THE EFFECT OF PRESCRIBED FIRE HISTORY ON BELOWGROUND PROCESSES IN TALLGRASS PRAIRIE

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ABSTRACT

Fire is an agent of ecosystem change that has played a critical role in shaping the tallgrass prairie ecosystem. Periodic prescribed fire maintains the prairie by removing woody and invasive plant species, and stimulating growth of native herbaceous species. Fire's beneficial effect on the tallgrass prairie aboveground community, in terms of aboveground productivity and plant diversity, has been well established. However, other parts of the ecosystem, especially the soil community, have been less thoroughly studied. The central question posed by this study was: what is the effect of fire frequency (frequent fire, infrequent fire, or no fire) in undisturbed tallgrass prairie on components of the tallgrass prairie soil community?

The soil characteristics investigated were nutrient levels (K, Mg, Ca, available P, available N, total N, and total C), mycorrhizal fungal activity, bacterial metabolic activity, and root biomass. These characteristics were investigated at six tallgrass prairie remnants in the Chicago region that have experienced three different types of fire management over the last 20 years. Any similarities between sites that had received the same frequency of fire were expected to reveal soil characteristics most influenced by fire.

All measured characteristics differed significantly between sites, but the variables most strongly affected by fire frequency were root mass, available nitrogen (N), and potassium (K^+). The two frequently burned sites, Skokie River and Somme, had the highest root mass per volume of soil and the lowest levels of available N. The two unburned sites, Morton Grove and Monee, had the highest K levels. All other variables did not follow any strongly discernible fire frequency-related pattern. The results imply that frequent burning increases belowground primary productivity in tallgrass prairie, while at the same time reducing levels of soil nutrients, especially nitrogen and potassium.

The results demonstrate that fire alters key soil characteristics. Although the results do not decisively point to a particular fire frequency recommendation based on these characteristics, they do not contradict a currently accepted recommendation of biennial burning in tallgrass prairie. Thus, it would be best for managers to burn tallgrass prairie preserves frequently, while monitoring those soil characteristics most likely to be strongly impacted.

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INTRODUCTION/BACKGROUND

Introduction and history

Tallgrass prairie is a floristically diverse grassland ecosystem that once occupied a vast part of the contemporary United States (Baer et al. 2002). Since European settlement, however, prairie has become one of the continent's most endangered ecosystems, with about 0.1% of native tallgrass prairie remaining in North America (Samson and Knopf 1994). Fire is an agent of ecosystem change that has played a critical role in shaping the tallgrass prairie ecosystem (Knops 2006). Historically, tallgrass prairie in the Midwest U.S. is thought to have been maintained by fires, ignited for a variety of reasons, for as long as the region has been inhabited by humans.

These causes of fire include both accidental (i.e. lightning) and human-caused fires. Native Americans may have deliberately set fires to aid in hunting (Daubenmire 1968, Vogl 1974), to increase visibility (Vogl 1974), to reduce insects (Vogl 1974), and to aid in warfare (Daubenmire 1968). Hunting by fire, specifically, may have been the single most important impetus, more than any other deliberate or accidental causes, for landscape-opening fire throughout the present-day United States (Pyne 1983). Based on typical precipitation and topography patterns in tallgrass prairie, it has been estimated that the natural (i.e., non-deliberate) frequency of fire in North American grasslands around the time when European settlement began was every 5-10 years in open prairie, and every 20-30 years in areas containing rivers and other natural fire obstacles (Wright and Bailey 1982). Although the majority of historic fires were probably set by (native) people, people have lived continuously on the land of the Midwest

United States since it was made available by the retreat of the glacier; thus, there is no pre-human, more “natural” state (Pyne 1982).

This periodic incidence of fire maintained the prairie by removing woody plants that cannot tolerate fire and by opening the ground to more light penetration, rejuvenating native prairie plant species (Copeland et al. 2002, Evans 1984). In order to maintain present prairie remnants and restore degraded areas, prescribed burning is a common modern management practice that is undertaken in prairies with the intent to mimic this historic natural phenomenon (Brye 2006).

Ecological effects of fire

Fire, like other types of disturbance and along with physical site characteristics like climate and topography, impacts an ecosystem primarily through its influence on the availability of three major resources: water, light, and nutrients (Baer et al. 2003). Fire’s direct effect on these three resources is discussed in the following pages. In addition, fire’s indirect effects on other elements of the ecosystem are mediated by changes in these three major resources. Along with fire’s effects on water, light, and nutrients, the effects upon aboveground elements of plant diversity and productivity and prairie animal diversity will be discussed, followed by a discussion of the belowground elements of root productivity and microbial diversity and activity.

When considering the ecological effects of fire, it is important to distinguish between the effects of a single fire event on an ecosystem, which can be especially dramatic after a period of no fire, and the effects of a long-term frequent fire regime. For some ecological characteristics, frequent fire may appear to have a completely opposite effect compared to the short-term impact of one fire. A good example of this is found in nitrogen cycling. Because nitrogen levels generally increase briefly immediately after a fire, this may be considered an effect of fire, even though over the long-term frequent burning will result in less total nitrogen in the ecosystem, for reasons discussed later.

Aboveground effects of fire

Perhaps most obvious is that fire immediately and dramatically affects the amount of light reaching the soil, as it removes all aboveground herbaceous vegetation. This increased light results in both an increase in the soil's average temperature and an increase in the light available at the ground surface, both of which can alter competitive dynamics between plant species (Knops 2006). Also, productivity of the entire ecosystem can be greater because all the plant matter receiving sunlight is living plant material, since there is no detritus from previous years intercepting sunlight (Brye et al. 2002). The ecological effects on the plant community during the growing season following a fire event include: increased productivity, a shift in species composition toward more fire-tolerant species (specifically, warm-season grasses), and increased reproduction for the warm-season grasses and some forbs (Hartnett 1991).

Plant productivity is considered an important measure of ecosystem functioning for grasslands of the Midwest (Baer et al. 2002). Productivity is a concept that signifies the amount of solar energy being transformed into physical biomass, and the rate of this transformation, in the system. Productivity can be measured as total dry plant biomass per unit area, consisting of either all aboveground biomass (aboveground net primary productivity), or total biomass including belowground parts. The latter provides a more complete picture of ecosystem productivity, but may be too invasive in a fragile preserve.

Aboveground net primary productivity (ANPP) has been observed to be higher on annually burned tallgrass prairie sites when there is sufficient moisture (Collins and Steinauer

1998), and has also been found to increase following fire (Baer et al. 2002). Fire causes an especially large increase in productivity in prairie after several years without fire (Knapp and Seastedt 1986). The mechanism of increased productivity following fire is related to a combination of increased light availability, increased soil temperature, and increased nitrogen use efficiency (NUE) by plants (Ojima et al. 1994).

Plant species richness has been observed to increase with increased prescribed fire frequency in tallgrass prairie (Bowles and Jones 2004, Bowles et al. 2003). At the same time, annually burned sites are more strongly dominated by C_4 grasses and thus have a less varied vegetation structure (Collins & Steinauer 1998). Therefore, fire's benefit to plant diversity depends to some extent on site management context. If encroachment by woody and invasive species is an immediate and urgent concern, frequent (annual or biennial) fires can help maintain the highest possible level of diversity. On the other hand, in a stable prairie with a low risk of invasion and a high baseline species richness, the number of native prairie forb species, and thus overall plant diversity, may be highest when it is less frequently burned (Howe 1994).

More frequent fire in tallgrass prairie is associated with lower species richness of grassland birds (Reinking 2005). This is attributed to the lower structural diversity found in the most frequently burned prairies. A patch-burning regime rotating on a 3-year cycle has been recommended to optimize conditions in the tallgrass prairie for birds (Reinking 2005). Insects in tallgrass prairie, too, have been found to experience increased species diversity as the time since fire increases. A study of grasshoppers at Konza Prairie in Kansas found significantly greater numbers of species on prairies that were burned every 4 years or were unburned, compared to annually burned prairies (Evans 1987). This was attributed mainly to the loss of forb-feeding grasshoppers, because forbs are less abundant in more frequently burned prairies. In contrast, a study of native earthworms at Konza found higher abundance on annually burned sites compared to unburned sites (James 1982). A Chicago-area study of fire's effect on 151 insect species found that 40% of the insect species experienced substantial population declines after fire, but all had recovered fully within two years following the fire, suggesting an optimum fire return interval of three years (Panzer 2002).

The soil community and belowground effects of fire

Soil provides ecosystem services. It is the base upon which the plant community forms its structure. It is the source of resources that allow plants and other soil organisms to grow: essential nutrients, water, organic matter, and temperature regulation. A well-structured soil provides drainage, water-holding capacity, and aeration (Sollins et al. 1999). The soil microbial community, including nematodes, bacteria, actinomycetes, protozoa, fungi, and microarthropods, serves roles that help maintain the soil's structure. One vital function is that of decomposition and recycling of soil materials (Brady 1984). Microbes are also regulators of soil nutrient cycling, through both the decomposition function and other means, such as fixation of atmospheric nitrogen (Paul 2007).

Belowground species diversity is generally correlated with aboveground diversity. For example, diversity of mycorrhizal fungi may directly increase the diversity of plants (Bever et al. 2001). Also, high aboveground productivity has been observed to correlate with high belowground productivity (Reich et al. 2001). However, belowground measures such as species diversity and overall productivity may not always mirror aboveground values in tallgrass prairie, where resources are disproportionately allocated belowground (Baer et al. 2002, Boerner 1982).

Physical soil characteristics. Underlying fire's effects on many biotic components of an ecosystem are direct, long-lasting impacts on abiotic components of the soil microclimate. At least temporarily, fire affects the temperature and moisture level of the soil. Mainly as a result of the increased exposure of black soil to sunlight, the soil temperature is higher and the moisture level is lower during the season following a burn (Gibson and Hetrick 1998, Ojima et al. 1994, Knapp and Seastedt 1986). Moisture is lower on annually burned tallgrass prairie sites at Konza (Collins and Steinauer 1998). Fire has also been shown to result in an increased soil pH in some ecosystems, such as conifer forest (Grogan et al. 2000, Deluca and Sala 2006), but a study of tallgrass prairie found no change of pH after twelve years of annual burning (Brye 2006). The same prairie study found that electrical conductivity of the soil decreased significantly as a result of annual burning.

