limits nutrient inputs into these systems and could affect the activity of the decomposer community.

Over the course of the experiment, soil enzyme activities showed seasonal patterns that were somewhat consistent between enzymes, with activities peaking during the second spring of sampling. Similar findings have been reported in a different system, where phosphatase activity peaked in early spring and showed lowest activity in early autumn over a 12 month period (Kang and Freeman, 1999). The restoration treatments did have an effect on phosphatase activity, where a reduction in mean phosphatase activity after the prescribed burn was observed in relation to an increase in soil organic matter in plots that were either burned infrequently or burned only. While this study looked at the immediate effects of burning on enzyme activity, the findings are consistent with other studies that have looked at enzyme activity in a historical context over a period after a fire. In those studies, phosphatase activity was inhibited by the combination of burning and thinning, but not affected by fire alone (Boerner and Brinkman, 2003; Boerner et al., 2004, 2008), however in this study phosphatase activity was also affected by fire alone. A

separate study found greater phosphatase activity after a lag period of a growing season in burned and thinned plots compared to that in control or burn only treatments (Boerner et al., 2006), suggesting that the long-term effects on phosphatase activity may be as or more important than short-term effects. Other studies have found phosphatase activity to increase in a Konza Prairie (Ajwa et al., 1999), and decrease in an oak-hickory forest (Eivasi and Bryan, 1996) as a result of fire. In this study, the reduction in phosphatase activity after the prescribed burn was related to an increase in soil organic matter and a reduction in soil moisture. Since fire can reduce soil moisture, this could indirectly affect extracellular enzyme production (Sardans and Penuelas, 2005), however soil moisture was not a variable distinguishing treated plots in this study. The increase in soil organic matter content after the burn was a response to the treatments, where the effect of fire on soil organic matter seems to depend in part on the amount of leaf litter present before the fire. The fire could have released inorganic phosphate (Boerner et al., 1988; Certini, 2005; Rau et al., 2007) and converted some of the surface litter into the soil organic matter pool, thereby reducing microbial demand for phosphorus and decreasing soil phosphatase production.

Neither ß-glucosidase nor NAGase activities were affected by the restoration treatments with sites combined, however the reduction in observed ß-glucosidase and NAGase activity after the prescribed burn was related to the change in soil moisture. Even though changes in soil moisture were not related to the treatment type, changes in ß-glucosidase were most prominent in plots that were burned and thinned, suggesting a possible effect that could not be observed due to the differences between sites. Other studies have reported decreases in ß-glucosidase activity

following fire (Boerner and Brinkman, 2003; Eivasi and Bryan, 1996; Ajwa et al, 1999); however there is no evidence that the burn treatments directly affected ß-glucosidase activity in this study.

ß-glucosidase and phenol oxidase are thought to be regulated primarily by substrate availability, whereas phosphatase and NAGase are regulated by the soil microclimate and chemical factors (Sinsabaugh et al., 1992, 1993). Therefore, ß-glucosidase and phenol oxidase could be used as indicators of substrate alteration by fire, and phosphatase and NAG as indicators of physicochemical alteration due to fire. Since both phosphatase and ß-glucosidase showed a greater reduction in activity in the treated plots than in the control plots, this would suggest that the soil microclimate and substrate inputs are being affected by the restoration treatments, thereby affecting the activities of these enzymes. As the changes in the activities of ß-glucosidase, phosphatase, and NAGase appeared to be related to changes in soil moisture and soil organic matter content, this suggests one mechanism by which fire could affect enzyme activity: burning can reduce soil moisture and possibly increase soil organic matter content, thereby increasing substrate availability and changing the soil microclimate which reduces the activity of ß-glucosidase, phosphatase, and NAGase. An alternative mechanism for the reduction of enzyme activity following fire could be increased microbial mortality due to fire, which was not examined in this study. However, other studies that have examined microbial biomass following fire have found that fire does not always lead to a strong reduction in soil microbial biomass (Michelsen et al., 2004), suggesting that increased microbial mortality may not be major factor.