Soil Nutrients. Fire affects soil levels of important plant nutrients. Nutrients may increase due to the transformation of plant materials into ash, and to warmer soil temperature leading to greater microbial activity (Anderson and Menges 1997, Blair et al. 1998). Alternatively, soil nutrient levels may decrease as a result of volatilized biomass or post-fire water leaching or wind erosion (Anderson and Menges 1997, Chorover et al. 1994). Lighter elements, including N, C, and S, are more likely to be sent into the atmosphere, while nutrients like P and cations are more likely to be re-deposited in the ash (Blair et al. 1998, Boerner 1982). However, in reality, fire's exact influence on nutrient levels in a particular location will depend on general site characteristics and history as well. Soil nutrients examined in this study, and their known relationships with fire, are enumerated below.

Although what is most at interest here is what happens under ideal conditions (i.e., large, undisturbed prairie), a concept that has been put forward concerning small prairie fragments is relevant to keep in mind: that small prairie fragments are more likely to be detrimentally affected by frequent fires through nutrient loss from the system because nutrient-rich ash is more likely to be blown off site, rather than re-deposited into the system (Brye 2006).

Nitrogen. Nitrogen exists in the soil in organic and inorganic forms. The majority of soil N is in organic forms associated with soil humus, which are not available for uptake by plants (Brady 1984). Plants can use the small amounts of the total nitrogen that are in the inorganic forms of ammonia (NH_4^+) or nitrate (NO_3^-), but these forms are more easily lost from the soil through leaching and immobilization. The process by which organic N is made available to plants is called mineralization and may be performed by microbes that release excess N while consuming detritus (Paul 2007). When the microbes are using more N than they are releasing, they are instead performing N immobilization. In tallgrass prairie, the native warm-season grasses have generally the lowest N requirements compared to forbs and cool-season grasses, and thus thrive most easily in an N-poor environment (Reich et al. 2001).

In most ecosystems, greater nitrogen availability is correlated with greater aboveground plant production, although the inverse relationship between nitrogen and plant productivity has been observed at the Konza Long Term Ecological Research (LTER) tallgrass prairie site in Kansas (Collins et al. 1998). This deviation from the norm is attributed to fire treatment.

Nitrogen use efficiency (NUE) is a plant characteristic that refers to the amount of C gained per unit of N that the plant utilizes (Ojima et al. 1994). In a study of the tallgrass prairie at Konza, frequent fire, both short-term and long-term, led to increased NUE due to greater photosynthesis and warmer root temperatures (Ojima et al. 1994). The greatest increase in NUE occurred belowground. The authors of this study theorized that high productivity was maintained in the prairie due to this increased NUE, despite overall declines of available N in the ecosystem, with fire (Ojima et al. 1994). In oak savannas in Minnesota, NUE was also found to increase as fire frequency increased (Reich et al. 2001).

Generally, researchers have observed that total soil nitrogen increases immediately following fire in a variety of ecosystems, including mixed-conifer forest (Chorover et al. 1994); tallgrass prairie (Knapp et al. 1998); and mixed-grass savanna (Ansley et al. 2006). However, this may depend on the season of burning (Ansley et al. 2006). Also, a pronounced “pulse” of nitrogen may be noticeable only after several years without fire (Knapp et al. 1998).

In contrast, studies of soil dynamics over many years of burning find decreased available N and decreased net N mineralization on annually burned prairies compared to unburned prairies (Ojima et al. 1994, Collins and Steinauer 1998, Blair et al. 1998). Lower levels of N mineralization were also observed in oak savanna as fire frequency increased (Reich et al. 2001). This was attributed to the higher NUE of warm-season grasses, which are increasingly dominant as fire frequency increases, and the consequent lower N turnover.

Available forms of N (mainly NH_4^+ and NO_3^-) have increased in many ecosystems of the world in recent years as a result of anthropogenic N fixation for agricultural use (Gilliam 2006). When there is an excess of N in a system, it can act as a pollutant. Effects of N deposition include: increased invasibility by exotic species; decreased availability of essential cations, such as Ca^{2+}; increased soil acidification, decreased plant species diversity, and decreased water quality (Gilliam 2006, Fenn et al. 1998, Suding et al. 2005). Thus, the overall effect that fire appears to have, of nitrogen removal from the system, is probably another service provided by fire.

Carbon. Total carbon pools are a relatively stable part of the ecosystem. Carbon enters the soil in organic forms as a result of decomposition of plant matter. Microbes (and plants) respire back into the atmosphere some of this organic C in the form of CO_2 (Paul 2007). The rest remains in stable C pools as humic substances that can persist in the soil for thousands of years (Paul 2007).

In a study in a ponderosa pine (*Pinus ponderosa*)/Douglas fir (*Pseudotsuga menziesii*) forest with a 120-year fire history, fire frequency had no influence on total C (Deluca and Sala 2006). However, in tallgrass prairie in Arkansas, total soil C was observed to increase significantly after twelve years of annual burning (Brye 2006). And in mixed-grass savanna in north-central Texas, soil organic carbon (SOC) increased significantly but slowly at frequently burned sites. (Ansley et al. 2006). SOC is a measure that differs from total soil C in that it does not include inorganic forms of carbon such as carbonates (Sollins et al. 1999).

Because soil microbes are highly sensitive and responsive to nutrient levels in the soil and represent a predictable proportion of total soil nutrient levels, measurement of microbial biomass is a good way to estimate the total amount of C (and N) in the soil (Paul et al. 1999). Microbial biomass C increased slightly but significantly after one or two years of burning at Konza prairie in Kansas (Ojima et al. 1994). However, the same study found that microbial biomass C was significantly *lower* on prairie sites that had been annually burned over a longer time period (>50 years) compared to sites that had not been burned for that time period (Ojima et al. 1994).

Phosphorus. The primary source of soil phosphorus is parent rock material (Paul 2007). Phosphorus exists in the soil in both organic and inorganic forms. Available P is inorganic, but most of the inorganic phosphorus present in the soil is unavailable for plant uptake because of this element’s tendency to adsorb to clay surfaces and aluminum and iron oxide surfaces (Paul

2007). Organic P is converted to available, inorganic P through microbial mineralization. As with nitrogen, soil microorganisms can also carry out P immobilization.

Phosphorus increased for a brief time following fire in sandhill scrub in Florida (Anderson & Menges 1997). In tallgrass prairie, no significant change in P was observed at Konza after either short-term or long-term burning (Ojima et al. 1994). A review of burn frequency studies at Konza found that while prairie sites that have received frequent burning do not have a higher level of total P than unburned sites, they have more P that has been converted to the inorganic, available form (Anderson 1990). Extractable P was observed to decrease significantly after twelve years of annual burning in tallgrass prairie in Arkansas (Brye 2006). Total P increased slightly following a one-time fire event in mixed-conifer forest (Chorover et al. 1994). Thus, the overall effect of fire on phosphorus is not straightforward.

Other nutrients. Cations like K^+, Mg^{2+}, and Ca^{2+} arrive into the ecosystem from weathering and are adsorbed by soil colloids (Brady 1984). Other cations found in the soil are Al^{2+}, H^+, and Na^+. Cations adsorbed to colloids can replace each other through a process called cation exchange (Brady 1984). The cation exchange capacity of a soil reflects the total amount of cations the soil can adsorb. A high cation exchange capacity helps plants, which need these nutrients, because adsorbed nutrients remain in a relatively available state but are less at risk of being leached (Brady 1984).

Potassium increased for a brief time following fire in sandhill scrub in Florida (Anderson & Menges 1997). Extractable K, Ca, and Mg levels were lower after twelve years of annual burning in Arkansas, but the difference was not significant (Brye 2006). Soil solution concentrations of K, Mg, and Ca increased 10 fold following a one-time fire event in mixed-conifer forest in the Sierra Nevada (Chorover et al. 1994). In glades of an oak-hickory forest in

Kentucky, no significant differences were found in leaching levels of K, Mg, and Ca between burned and unburned sites (Trammell et al. 2004). A review of many studies in temperate ecosystems found that in temperate grasslands, only transient increases in cations occur after fire (Boerner 1982).

Other nutrients, not addressed in this study due to limitations of time and scope, have also been investigated for their relationship to fire in various ecosystems. Nutrients that have been observed to increase following fire include Fe (Anderson and Menges 1997) and SO_4^{2-} (Chorover et al. 1994). Elements that have decreased significantly following fire include Na (Brye 2006), Fe (Brye 2006), and Mn (Brye 2006).

Mycorrhizal Fungi. Arbuscular mycorrhizae are mutualistic associations between plant roots and fungi that have been estimated to form in 70% of all land plants (Paul 2007) and 84% of all grass species (Newman and Reddel 1987). The mutualism functions because the mycorrhizal fungus provides nutrients, especially phosphate, and water to the plant roots, while the plant provides carbon to the fungus. The presence of mycorrhizal fungi has been shown to have a significant role in maintaining vegetation composition, productivity, and the course of succession (Bever et al. 2001). Arbuscular mycorrhizal (AM) fungi are characterized by multinucleate hyphae and a number of other structures that reside inside the plant cortical cells (Johnson et al. 1999). AM fungi also contribute to a stable soil structure by binding soil particles together (Paul 2007). There is evidence that AM fungal growth rates can be host specific, and that increased AM fungal species diversity leads to increased plant diversity (Eom et al. 2000, Bever et al. 2001).

Other, less ubiquitous types of mycorrhizae include ectomycorrhizae and ectendomycorrhizae. Different types of mycorrhizal fungi differ in the plant species with which they associate, the nutrients that they primarily facilitate for plant uptake, and their general physical structure. This study focuses on arbuscular mycorrhizae due to their ubiquity, especially in grasslands.

To examine how an independent variable, such as fire, affects mycorrhizal fungi in an ecosystem, there are a number of potential measures of the mycorrhizal fungal community. Activity and abundance can be observed by quantifying the amount of fungal colonization on stained roots (Anderson and Menges 1997, Johnson et al. 1999). Another way to quantify fungal activity is to measure soil levels of fungus-produced compounds. For example, glomalin is an AM fungus-specific glycoprotein that can be reliably extracted from soil and can be used to quantify AM fungal activity (Knorr et al. 2003). Also, species diversity can be calculated after identifying fungal species by visual inspection of spores (Anderson and Menges 1997) or by amplifying distinctive DNA or rRNA fragments (Eom et al. 2000, Johnson et al. 1999).

There are a few studies that have focused specifically on the effects of fire frequency on AM fungi. Of these, there has been observed: no effect on fungal root colonization activity after ten years of annual burning in tallgrass prairie at Konza (Eom et al. 1999); no difference in glomalin levels between frequently burned and unburned oak-hickory sites in Ohio (Knorr et al. 2003); and no difference in AM fungal root colonization observed after a one-time fire event in sandhill scrub in Florida (Anderson and Menges 1997).

Meanwhile, other studies have found significant changes to the mycorrhizal community. AM fungal root colonization was greater in frequently burned dry sclerophyll compared to unburned sites in the Sydney region of Australia (Torpy et al. 1999). Root colonization by AM fungi at Konza was significantly greater after a one-time fire event, but only for the first month after the fire (Bentivenga and Hetrick 1991).

AM fungal species diversity calculated by spore identification decreased after ten years of annual burning at Konza (Eom et al. 1999). Based on spore identification, annual burning (compared to infrequent burning) had no effect on species richness of AM fungi at Konza tallgrass prairie, although the abundances of three AM fungal species were affected by a topography-burning interaction (Gibson and Hetrick 1998). A review of many studies that have been conducted at Konza found that most mycorrhizal fungal species have increased spore abundance with annual burning (Rice et al. 1998).

Studies of ectomycorrhizal (EM) fungi and fire have also had mixed results. Frequent burning in wet sclerophyll forest in Queensland, Australia resulted in a significantly different EM species assemblage as measured by rDNA profiles (Bastias et al. 2006). In ponderosa pine stands in Oregon, fall burning (compared to spring burning or no burning) resulted in significantly lower species richness of EM fungi, as determined by RFLP analysis (Smith et al. 2004). Fire may impact the mycorrhizal fungal community indirectly (through effects on host plants) or directly, by changing the soil microclimate (Eom et al. 1999).

Bacteria. The soil microbial community includes many other organisms in addition to fungi, for example bacteria, actinomycetes, protozoa, and microarthropods. Some ecosystem services provided by microorganisms include serving as sources and sinks of soil nutrients and maintaining soil structure (Hart et al. 2005). They also serve as essential agents of decomposition and recycling of ecosystem resources. Bacteria are probably the most speciose and numerous organisms on earth and the majority of them live below the earth’s surface (Coleman et al. 2004). Along with other soil microbes, bacteria can form mutually beneficial (e.g. rhizobia), neutral, or negative relationships with plants (Kang and Mills 2004).

Fire can affect the structure of the bacterial community because not all bacterial groups have the same degree of heat sensitivity. (Hart et al. 2005). After a one-time fire event at a temperate tallgrass restoration in Virginia, bacterial abundance increased as measured by direct counts (Kang and Mills 2004). The same study also found that the bacterial community structure had drastically changed two weeks after the fire, as measured by DNA polymorphism analysis.

A 1965 study of microorganisms in Minnesota jack pine stands found that bacteria decreased immediately after a one-time fire event, as measured by direct counts, but then experienced a very large increase after the first rainfall following the burn, where the increase was significantly greater on plots that had been burned compared to unburned plots (Ahlgren and Ahlgren 1965). Fire's indirect effects on the bacterial community are attributed to its more direct effects on the temperature, moisture, and organic content of the soil microclimate (Kang and Mills 2004).

Roots. As discussed earlier, productivity is a fundamental ecosystem characteristic that is likely impacted by fire and is often expressed as total dry weight of plant parts. Just as total aboveground dry biomass represents aboveground productivity, belowground productivity may be quantified with total belowground dry biomass. Belowground productivity may be especially of interest in tallgrass prairie, since the majority of growth happens belowground in this ecosystem (Johnson and Matchett 2001).

Although using root biomass as a proxy for belowground productivity is somewhat problematic due to its many assumptions (see Fahey et al. 1999), it is an easily measured variable that was used in this and other studies (Baer et al. 2002). Root biomass is greater in frequently burned prairie compared to unburned prairie (Rice et al. 1998). This is attributed to increased root production rate rather than a decreased root senescence rate. At Konza prairie, frequently burned plots had greater root growth, as measured by root ingrowth cores, compared to infrequently burned plots (Johnson and Matchett 2001). This observed increase was explained by decreased N availability, which leads to production of new roots with lower tissue N concentration.

In a Minnesota oak savanna, frequently burned plots had greater fine root biomass than infrequently burned or unburned plots (Reich et al. 2001). However, the authors attributed this difference not to increased belowground net primary productivity, but to lower root turnover rates. Based on calculations of productivity involving measured N mineralization rates and tissue N concentrations, both aboveground (ANPP) and belowground (BNPP) productivity were found to decrease as fire frequency increased in the oak savanna plots (Reich et al. 2001).

Interactions

Although many components of the ecosystem that can be impacted by fire have been discussed in turn, each in its own separate category, these components are, of course, not independent of one another. Aboveground plant diversity can influence belowground productivity, N availability can influence aboveground productivity, grazing or mowing can alter the way that fire affects nutrient cycling, and so on. Some of these interactions have been alluded to, but most of them are beyond the scope of this review. However, it is relevant to keep in mind that there are many potentially confounding factors involved in the question of how fire impacts the ecosystem.

Importance of Management

Fire is an important, imperfectly understood tool of managers of tallgrass prairie and many other ecosystems. Since the time of European settlement in the 1800s until the second half of the 20th century, management mostly consisted of fire suppression because fires were viewed as harmful to people and nature (Wan et al. 2001). But since the 1960s, researchers have shown that fire is essential for maintaining native ecosystems such as tallgrass prairie, and management practices have slowly begun to respond (Anderson 1990). Although there are legitimate issues that arise with respect to modern prescribed fire, including concerns about detrimental effects in highly fragmented ecosystems (Brye 2006) and difficulties of burning near urban areas, overall prescribed fire is viewed as a very beneficial tool.

In 2001, a study in the Chicago region looked at the effect of fire frequency on species diversity in tallgrass prairie (Bowles and Jones 2004). This study re-sampled prairie sites that had originally been sampled for species diversity in 1976. The authors found that of those prairies that had received fire management, high-quality prairie sites (those with high species diversity) remained high-quality, and lower quality sites increased in quality (Bowles and Jones 2004). Based on this finding, the authors recommended biennial burning to maintain the integrity of the high-quality tallgrass prairie sites. The current study took place at six of the 62 prairie stands investigated by Bowles and Jones (2004). The intention here was to supplement the 2001 vegetation data from these sites with belowground data, and either strengthen or contest the recommended biennial burning regime.

Goals and Expectations

The goal of this study was to expand knowledge about how anthropogenic fire management affects ecosystem functioning in tallgrass prairie. The Midwestern tallgrass prairie is one of the most endangered ecosystems in the world, with only a tiny fraction of a percent of its original expanse still remaining as native remnant prairie (Samson and Knopf 1994). Prescribed burning is one important tool managers use to maintain and protect tallgrass prairie. Even though management techniques such as fire are undertaken for the sake of the health of the ecosystem, it is very difficult to obtain a complete view of all the ecological changes that can result, due to the complex and interconnected nature of ecological processes.

In general, belowground processes are less well understood than ecosystem components that are easier to see, even though they have important relationships with the state of the plant community. It was hoped that the information provided by this study would shed light on whether frequent fire regimes that have been recommended in view of other aspects of the ecosystem (e.g. plants and insects) optimize the health of the belowground community as well.

This study asked: what is the effect of fire frequency (either frequent fire, infrequent fire, or no fire) in undisturbed tallgrass prairie sites in the Chicago region on components of the tallgrass prairie soil community? The components that were considered, and the accompanying expectations, are listed below.

(1) I expected soil levels of both available N and total N to decrease with increased fire frequency, based on other studies in tallgrass prairie (Ojima et al. 1994). I expected soil levels of total C to be unaffected by fire frequency, due to the relative inertness of this element in the ecosystem (Paul 2007). I expected to see no strong trend for soil levels of the cations that were measured in this study (K, Mg, Ca) because of contradicting results from other studies (Anderson

and Menges 1997, Brye 2006). I also expected to see no strong trend for soil levels of available P for the same reason (Anderson and Menges 1997, Brye 2006).

(2) For arbuscular mycorrhizal (AM) fungi, I expected to see both species richness, as determined by molecular DNA analysis, and activity level, as measured both by amount of colonization on stained roots and amount of colonization on nitrocellulose membranes, to increase with increasing fire frequency. I expected this based on several tallgrass prairie studies that had similar findings (Torpy et al. 1999, Bentivenga and Hetrick 1991), and because of an expectation that the fungal community would experience a parallel effect to that experienced by the plant community (Bever et al. 2001).

(3) I expected the structure of the soil bacterial community, as measured by level of metabolism of different carbon sources, to be significantly different at sites with different fire treatments. This expectation was due to previous studies on fire and microbes (Hart et al. 2004, Kang and Mills 2004).

(4) Finally, I expected root biomass per unit area to increase with increasing fire frequency, due to an expected increased belowground productivity on sites with higher fire frequency (Rice et al. 1998, Johnson and Matchett 2001). All of these variables were expected to provide potentially important information about the functioning of the ecosystem.

MATERIALS AND METHODS

Description of study sites

The six sites used in this study were chosen because they all contain remnant prairies with similar moisture, soil texture, and floristic quality ratings, and are within 40 miles of downtown Chicago. With the exception of McLaughlin (a part of the Skokie River Nature Preserve site), all areas used were grade A mesic prairies with sandy loam or loamy sand soil. McLaughlin is a grade B (signifying late-successional) mesic prairie with sandy clay loam soil. Grades reflect the assignment made by the Illinois Natural Areas Inventory based on the floristic quality of the habitat, where “Grade A” is defined as stable or undisturbed (Bowles and Jones 2004). Five of the six study sites have been dedicated as Illinois Nature Preserves. This designation, granted by the Illinois Nature Preserves Commission, signifies permanent state protection for natural areas in Illinois that contain rare, valuable, and/or threatened native habitat. The study was limited to Grade A prairie because of its high conservation value and consequent ability to represent a healthy ecosystem.

Fire frequency was the independent variable. Skokie River Nature Preserve and Somme Prairie Nature Preserve have a management regime of **frequent fire** (more than 10 fires in the last 20 years); Glenbrook North High School Prairie and Santa Fe Prairie Nature Preserve have a management regime of **infrequent fire** (fewer than 10 fires in the last 20 years); and Site #901 (Monee) and Morton Grove Nature Preserve have a management regime of **no fire** (no record of fire in the last 20 years). In order to ensure that fire *frequency* was the variable being tested, rather than the immediate effects of a single recent fire, all transects were placed in areas where the most recent burn had been in 2006 or earlier. The locations of these six sites in Illinois are shown in Figure 1. Each of the six is described in greater detail below.