Activities of the oxidative enzymes, particularly phenol oxidase, exhibited a large amount of variation over the study period, which is consistent with other studies (Boerner et al., 2008). Changes in the activities of phenol oxidase and lignin-peroxidase were generally not similar to each other over the course of the study, or to patterns in the activities of the hydrolytic enzymes. However, reduced phenol oxidase activity following the prescribed burn at Site 2 was related to the reduction in litter depth following the fire, as was a reduction in NAGase activity. In a study in a loblolly pine forest in South Carolina, neither mechanical thinning nor burning had an effect on phenol oxidase activity when samples were taken one year post-treatment, (Boerner et al., 2006). However, mechanical thinning stimulated phenol oxidase activity in samples taken four years post-treatment (Boerner et al., 2006), suggesting that restoration treatments may result in long-term effects on soil phenol oxidase activity. Lignin-peroxidase was not affected by the restoration treatments with sites combined or separated, and the change in its activity was most prominent in the control plots, suggesting that either the restoration treatments tended to stabilize lignin-peroxidase activity, or had no effect.

The relationship between pre-fire and post-fire phenol oxidase activity and the reduction of litter after the prescribed burn was most likely influenced by the many sampling dates where phenol oxidase showed no activity in some plots. However, changes in NAGase activity also showed a strong negative relationship with litter reduction at Site 2. Litter depth was the environmental variable that most distinguished the treatment plots from the control plots, indicating that changes in NAGase activity were related to the restoration treatments, where a large reduction in leaf litter corresponded to only a slight reduction in NAGase activity.

Restoration treatments have been shown to increase annual net nitrogen mineralization and nitrification which could lead to greater nitrogen availability (Kaye and Hart, 1998). Given that NAGase is tied to the acquisition of both carbon and nitrogen by microorganisms, an increase in nitrogen availability would suggest that microorganisms may not need to produce as much NAGase to acquire nitrogen, potentially accounting for a reduction in NAGase activity. However, the negative relationship between NAGase activity and litter depth suggests that NAGase activity may be increasing somewhat, with comparatively little reduction in activity in relation to litter depth.

Comparing the actual values of enzyme activities in woodland soils across studies is difficult due to a lack of a standard methodology for enzyme assays (Wallenstein and Weintraub, 2008). Other difficulties arise when comparing sites that differ ecologically, as enzyme activities often vary depending on ecosystem variables (Sinsabaugh et al., 2008). For example, as part of a larger study examining the effects of fire suppression, activities for ß-glucosidase, NAGase, phosphatase, and phenol oxidase in soils of dry forests in the northeastern Cascades (Agee and Lehmkuhl, 2009) were one to two orders of magnitude higher than those obtained in the current study. In contrast, the activities of phosphatase and NAGase in soils of *Quercus* dominated forests of Ohio (Boerner et al., 2005) were an order of magnitude lower than those found in the current study, while activities of phenol oxidase were similar. Values for the activities of soil ß-glucosidase and NAGase observed in this study were similar to activities reported for forests of Michigan (Waldrop et al., 2004), but the activities of phenol oxidase and lignin-peroxidase were much lower, likely because of dates when entire plots recorded zero activity. Overall, activities

of soil enzymes recorded in this study are likely comparable to those in the literature, but absolute comparisons of soil enzyme activity across studies is difficult.