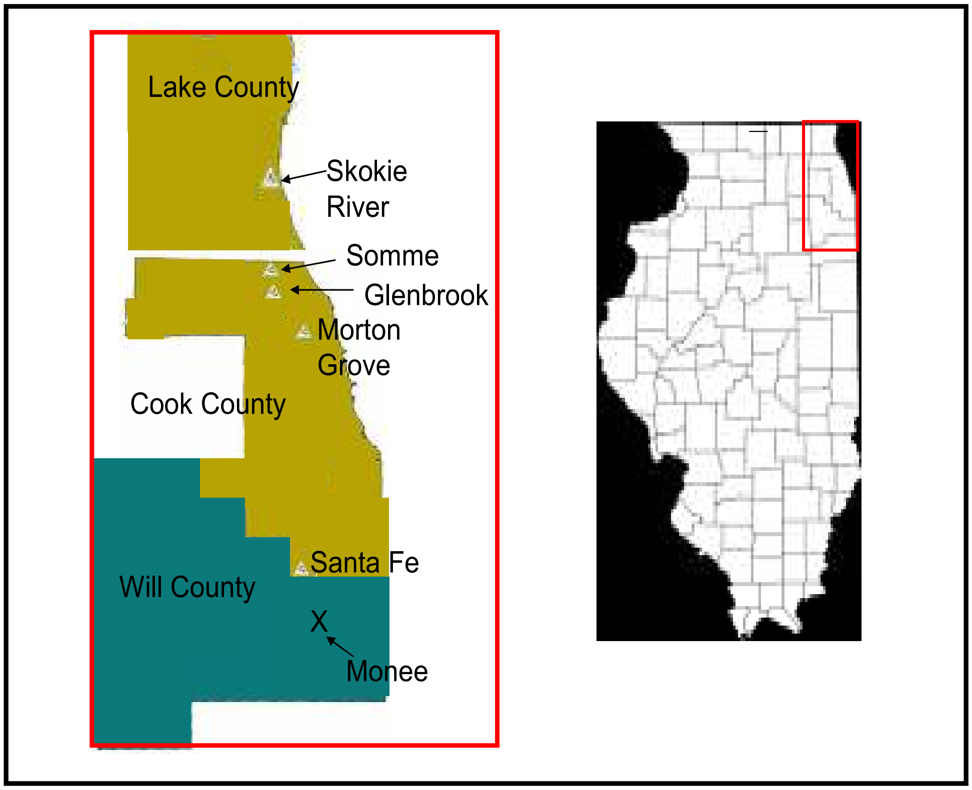


Figure 1. Location of the six study sites in Lake County, Cook County, and Will County in Illinois. Illinois on right and detail of inset on left. Modified images from the website of the Illinois Department of Natural Resources, http://dnr.state.il.us.

Skokie River Nature Preserve. Skokie River Nature Preserve is a 49.8-hectare Illinois Nature Preserve located in Lake Forest. The preserve includes remnant and restored mesic prairie, among other rare and valuable habitat types. The preserve is owned and managed by the Lake Forest Open Lands Association, a non-profit land conservation organization. Two high-quality sections of the preserve were used in this study: Shaw Prairie, a pristine 6.07-hectare tallgrass prairie with sandy loam soil; and McLaughlin Meadow, a 4.45-hectare restored tallgrass prairie with a sandy clay loam soil. Both Shaw and McLaughlin were burned approximately 13 times between 1987 and 2007 (all spring burns). At the time of this study, the most recent burn had been in spring 2006. Transects are shown in Figure 2.

Somme Prairie Nature Preserve. Somme Prairie is a 28.3-hectare Illinois Nature Preserve located in Northbrook. The preserve is a combination of remnant and restored mesic prairie (among other habitat types) with a loamy sand soil. Somme is owned by the Forest Preserve District of Cook County and has been managed by the District and the North Branch Restoration Project since 1977. This study's transect (Figure 3) at Somme is a remnant area that was burned 12 times between 1987 and 2007. Five of these burns were in the fall; the rest were in the spring. At the time of this study, the most recent burn had been in spring 2005.

Glenbrook North High School Prairie Nature Preserve. Glenbrook North High School Prairie is a 0.61-hectare Illinois Nature Preserve located in Northbrook. It has been managed by the North Branch Restoration Project since 1997. The preserve is remnant mesic prairie with a sandy loam soil. Glenbrook was burned regularly from the late 1960s to the early 1980s by a science teacher at the high school. Glenbrook received no burning from the mid-1980s to the late 1990s. A burn regime was resumed by the North Branch Restoration Project in the late 1990s, and it has continued since then. This study's transect at Glenbrook (Figure 4) was burned in 1991, 2001, and 2003, all in the spring. Thus, Glenbrook effectively was burned three times during the period from 1987-2007.

Santa Fe Prairie Nature Preserve. Santa Fe Prairie is a 4.39-hectare Illinois Nature Preserve located in Hodgkins. The preserve is remnant mesic to dry mesic gravel prairie with a sandy loam soil. It was owned by the Burlington Northern Santa Fe Railway until 1998, when the Illinois-Michigan Canal National Heritage Corridor Civic Center Authority, which had been managing the property for the previous ten years (without burning), became the owner. Prescribed burns were attempted but failed to ignite substantially in 1998 and 1999, following which grasses were seeded on the site. As a result of the increased grass cover, the first successful burn was conducted in 2000. Some part of the site has been burned every year since then (2001-2007). This study's transect at Santa Fe (Figure 5) was burned in 2000, 2001, and 2003, all in the spring. Thus, Santa Fe effectively was burned 3 times during the period from 1987-2007.

Site #901. This site is a 1.26-hectare remnant tallgrass prairie in Monee with a loamy sand soil. There are no records of it having received any management between 1987-2007, but it was surveyed by the Illinois Natural Areas Inventory in 1976 and assigned its identification number of 901. This site is located directly adjacent to Canadian National Railway tracks and is assumed to be owned by the Railway (Marlin Bowles, personal communication). The proximity to the rail tracks probably maintained this area as prairie by preventing this land from being developed or plowed and by potentially sparking occasional fires. Historically, wood-powered locomotives were a constant source of stray embers that would start fires (Pyne 1982). This study's transect at 901 (Figure 6) covered the entire 0.24-hectare grade A section.

Morton Grove Nature Preserve. Morton Grove Prairie is a 0.53-hectare Illinois Nature Preserve owned by the Morton Grove Park District located in Morton Grove. The preserve is remnant mesic to wet mesic tallgrass prairie with a sandy loam soil. It became a preserve in 1979 and was burned every other year from 1979-1987. In 1987, a volunteer steward began a new management program of fire suppression on the site. Management was turned over to two new volunteer stewards in 2007, who conducted the first burn in 20 years on the south half of the site in spring 2007. This study's transect at Morton Grove (Figure 7) was in the north half, the section that had not been burned for 20 years.

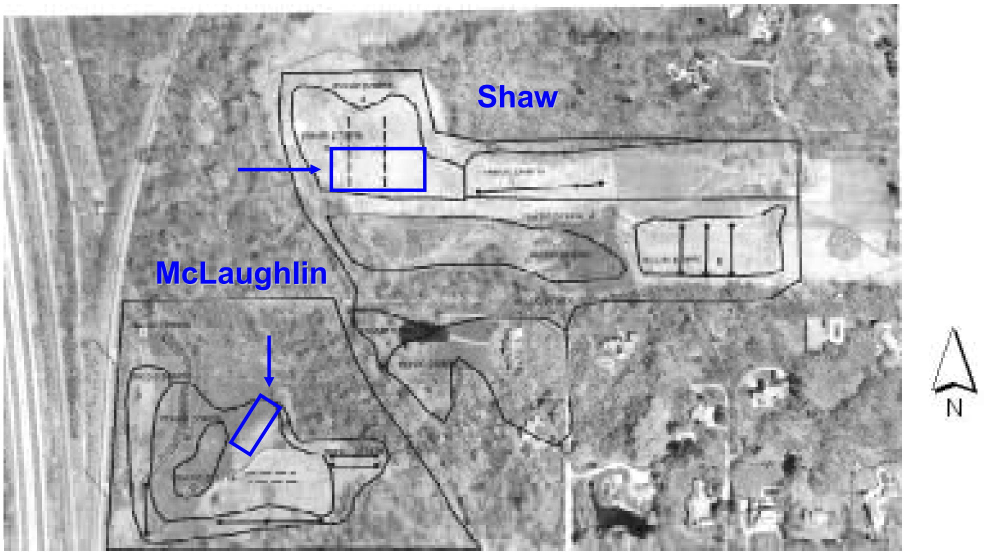


Figure 2. Skokie River Nature Preserve. Arrows and boxes indicate the transects at Shaw and McLaughlin. Modified from a modification by Jenny McBride at Morton Arboretum

Figure 3. Somme Prairie Nature Preserve. Arrow and box indicate the transect area. Modified from a modification by Jenny McBride at Morton Arboretum

Figure 4. Glenbrook North High School Prairie Preserve. Arrow and box indicate the transect area. Modified from Google Earth.

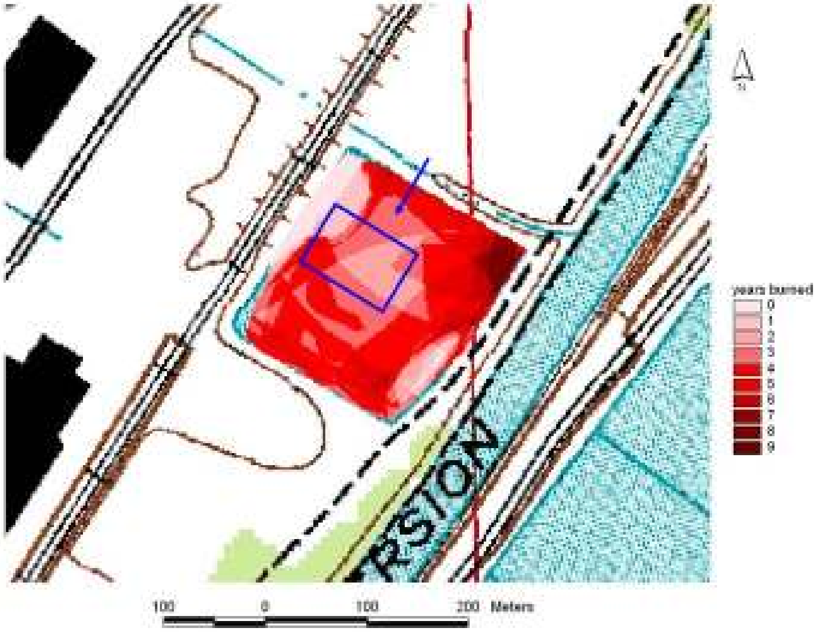


Figure 5. Santa Fe Prairie Nature Preserve. Arrow and box indicate the transect area. Modified from a modification by Jenny McBride at Morton Arboretum.

Figure 6. Site #901 (Monee). Arrow and box indicate the transect area. Modified from Google Earth.

Figure 7. Morton Grove Nature Preserve. Arrow and box indicate the transect area. Modified from Google Earth.