Activities of microbial extracellular enzymes on leaf litter were similar to those reported in other systems. ß-glucosidase activities associated with *Q. falcata* leaves were similar to those reported for red oak leaves (Carreiro et al., 2000), however the activities in this study were closer to those reported for leaves in experimental plots subject to nitrogen addition (Carreiro et al., 2000). The observed patterns in leaf litter ß-glucosidase were similar to those reported in another study in a Black Alder forest, where initial ß-glucosidase activity was high but decreased rapidly, and then leveled off (Dilly and Munch, 1996). Cellulose activity on deciduous litter is thought to increase rapidly, reach maxima within weeks, and then slowly decline (Sinsabaugh et al., 1981; Linkins et al., 1990), as was seen with ß-glucosidase in this study. Phenol oxidase and lignin-peroxidase activities on decaying leaves from this study were much lower than those reported by others (e.g. Carreiro et al., 2000). Specific comparisons of litter enzyme activity in relation to burning are not possible as to date no other studies have related leaf litter enzyme activity or decomposition to the practice of prescribed burning.

Decomposition rates of oak leaf litter were consistent with those of other studies. In a study of *Quercus robur L.* (English oak) litter in an oak forest in the Netherlands, 94% of leaf mass remained four weeks after litterbag placement, and 91 % after six weeks (Tietema and Wessel, 1994), values that were similar to those obtained for *Q. falcata* in this study. In a longer study, 56 % of the mass remained after 18 months for red oak leaves in a mixed hardwood forest in North Carolina (Hansen, 1999), equivalent to a decay rate of 0.08 % loss per day, which

closely matches the rates obtained in this study. Other studies have reported slower decay rates for oak leaves (Conn and Dighton 2000), but overall the decomposition dynamics of oak leaf litter in this study followed those seen in other systems.

As the decomposition of organic material progresses, the remaining organic matter is thought to become progressively more recalcitrant to decay, with the more labile material being consumed early in the decomposition process (Couteaux et al., 1995). This accounts for the exponential (first order) decay models often reported in the literature, and likely explains why mass loss rates were higher initially but tended to level out as the study progressed. However, the time when mass loss leveled out (after approximately 60 days in the field) coincides with mid-winter, and lower ambient temperatures could also explain the lower rates of mass loss over this time period. It's notable that mass loss rates increased a few months later, coinciding with the onset of spring, and likely warmer temperatures. Because of this sigmoidal decay pattern (fast initially, slower mid-winter, and faster again in the last third of the study), a second order polynomial decay model described the data better than the single exponential term. However, polynomial models were only slightly better fits than linear decay models for almost all plots, therefore, linear models of decomposition were used, as a simplified method of comparing decomposition rates between plots and to explore relationships between decomposition and microbial activity,

Decomposition of *Q. falcata* leaves in both burned and burned and thinned plots was accelerated as compared to control plots however not significantly so. Fire-produced charcoal can accelerate the decomposition of organic material in soil (Wardle et al. 2008), potentially by

binding phenolic and aromatic compounds which could otherwise inhibit enzymatic activity (Wetzel, 1992; Zackrisson et al., 1996). It's likely that soil charcoal content was higher initially in the treatment plots (because of past burn history) and increased further following the April burn. Theoretically, this could increase decomposition rates in those treatments by removing potential inhibition of degradative enzymes, although no increase in leaf litter enzyme activity following the fire was detected. *Q. falcata* leaves from control plots were used to examine decomposition rates in all treatments to avoid the potential increases in leaf C:N content that can occur because of fire history (Hernandez and Hobbie, 2008). However, this could also result in differences in decomposition rates as the control plot leaves may have served as a better resource for the decomposer community (because of lower C:N content) than the leaves that are naturally found within the treatment plots. Essentially, adding litter of increased quality to treatment sites that could be pre-adapted to lower quality material might result in faster rates of decay. While possible, this explanation is probably unlikely given that plots were close together and natural patterns of leaf fall likely result in a mixture of leaves from different plots falling together. Thus, the observation that litter decomposition was not significantly increased in the treatment plots is not necessarily surprising.

The slopes of regressions of % mass remaining as a function of cumulative enzyme activity for each enzyme can be used as a measure of a particular enzymes apparent efficiency at degrading a particular substrate (Sinsabaugh et al., 1994, Jackson and Vallaire, 2007). Phosphatase activity had the closest relationship to mass loss for all plots, which, given that phosphatase isn't directly related to leaf litter decay, suggests that phosphatase activity may be

reflective of overall microbial activity. A repression-derepression mechanism exists for phosphatase production, which represses enzyme synthesis when substrate is readily available (Sinsabaugh et al., 1993), suggesting that the decomposition of leaf litter at these sites may be phosphorus limited. Nitrogen has long been considered an important regulator of decomposition rates (Harmon et al., 1986, Fog, 1988) and is often used as a predictor of decomposition rates via indices of litter quality. In this study, the relationship between NAGase activity (an enzyme potentially associated with N acquisition) and mass loss was strong, but less than that of phosphatase. NAGase is tied to the cycling of both nitrogen and carbon (Olander and Vitousek, 2000; Kang et al., 2005), so its role in organic matter decay may be more important than nitrogen acquisition in this system. Individual enzymes, however, are only one component of a complex decomposition system and individual enzyme efficiencies ignore interactions between enzymes (Jackson and Vallaire, 2007). For this reason, all enzymes were combined into an integrated global index of microbial activity (Jackson et al., 1995a; Alvarez and Guerrero, 2000). Global indices revealed that microbial enzymes in the burned and thinned plots were more efficient at degrading *Q. falcata* leaf litter than those in other plots, suggesting that burned and thinned plots could contain different microbial populations, or have altered environments resulting in more effective organic matter degradation.

In this study, enzyme activity and decomposition were examined in the context of forest restoration treatments. A major effect of burning is on the soil litter layer, which in this study was completely consumed by the fire. This large reduction in leaf litter led to a relatively small overall reduction in the activity of the enzyme NAGase, an enzyme that is related to both

nitrogen and carbon cycling (Olander and Vitousek, 2000; Kang et al., 2005). The addition of soil organic matter to treated plots after the burn was related to a reduction in phosphatase activity, and more available phosphate could have been released by burning (Boerner et al., 1988; Certini, 2005; Rau et al., 2007). The reduction in litter could also be tired to the increase in soil organic matter, as burning could be converting leaf litter material into the soil organic matter pool. What this suggests is that burning leads to a decrease in soil nitrogen, as nitrogen is easily volatilized and has many gaseous forms (Caldwell et al., 2002), thereby increasing nitrogen demand and microbial NAGase activity to acquire nitrogen. At the same time, burning may have increased the amount of available phosphate, which has been shown to increase after fire (Rau et al., 2007) because phosphorus has no gaseous forms and cannot be volatilized by fire. This increase in available phosphate could lead to a reduced microbial demand for phosphorus, thereby reducing phosphatase activity. Overall, these results suggest that after a fire, this system is shifting from being limited by nitrogen to being limited by phosphorus.

While there was no significant difference in litter decomposition rate between the treatments, the decomposition of *Q. falcata* leaves was slightly faster in the treatment plots as compared to controls. Even small increases in litter decomposition rates could have implications for ecological restoration, as less leaf litter can aid in the germination of desired herbaceous species that are shade-intolerant (Molofsky and Augspurger, 1992). Leaf litter enzyme activity also seemed to be affected by the treatments, specifically in the burned and thinned plots, where the apparent efficiency of microbial enzymes in degrading leaf litter was higher. Together, these changes suggest a possible implication for restoration treatments related to the promotion of

shade-intolerant understory species. Thinning of the canopy reduces the amount of litter on the forest floor, and microbial enzymes in these plots are also more efficient at degrading leaf litter, further reducing the amount of litter. Thus, the combination of burning and thinning can further a restoration project by reducing the amount of litter through both a reduction in inputs and more efficient decomposition of litter material. This reduction in the litter layer allows for increased solar penetration to the soil, and could allow the shade-intolerant species that once dominated the understory to proliferate.