Methods

Sampling

At each study site, I established transects in late June 2007. Transects were established by laying a 50-m measuring tape through the center of the area of interest. At smaller sites, the area of interest was the entire site. At the larger preserves, the area of interest was the section that constituted the highest quality prairie with the desired fire frequency history. At all sites except Skokie River, I flagged 15 study plants, five each of three different species, along the transect line. At Skokie River, due to permit constraints, nine study plants, three each of three species, were flagged at Shaw, and nine more at McLaughlin.

The study plants were identified by choosing five (or three) random points along the transect line. At each point, the nearest individual of each of the three species was located. The three plant species chosen for each site varied depending on which species appeared most dominant at each site (by visual inspection) and were readily identifiable by a non-expert in June. Table 1 illustrates the study species for each site. *Silphium terebinthinaceum* Jacq. was the only plant species that was used at every site.

Skokie River		Somme	Glenbrook	Santa Fe	901 (Monee)	Morton Grove
Shaw	McLaughlin					
Andropogon	*Andropogon*	*Andropogon*	*Eryngium*	*Andropogon*	*Monarda*	*Andropogon*
Sporobolus	*Monarda*	*Sporobolus*	*Monarda*	*Sporobolus*	*Sporobolus*	*Sporobolus*
Silphium	*Silphium*	*Silphium*	*Silphium*	*Silphium*	*Silphium*	*Silphium*

Table 1. Study plant species used at each study site. Genera are listed in table. These include the following species: *Andropogon gerardii* Vitman, *Eryngium aquaticum* L., *Monarda fistulosa* L., *Silphium terebinthinaceum* Jacq., *Sporobolus heterolepis* (A. Gray) A. Gray.

At the time of transect establishment, I inserted hyphal growth pouches into the surface of the soil near the root zone of each study plant. The pouches consisted of one piece of fine mesh (1 mm openings), folded over and heat sealed on three sides, with a second piece of mesh

(3 mm openings) inside. The pouches were about 5 cm wide and 15 cm long. When inserted into the soil, with the aid of a craft stick, the top of the pouch was 1-2 cm above the surface of the soil, so the bottom of the pouch reached to a depth of 13-14 cm below the surface.

Each pouch contained two nitrocellulose membranes, placed on either side of the inner mesh. The membranes, 25 mm in diameter, have a pore size of 0.45 µm. The membranes capture growing fungal hyphae in the soil. The pouches were collected and replaced every month from July through October 2007, with the final collection in November 2007. In addition, at the time of each visit from June through November, one moisture reading was taken at the 0 point of the transect using a portable time domain reflectometer (Hydrosense, Campbell Scientific, Inc., Logan, UT, USA).

Soil cores were collected in July and October. At both sampling times, two cores, 10 cm in depth and 6 cm in diameter, were taken near the root zone of each study plant, for a total of 30 (or 36) cores per site. The soil was immediately weighed and air-dried at room temperature. For most October samples, before air-drying, a subsample was separated and frozen at -17°C. For most July samples, a subsample was separated and refrigerated at 3°C. The air-dried soil was then re-weighed for a measure of field moisture conditions. All the roots that could be hand-removed from a 200g sample in 10 minutes were removed from the wet soil after the initial weighing and before separating out a subsample. This generally included all root pieces with a diameter >1 mm. The roots were rinsed in tap water and dried separately in a 70° C oven. The roots were then re-weighed to obtain dry biomass.

Analyses

Physical soil characteristics. Soil texture was measured by adding 100 mL of 5% Calgon solution to 50 g soil and agitating the solution for 5 minutes. Distilled water was then added to the solution until the total solution volume was 1000 mL. After 20 seconds of agitation, a Fisher brand Hydrometer was inserted into the solution. After 40 seconds, a sand reading was taken. After two hours, a clay reading was taken. Percent silt is calculated by subtracting the percent sand and clay. This analysis was the source of the soil texture types listed in the site descriptions. Refrigerated soil samples collected in July were used to measure the pH and electrical conductivity of each site. Soil samples were diluted in deionized water in a 1:5 solution, and were measured using a Daigger 5500 pH meter and a Corning EC/conductivity meter.

Soil nutrients. Air-dried soil samples were passed through a 2-mm sieve in preparation for chemical analyses. I sent 186 soil subsamples to the Kansas State University Soil Testing Lab, and 18 subsamples to the Research and Development Department of the Metropolitan Water Reclamation District of Greater Chicago. The 204 subsamples came from 204 of the ~360 soil samples, and included samples collected in both July and October, from all sites, and under all plant species. These labs tested the soil for levels of three exchangeable cations: Potassium; Calcium; and Magnesium. These three exchangeable cations were extracted at both labs using 1 M Ammonium Acetate (NH_4OAc). NH_4OAc is a strong salt solution which displaces ions from soil exchange sites. Soil samples were combined with NH_4Oac, shaken for 5 minutes, and filtered through a Whatman No. 2 filter. Cation concentrations were determined using an adsorption/emission spectrometer. The KSU Testing lab also tested the samples for levels of available P and available N in the forms of Phosphorus (Bray-1 Extraction); NH_4-N (KCl extraction); and NO_3-N (KCl extraction).

For analysis of total C and total N, I ground 130 subsamples (from all sites, from both July and October) at DePaul University in a Spex Certiprep mill for one minute to a consistent texture. The ground samples were packaged into tin capsules, each containing 50-100 mg of soil.

The capsules were then loaded into the N/C Element Analyzer at the Environmental Sciences lab at DePaul University. When each capsule was dropped into the combustion tube of the machine, which is kept at 900° C, it came into contact with a strongly oxidizing environment, triggering an exothermic reaction. This caused the temperature to rise to 1800° C, resulting in combustion of the capsule and the soil sample inside. The combustion reaction produced a mixture of N_2, CO_2, H_2O and SO_2, which was conveyed across the reactor to a second reactor. In the second reactor, any nitrogen oxides that may have formed were converted to elemental nitrogen. After the gas mixture crossed an adsorption filter, retaining the water, it was sent to a thermal conductivity detector, which produced an electrical signal proportional to the N or C present.

Mycorrhizal activity. I quantified mycorrhizal activity by looking at the nitrocellulose membranes from the hyphal growth pouches and at stained roots from the soil samples. I mounted one membrane from each of 463 hyphal growth pouches (from all months, July through November) on a glass slide in Polyvinyl Lacto-Glycerol (PVLG). Fungal activity was measured by examining each membrane under a microscope at 100X magnification. The number of hyphal structures per viewing field was observed for 30 fields per membrane.

Rate of growth (R), meters of hyphae per m^2 of soil, was calculated for each membrane using the formula

$$R = n * 0.00418 * 605681 * (1/1000)$$

where n is the number of hyphal structures, 0.00418 is a constant representing the length of hyphae per field of view for the microscope that was used, 605681 is a constant representing the size of the filter and the number of fields of view that were used, and 1/1000 is a constant that converts the unit to meters.

Fungal productivity (P_F), defined as length of hyphal growth per day, was calculated using the formula:

$$P_F = R/d$$

where R is the rate of growth and d is the number of days that the membrane was buried in the soil.

For 123 soil samples (88 from July and 35 from October), a portion of the roots was stained with Trypan blue and mounted onto a glass slide in PVLG. The staining procedure was modified from Koske and Gemma (1989). For each slide, between 30 and 40 fields of view were observed. In each field of view, the number of hyphae, coils, vesicles, and arbuscles (all arbuscular fungal structures) was noted. The number of empty fields was also noted. Mycorrhizal activity (T) for each slide was calculated using the formula

$$T = (H + V + A + C)/(H + V + A + C + N)$$

where H is the total number of hyphae observed, V is the total number of vesicles, A is the total number of arbuscles, C is the total number of coils, and N is the total number of blank fields.

Fungal diversity. In order to investigate species diversity of arbuscular mycorrhizal fungi at the study sites, fungal DNA was extracted from frozen soil (from October) using a FastDNA SPIN kit for soil (Qbiogene) according to the manufacturer's protocol using 500 mg soil per sample. DNA was extracted from ten samples from Morton Grove, ten samples from Somme, and ten samples from Santa Fe. The samples were chosen in order for each of the three fire treatments to be represented in this relatively small analysis. Arbuscular mycorrhizal fungal DNA was amplified using a nested PCR following the design of Redecker (2000). First two fungus-specific primers, ITS1F and ITS4, were used, followed by combinations of one of these two primers and one of the following AM fungus-specific primers: GLOM5.8R, GIGA58.R, LETC1670, ACAU1660, and ARCH1311. PCR products were run on a 1.5% agarose gel and gels were documented using Kodak Molecular Imaging Software. The PCR products were digested with two endonucleases, HinfI and AluI, for one hour at 37˚C. The resultant digests were run on a 1.5% agarose gel, and banding patterns were documented and analyzed using Kodak Molecular Imaging Software. Arbuscular mycorrhizal identities were determined by comparing the banding patterns from the samples against those for known AM taxa.

Bacterial activity. Activity of the bacterial community can be visualized using Biolog Microplate analysis, which uses a microarray of carbon substrates that can each be metabolized only by some groups of bacteria (Garland and Mills 1991). The degree of metabolic activity in each well can be quantified by a spectrophotometer, because each well contains tetrazolium violet, which changes color when it is reduced as a result of respiration of the carbon source. This technique gives information about the functional diversity of the bacterial community.

For this study I used GN2 MicroPlates, which contain 95 different carbon substrates (see Appendix) in 95 wells, and water as a control in the 96th well. First, I inoculated duplicate MicroPlates with one sample each from each of the six study sites. For Skokie River, a sample from McLaughlin was used. All samples used for the Biolog analyses were frozen subsamples of soil collected in October. The plates were inoculated with dilutions of the soil samples. The dilutions were prepared by thawing frozen soil at room temperature for 24 hours. I dissolved 5 g of soil in 45 mL of sterile water to create a 10^{-1} dilution, transferred 1 mL of this solution to 9 mL of sterile water to create a 10^{-2} dilution, and transferred 3 mL of this solution to 27 mL sterile water to create a 10^{-3} dilution. I pipetted 100 µL of the 10^{-3} dilution into each well of each microplate.

I used a Biolog MicroStation Reader spectrophotometer at the Environmental Sciences Lab at DePaul University to quantify activity in the plates on Days 3, 4, 5, and 6 after inoculation. Due to a problem with the spectrophotometer, readings were obtained only for Days 3 and 4, and data was collected for Days 5 and 6 manually as binary data—presence or absence of color in the well. Duplicate MicroPlates were inoculated a second time with the same six samples, and spectrophotometer readings were obtained for Days 3, 4, 5, and 6 after inoculation.