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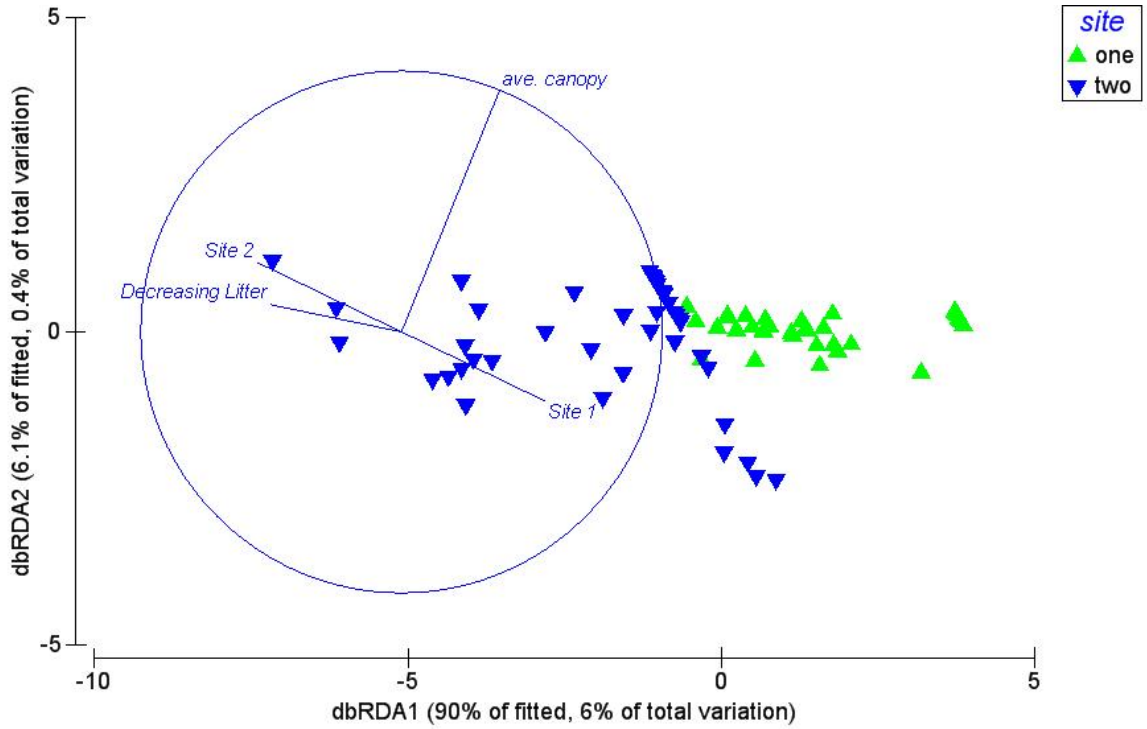
APPENDICES

Appendix A

Results of PERMANOVA test of changes in mean canopy density, litter depth, after the prescribed burn and Site. AV: canopy density. De: litter depth. Si: Site.



PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
av	1	129.77	129.77	1.2913	0.251	999
De	1	130.58	130.58	1.2994	0.254	998
si	1	391.38	391.38	3.8944	0.046	999
avxDe	1	202.82	202.82	2.0181	0.147	999
avxsi	1	147.91	147.91	1.4717	0.21	999
Dexsi	1	354.09	354.09	3.5233	0.05	998
avxDexsi	1	148.1	148.1	1.4736	0.25	999
Res	82	8240.9	100.5			
Total	89	9745.5				

Appendix B

A multi-source regression analysis of variance (ANOVA) for each change in enzyme activity after the prescribed burn with changes in mean canopy density, litter depth, soil organic matter content, and soil moisture after the prescribed burn with Site 1 and 2 combined

β-glucosidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	51.64572	10.3291	2.2754
Error	84	381.309	4.5394	
C. Total	89	432.9547		
Prob > F	0.0543			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	2.53558	2.705902	0.94	0.3514
Litter depth	-0.12822	0.11913	-1.08	0.2849
Average canopy	-0.0441	0.023121	-1.91	0.0599
Site	-0.60565	0.668642	-0.91	0.3676
Organic matter	-0.05027	0.048571	-1.03	0.3037
Moisture	-0.16304	0.076606	-2.13	0.0362