Duplicate microplates were inoculated a third time using seven samples from four sites: two from Monee, one from Morton Grove, two from Shaw, and two from Somme. Readings from the MicroStation reader were obtained on Days 1, 2, 5, and 6 after inoculation. The samples used in the first two Biolog rounds were chosen in order for all sites to be represented on a limited number of plates. The samples used in the third Biolog round were chosen with the goal of highlighting any differences between the two ends of the burn treatment continuum, the frequently burned sites and the unburned sites, by having as many samples as possible from these 4 sites on a limited number of plates.

For each plate, several values were calculated. Community Metabolic Diversity (CMD) is the number of wells (out of a possible 95) that had at least some activity in the plate. CMD was calculated by assigning each well either a +, indicating activity, or a -, indicating no activity. The CMD value for each plate was the total number of +'s in the plate. Average Metabolic Response (AMR) is an average of the optical density readings of all of the wells in the plate. AMR was calculated by first zeroing all values by subtracting the control well reading. The corrected values for the 95 active wells were used to calculate an average value for each plate—the AMR. AMR was calculated only for Rounds 2 and 3, because of errors in the numerical data from Round 1.

Statistical Analysis

Values for total C and total N were analyzed for a site effect, group effect, and month effect using a one-way ANOVA (SYSTAT 12). Values for Ca, Mg, K, and October moisture were analyzed for a site effect and group effect using one-way ANOVA. Differences between sites were analyzed using post-hoc Fisher's LSD pair-wise comparisons (SYSTAT 12). Values for available P and for October root masses were log-transformed to attain a normal distribution and analyzed for a site effect, group effect, and month effect using a one-way ANOVA with post-hoc Fisher's LSD pair-wise comparisons. All other variables (NH_4, NO_3, pH, conductivity, July root masses, July moisture, mycorrhizal activity [T], fungal productivity [P_F], and Biolog Average Metabolic Response [AMR]) could not be normalized by any transformation, and were thus analyzed for site, group, and month effects by the nonparametric Wilcoxon signed-rank test, which does not assume a normal distribution. Fungal productivity P_F data was also analyzed for a plant species effect by the Wilcoxon signed-rank test.

The Biolog data was also analyzed using a Multidimensional Scaling (MDS) analysis (SYSTAT 12), which is a multivariate ordination test that clusters samples in two-dimensional space based on how similar they are to each other overall, taking into account multiple variables. MDS does not make any assumptions about the distribution of the data. Because each of the twelve or fourteen samples that were used for each Biolog run had 95 data points (for the 95 carbon sources in a Biolog plate) for each of the four days that optical density readings were taken, a test like MDS was the best way to pull out the overall relationships between the bacterial communities of each of the sites. Pearson correlations (SYSTAT 12) were run comparing each of the 95 data points (for each sample, for each day) to the two dimensions (DIM1 and DIM2) created by the MDS analysis. The carbon sources which correlated most strongly with DIM1 or DIM2 were the ones that contributed the most to the spatial distribution of the sites in the MDS analysis.

Because the question of ultimate interest was how similar or different the six sites were to each other, overall, variables were combined in two additional MDS analyses. The first overall MDS analysis combined all of the nutrient variables: NH_4, NO_3, available P, total N, total C, Ca, Mg, and K. The second overall MDS analysis combined all the data of the mycorrhizal variables, activity (T) and productivity (P_F). For all MDS analyses, distances between coordinates were quantified using pairwise Euclidean distances (SYSTAT 12).

RESULTS

Results are discussed here first in terms of within-group variation (the six sites compared to each other), followed by between-group variation (the three fire frequency groups compared to each other). For both within-group and between-group comparisons, data from Shaw only was used to represent Skokie River, since McLaughlin had important characteristics other than fire frequency differentiating it from the rest of the sites. However, for the between-group comparisons, a fourth group, "Frequent + M," representing the average value of McLaughlin, Shaw, and Somme, is also included. In addition, McLaughlin is discussed individually.

Within-group variation

Physical Soil and Root Characteristics.

Soil moisture as determined by gravimetric analysis of soil samples (Figure 8) differed significantly by site ($p<0.05$) and by month ($p<0.01$). In July, the wettest samples came from Shaw, and in October, the wettest samples came from Shaw and Somme. In July, the driest samples came from Monee and Somme, and in October, the driest samples came from Monee. Moisture results from the reflectometer, from which readings were obtained for all five months, are shown in Figure 9, confirming that there was much month-by-month variation.

Electrical conductivity (EC) and pH, shown in Figure 10, were measured using only soil samples that had been collected in July. The pH was significantly different by site ($p<0.05$), with Santa Fe measuring as the most basic with an average pH of 7.25, and Glenbrook measuring as the most acidic with an average pH of 6.63. EC differed significantly by site ($p<0.05$). Santa Fe had the highest EC with an average value of 94.83 μS, while Glenbrook had the lowest EC with an average of 43.13 μS.

Dry root biomass (Figure 11) differed significantly by site ($p<0.05$) and by month ($p=0.01$). The greatest root masses in July were found at Somme and Shaw, while the lowest root masses were found at Santa Fe and Monee. In October, dry root mass at Shaw, with a mean of 3.122 g per sample, was significantly greater than root biomass at all other sites. Root biomass at Monee had the lowest root mass, with a mean of 0.875 g per sample.

Soil Nutrients.

Cation values are from July only (Figure 12). Calcium, potassium, and magnesium all differed significantly between sites ($p<0.05$). Calcium levels were highest at Somme, with a mean of 5296 mg/kg, and lowest at Monee, which had a mean of 3613 mg/kg. Potassium levels were highest at Morton Grove, with a mean of 591 mg/kg, and lowest at Shaw, which had a mean of 118 mg/kg. Magnesium levels were highest at Santa Fe, with a mean of 1385 mg/kg, and lowest at Monee, which had a mean of 792 mg/kg.

Available N in the form of NH_4 (Figure 13) was significantly different by site ($p<0.05$) but not by month ($p>0.1$). Because they were statistically equivalent, NH_4 values from July and October were combined for the site analysis. NH_4 values were highest at Monee, with a mean of 22.57 mg/kg, and were lowest at Morton Grove, with a mean of 10.19 mg/kg. Available N in the form of NO_3 (Figure 14) was significantly different by site ($p<0.05$) and by month ($p<0.01$). Because they were statistically different, NO_3 values were analyzed separately for July and for October. NO_3 values were highest in July at Monee with a mean of 75.99 mg/kg, and lowest at Morton Grove, with a mean of 8.5 mg/kg. In October, the highest NO_3 values were found at Monee, Santa Fe, and Glenbrook, while the lowest values were found at Somme.

Available P (Figure 15) was significantly different by site ($p<0.01$) but not by month ($p>0.1$). Because they were statistically equivalent, values from July and October were combined for the site analysis. Available P was highest at Somme and Glenbrook, with means of 16.37

mg/kg and 16.8 mg/kg, respectively. Available P was lowest at Shaw and Santa Fe, with means of 10.12 mg/kg and 9.78 mg/kg, respectively.

Total carbon and total nitrogen as measured by combustion (Figure 16) were significantly different by site ($p<0.05$) but not by month ($p>0.1$). All values from July and October were combined for the site analysis. Total nitrogen was highest at Monee and Santa Fe, with mean values of 1.04% and 1.01%, respectively, and was lowest at Morton Grove, with a mean value of 0.63%. Total carbon was highest at Monee, with a mean value of 19.83%, and lowest at Morton Grove, with a mean of 8.90%.

Mycorrhizal Activity.

Mycorrhizal productivity (P_F), the measure of hyphal growth rate (Figure 17), varied by site ($p<0.05$) and by month ($p<0.05$) but not by plant species ($p>0.05$). Productivity was greatest in August and November, with overall means of 6.70 m/m^2/day and 5.83 m/m^2/day, respectively, and lowest in October, which had an overall mean of 2.30 m/m^2/day. By site, productivity was highest at Glenbrook and Monee, with overall means of 8.02 m/m^2/day and 5.36 m/m^2/day, respectively, and was lowest at Morton Grove, with an overall mean of 1.78 m/m^2/day. There were few significant differences between sites for each individual month, however (Figure 18). For July, there were no significant differences at all between sites. For the other months, Glenbrook or Monee tended to have the highest values for productivity.

Mycorrhizal colonization (T) on stained roots was significantly different between sites ($p<0.05$) but not months ($p>0.1$), so values from July and October were combined for the site analysis. Colonization was greatest on stained roots from Shaw, with a mean value of 48% colonization (Figure 19) .

Fungal Diversity.

Each sample used in the nested PCR demonstrated the presence of at least one band, meaning that each sample had AM fungal DNA that was amplified by at least one of the primer combinations. Qualitatively, the data demonstrated differences in species richness between frequently, infrequently and unburned sites (Appendix 2). Unburned sites contained highest species richness (30 AM taxa) compared with 13 and 16 taxa for frequently or infrequently burned sites, respectively. The biggest differences in community composition occurred between unburned and frequently burned sites. Unburned sites contained more members of the Gigasporaceae (7 taxa) and Acaulosporaceae (4 taxa) than the frequently burned sites (1 taxa each in the Gigasporaceae and Acaulosporaceae). Glomaceae were well represented on all sites.

Bacterial Activity.

Values for Community Metabolic Diversity (CMD), the total number of carbon substrates utilized by a given Biolog plate, are shown in Figures 20 and 21. The arrangement of the samples used in the second Biolog analysis (using one sample each from all six sites), as placed by the Multidimensional scaling (MDS) analysis, is shown in Figure 22. The analysis shown used the spectrophotometer readings from Day 3 (72 hours). The two plates from the Somme sample had the most distinct placement. Normalized Euclidean distances between the coordinates in Figure 22 quantify how close the samples were to each other (how similar they were). The Euclidean distance between SO-x and SO-y was 0.29 units, while the next nearest sample to both Somme samples was GL-x (.896 units from SO-x, 1.184 units from SO-y).

GL-y was also placed far from all the other samples. Its nearest neighbor was MC-y, at a Euclidean distance of 0.912 units away, and the other Glenbrook sample, GL-x, was 1.275 units away from GL-y. The carbon sources which contributed most to the arrangement shown in Figure 22 are listed in Table 2. These carbon sources had high Pearson correlations (a high R^2) with either Dimension 1 or Dimension 2 in Figure 22.

The arrangement of the samples used in the third Biolog analysis (using two samples each from four sites), as placed by the Multidimensional Scaling analysis, is shown in Figure 23. This analysis shows spectrophotometer readings from Day 2 (48 hours). In this analysis, the most similar sample pairs, as quantified by Euclidean distance, were F-x/F-y (0.029 units), F-y/G-x (0.073), and D-x/D-y (0.081). The four samples from Somme, D-x, D-y, E-x, and E-y, were more similar to each other than to any other sample, with the longest Euclidean distance among them being 0.36 units between D-y and E-x, and the nearest Euclidean distance to another sample being 0.47 units between D-x and A-x. The two samples from Monee ND3, B-x and B-y, were also quite distinct. The Euclidean distance between B-x and B-y was 0.472 units, while the distance from B-x to A-y was 0.93 units, and the distance from B-y to C-y was 0.441 units. The carbon sources which contributed most to the arrangement of the Round 3 samples, based on Pearson correlations, are listed in Table 3.

The carbon sources that were highly significant for both the second and third Biolog runs were N-acetyl-D-glucosamine, sucrose, and D-mannose. As an example of a typical sequence, the activity of the 14 plates in Round 3 in the sucrose well over the 3 days of measured activity (excluding the first day of measurement, which indicated very little activity) is shown in Figure 24.

Average Metabolic Response (AMR), the value which expresses the total amount of bacterial metabolic activity on a plate, was statistically compared (Wilcoxon) for the 12 plates in Round 2 and the 14 plates in Round 3. Both comparisons used Day 6 readings. One of the Glenbrook plates, GL-y, had the highest AMR in Round 2, while plates from Somme, Santa Fe, and Monee had the lowest AMRs in Round 2 (Figure 25). Plates from Monee, B-y and C-y, had the highest AMR in Round 3, while a plate from Somme, E-x, had the lowest AMR in Round 3 (Figure 26).

Between-group variation

There were significant differences between the fire frequency groups for soil moisture, pH, Electrical Conductivity, average root mass, and soil levels of calcium, magnesium, potassium, NH_4, NO_3, total nitrogen, total carbon, and November mycorrhizal productivity (P_F). There were no significant differences between groups for phosphorus levels, mycorrhizal productivity (P_F) of all other months, or mycorrhizal root colonization (T). Parameters that were significantly different between groups are shown in Figures 27 through 34.

The "frequent" group (comprising Shaw and Somme) and the "frequent + M" group (comprising Shaw, Somme, and McLaughlin) were significantly different from each other for the parameters of NH_4 and total N.

Of the three principal frequency groups (excluding "frequent + M") the infrequent group had the highest soil moisture in July and the frequent group had the highest soil moisture in October. The infrequent group had the highest average pH, while the infrequent and unburned groups had the highest electrical conductivity (EC). Average dry root mass was highest in the frequent group for both July and October. Calcium was highest in the frequent and infrequent groups, magnesium was highest in the frequent group, and potassium was highest in the unburned group. Average NH_4 was highest in the unburned group. Average NO_3 was highest in the infrequent and the unburned groups in July, and in the infrequent group in October. Average total carbon was highest in the unburned group, and average total nitrogen was highest in the infrequent group. November mycorrhizal productivity (P_F) was highest in the infrequent group.

McLaughlin.

Each soil parameter measured is shown for McLaughlin in Table 4, where average values are compared against the other Skokie River site, Shaw. Shaw had significantly higher moisture in both July and October. Dry root mass was significantly higher at Shaw in October. Calcium and magnesium levels were significantly higher at Shaw. NO_3 levels were significantly higher at Shaw in both July and October. Total nitrogen and total carbon were both significantly higher at Shaw. Mycorrhizal colonization (T) was significantly higher at Shaw. All other soil parameters were not significantly different between Shaw and McLaughlin.

Overall Site Comparisons.

The nutrient MDS (Figure 35) placed McLaughlin the furthest from all the other sites, while Somme and Santa Fe were the closest site pair. In this analysis, the unburned sites, Monee and Morton Grove, were placed relatively close to each other, while the pattern of the remaining 5 sites did not particularly reflect their burn treatments. In the mycorrhizal MDS (Figure 36), the closest site pairs as quantified by Euclidean distance were Somme/McLaughlin (0.44 units), Morton Grove/McLaughlin (0.495 units), Santa Fe/Shaw (0.513 units), and Santa Fe/Somme (0.608 units).

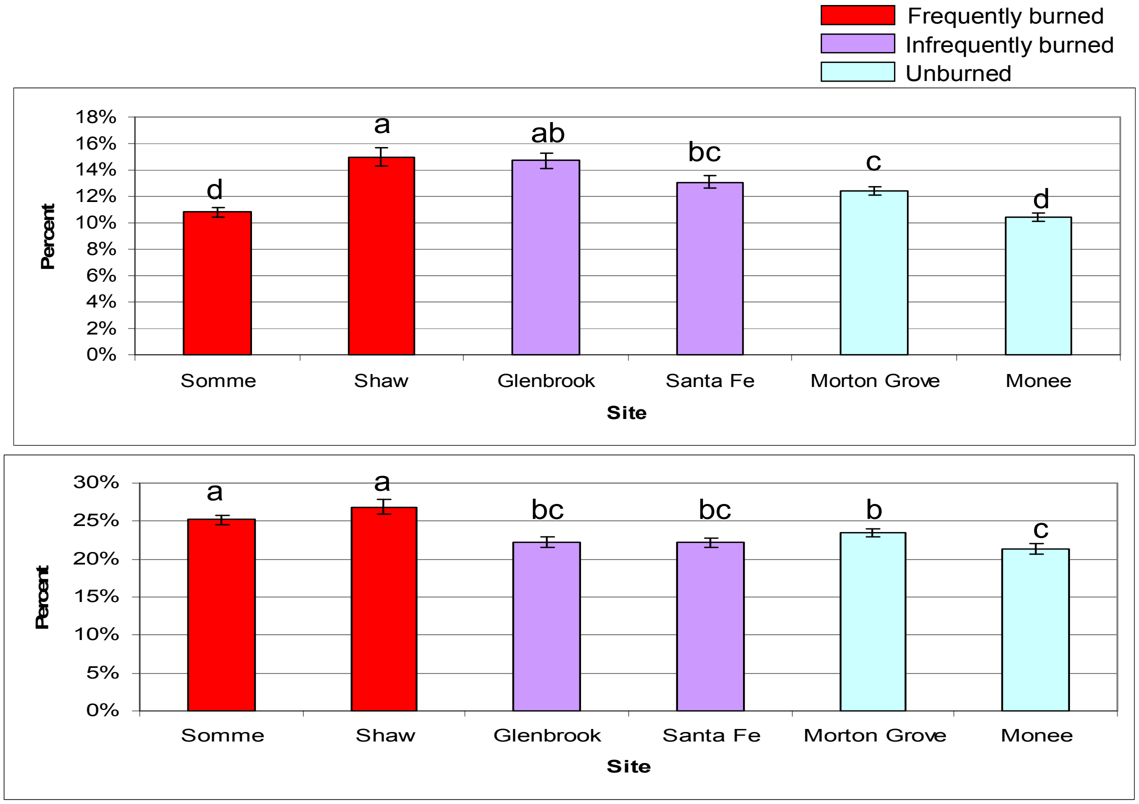


Figure 8. Average soil moisture as determined by gravimetric analysis in July (top) and October (bottom). Letters indicate significant differences between sites ($p<0.05$).

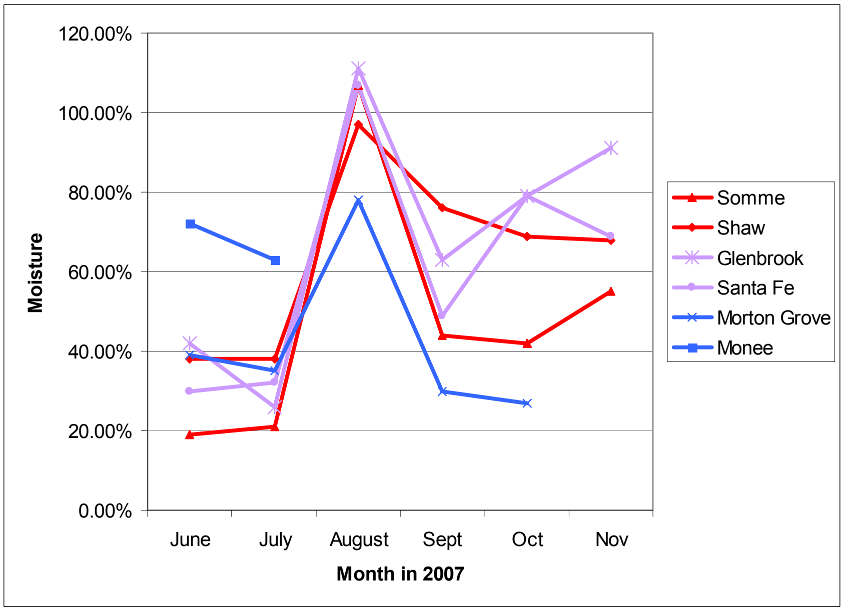


Figure 9. Moisture for each month as measured by portable time domain reflectometer. Missing values are due to instrument malfunction.

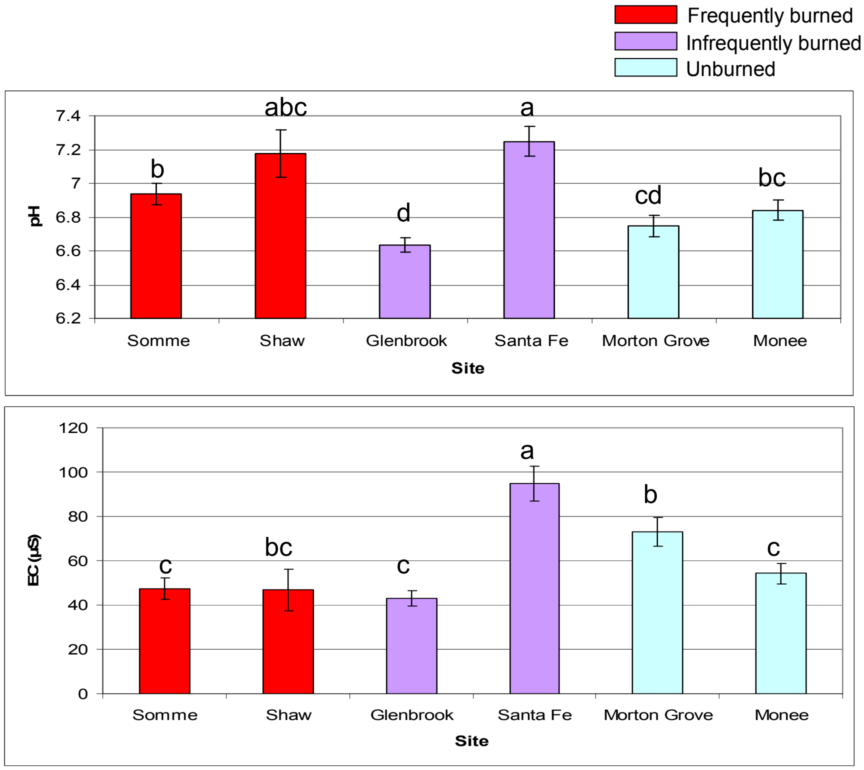


Figure 10. pH (top) and Electrical Conductivity (bottom) of soil collected from each site in July. Letters indicate significant differences ($p<0.05$).