Phosphatase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	1337.112	267.422	3.1004
Error	84	7245.374	86.254	
C. Total	89	8582.485		
Prob > F	0.0129			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	6.160476	11.79516	0.52	0.6028
Litter depth	-0.11893	0.519292	-0.23	0.8194
Average canopy	-0.13046	0.100787	-1.29	0.1991
Site	-8.89887	2.914643	-3.05	0.003
Organic matter	-0.40504	0.211722	-1.91	0.0591
Moisture	-0.75222	0.333932	-2.25	0.0269

NAGase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	40.28129	8.05626	2.3794
Error	84	284.4074	3.3858	
C. Total	89	324.6887		
Prob > F	0.0454			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	0.36424	2.336921	0.16	0.8765
Litter depth	-0.1657	0.102885	-1.61	0.111
Average canopy	-0.00851	0.019969	-0.43	0.6711
Site	-1.02159	0.577465	-1.77	0.0805
Organic matter	-0.01557	0.041948	-0.37	0.7115
Moisture	-0.12991	0.06616	-1.96	0.0529

Phenol oxidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	1.492803	0.298561	0.9539
Error	84	26.29206	0.313001	
C. Total	89	27.78486		
Prob > F	0.4509			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	0.396032	0.710536	0.56	0.5788
Litter depth	-0.00328	0.031282	-0.1	0.9168
Average canopy	-0.00781	0.006071	-1.29	0.2021
Site	0.057418	0.175577	0.33	0.7445
Organic matter	-0.00031	0.020116	-0.02	0.9877
Moisture	-0.00804	0.012754	-0.63	0.5300

Lignin-peroxidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	28.62812	5.72562	1.3782
Error	84	348.9773	4.15449	
C. Total	89	377.6054		
Prob > F	0.2407			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-4.30785	2.588643	-1.66	0.0998
Litter depth	-0.13717	0.113967	-1.2	0.2321
Average canopy	0.040953	0.022119	1.85	0.0676
Site	0.521505	0.639666	0.82	0.4172
Organic matter	-0.01478	0.073287	-0.2	0.8407
Moisture	-0.00252	0.046466	-0.05	0.9569

Appendix C

A multi-source regression analysis of variance (ANOVA) for each change in enzyme activity after the prescribed burn with changes in mean canopy density, litter depth, soil organic matter content, and soil moisture after the prescribed burn for Site 1.

β-glucosidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	19.15217	4.78804	1.2002
Error	40	159.58036	3.98951	
C. Total	44	178.73253		
Prob > F	0.3257			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	3.5455227	7.439544	0.48	0.6363
Litter depth	-0.015281	0.230461	-0.07	0.9475
Average canopy	-0.063852	0.076984	-0.83	0.4118
Organic matter	-0.072148	0.050405	-1.43	0.1601
Moisture	-0.215787	0.108319	-1.99	0.0532

Phosphatase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	329.3437	82.3359	1.3324
Error	40	2471.7583	61.794	
C. Total	44	2801.102		
Prob > F	0.2747			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-6.048227	29.27922	-0.21	0.8374
Litter depth	0.928342	0.907006	1.02	0.3122
Average canopy	-0.116527	0.302981	-0.38	0.7026
Organic matter	-0.349656	0.198376	-1.76	0.0856
Moisture	-0.6381	0.426302	-1.5	0.1423

NAGase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	13.414	3.3535	0.8931
Error	40	150.18756	3.75469	
C. Total	44	163.60156		
Prob > F	0.477			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-9.800617	7.21728	-1.36	0.1821
Litter depth	0.3784071	0.223576	1.69	0.0983
Average canopy	0.0765131	0.074684	1.02	0.3118
Organic matter	-0.00374	0.048899	-0.08	0.9394
Moisture	-0.092177	0.105083	-0.88	0.3856

Phenol oxidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	2.300506	0.575126	1.0243
Error	40	22.458662	0.561467	
C. Total	44	24.759168		
Prob > F	0.4065			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	2.8788519	2.790927	1.03	0.3085
Litter depth	0.0072992	0.086457	0.08	0.9331
Average canopy	-0.035306	0.028881	-1.22	0.2287
Organic matter	-0.011843	0.018909	-0.63	0.5347
Moisture	0.0294319	0.040636	0.72	0.4731

Lignin-peroxidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	27.77412	6.94353	1.5213
Error	40	182.56902	4.56423	
C. Total	44	210.34315		
Prob > F	0.2144			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-4.39401	7.95738	-0.55	0.5839
Litter depth	-0.281398	0.246502	-1.14	0.2604
Average canopy	0.0509811	0.082343	0.62	0.5393
Organic matter	-0.007259	0.053914	-0.13	0.8936
Moisture	-0.067331	0.115859	-0.58	0.5644

Appendix D

A multi-source regression analysis of variance (ANOVA) for each change in enzyme activity after the prescribed burn with changes in mean canopy density, litter depth, soil organic matter content, and soil moisture after the prescribed burn for Site 2.

β-glucosidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	41.1412	10.2853	1.9531
Error	40	210.64634	5.2662	
C. Total	44	251.78754		
Prob > F	0.1204			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	1.4618365	1.920966	0.76	0.4511
Litter depth	-0.30305	0.174388	-1.74	0.0899
Average canopy	-0.039672	0.022031	-1.8	0.0793
Organic matter	-0.012745	0.176048	-0.07	0.9426
Moisture	-0.120721	0.117286	-1.03	0.3095

Phosphatase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	1413.4159	353.354	3.503
Error	40	4034.9237	100.873	
C. Total	44	5448.3396		
Prob > F	0.0152			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-16.67609	8.407375	-1.98	0.0542
Litter depth	-1.206966	0.763233	-1.58	0.1217
Average canopy	-0.030386	0.096422	-0.32	0.7543
Organic matter	-1.713033	0.770498	-2.22	0.0319
Moisture	-1.104344	0.51332	-2.15	0.0375

NAGase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	73.29875	18.3247	8.3818
Error	40	87.4494	2.1862	
C. Total	44	160.74815		
Prob > F	<.0001			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-2.05018	1.237716	-1.66	0.1055
Litter depth	-0.43547	0.112362	-3.88	0.0004
Average canopy	0.004632	0.014195	0.33	0.7459
Organic matter	-0.186404	0.113431	-1.64	0.1082
Moisture	-0.175545	0.07557	-2.32	0.0254

Phenol oxidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	0.28309124	0.070773	4.7674
Error	40	0.59380808	0.014845	
C. Total	44	0.87689932		
Prob > F	0.0031			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	0.0761426	0.101992	0.75	0.4597
Litter depth	-0.020588	0.009259	-2.22	0.0319
Average canopy	-0.001449	0.00117	-1.24	0.2226
Organic matter	0.0038441	0.009347	0.41	0.6831
Moisture	-0.016564	0.006227	-2.66	0.0112

Lignin-peroxidase

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	14.38266	3.59566	0.9408
Error	40	152.87231	3.82181	
C. Total	44	167.25497		
Prob > F	0.4503			

Term	*Estimate*	*Std Error*	*t Ratio*	*Prob>|t|*
Intercept	-2.763331	1.636467	-1.69	0.0991
Litter depth	-0.022696	0.148561	-0.15	0.8793
Average canopy	0.0325651	0.018768	1.74	0.0904
Organic matter	0.112375	0.149975	0.75	0.4581
Moisture	0.0513393	0.099916	0.51	0.6102

VITA

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