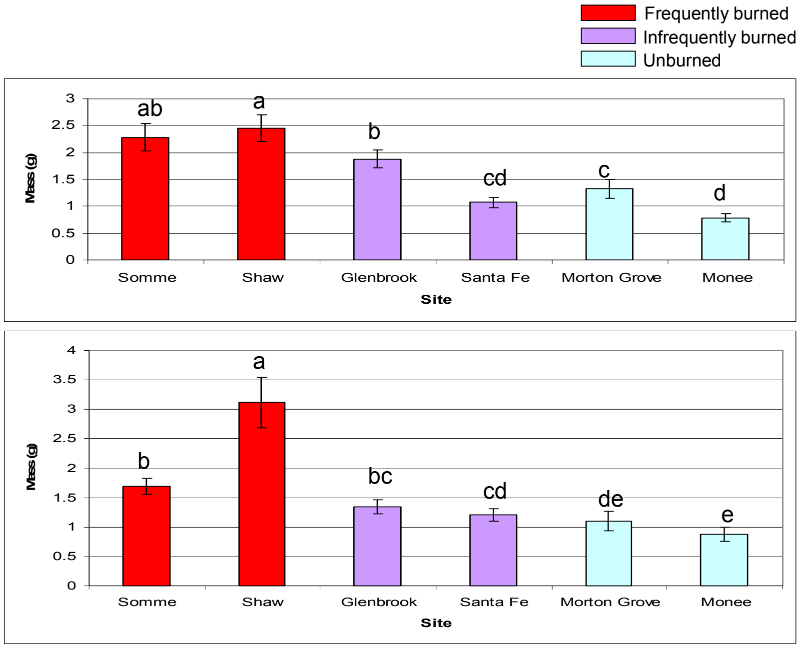


Figure 11. Average dry root mass at each site in July (top) and October (bottom). Letters indicate significant differences ($p<0.05$).

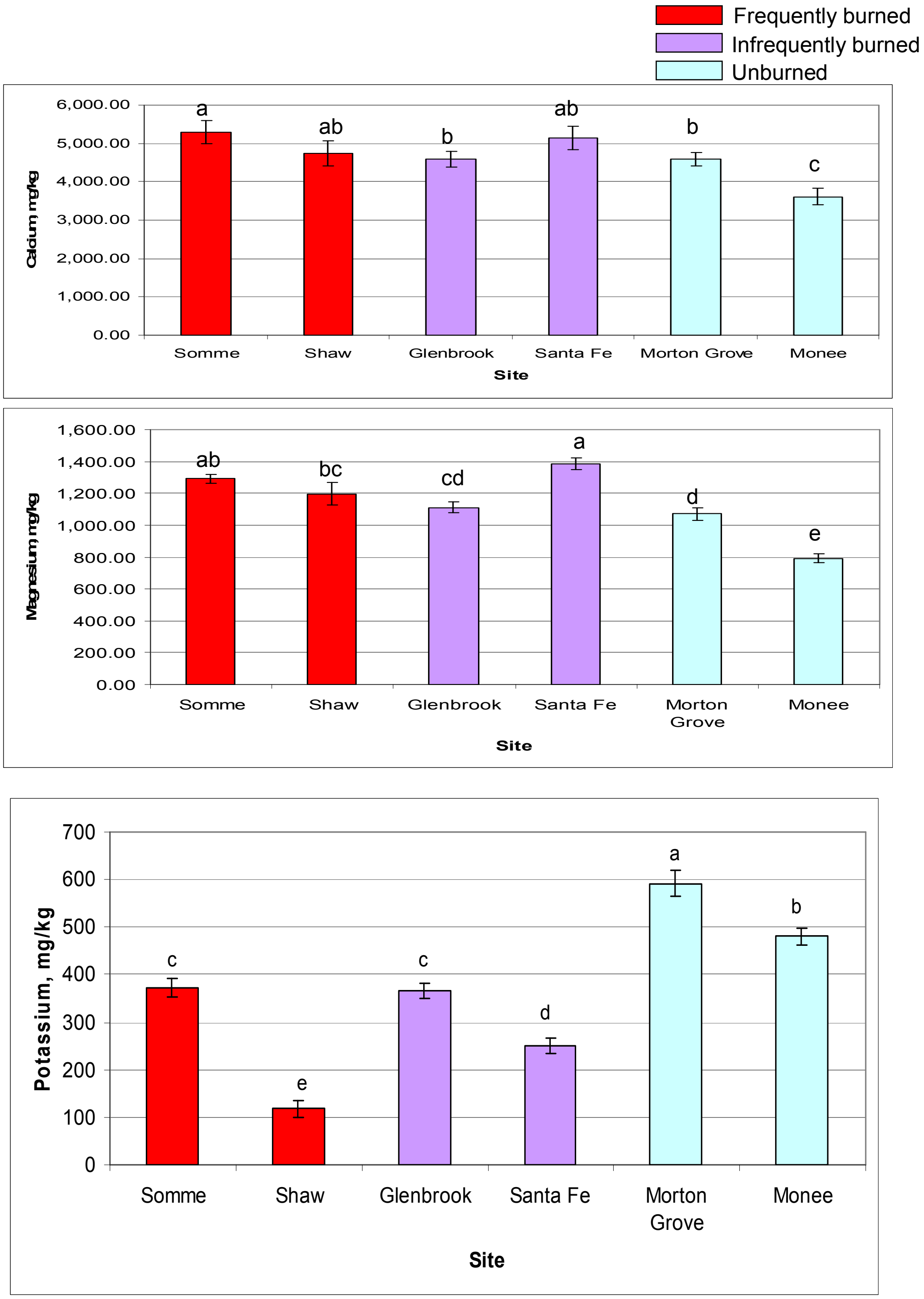


Figure 12. Calcium (top), magnesium (middle), and potassium (bottom) levels at all sites. Letters indicate significant differences between sites ($p<0.05$).

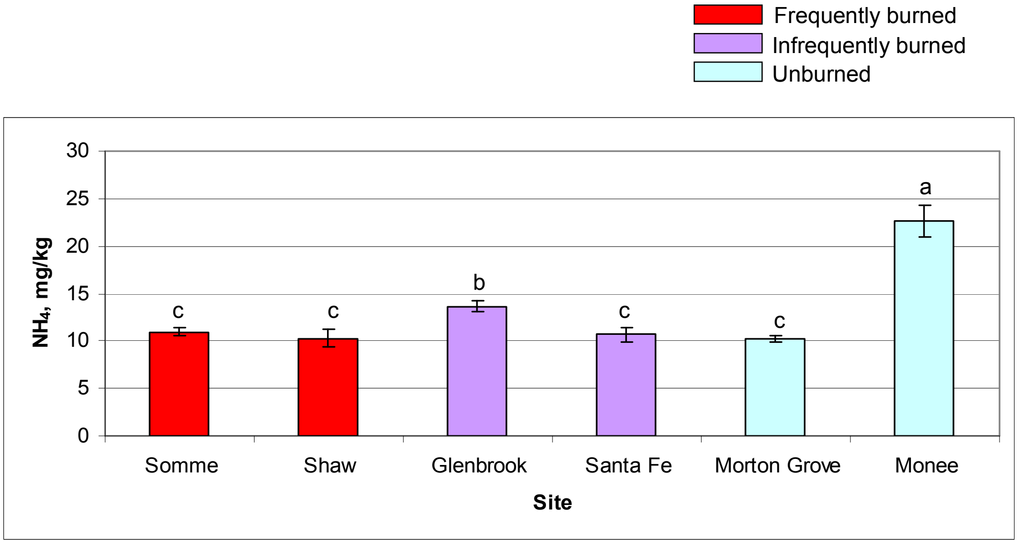


Figure 13. NH_4 levels at all sites (combined values from July and October). Letters indicate significant differences ($p<0.05$).

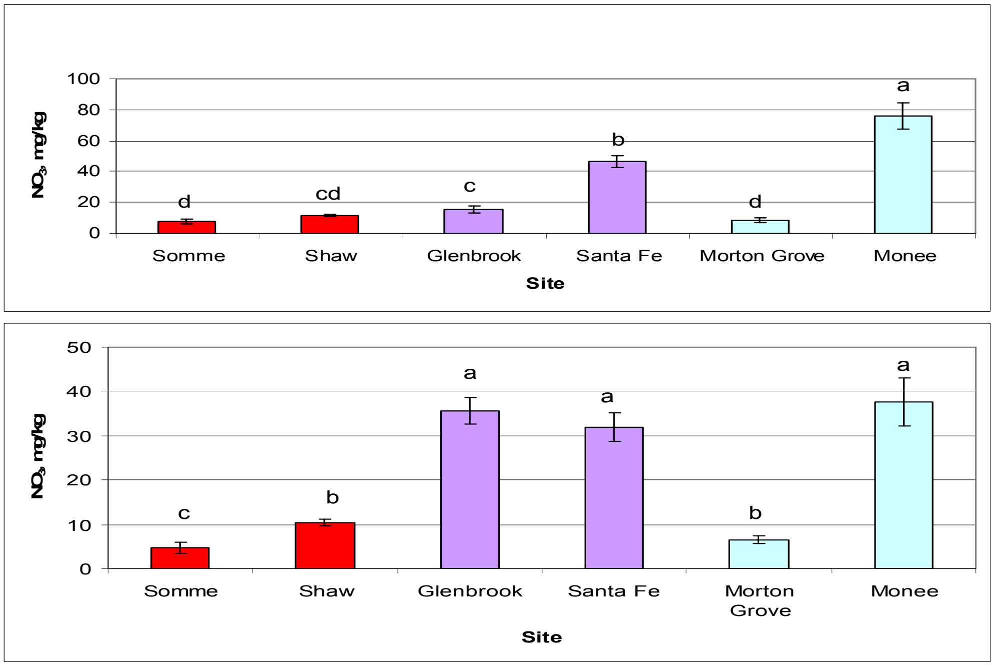


Figure 14. NO_3 levels at all sites in July (top) and October (bottom). Letters indicate significant differences ($p<0.05$).

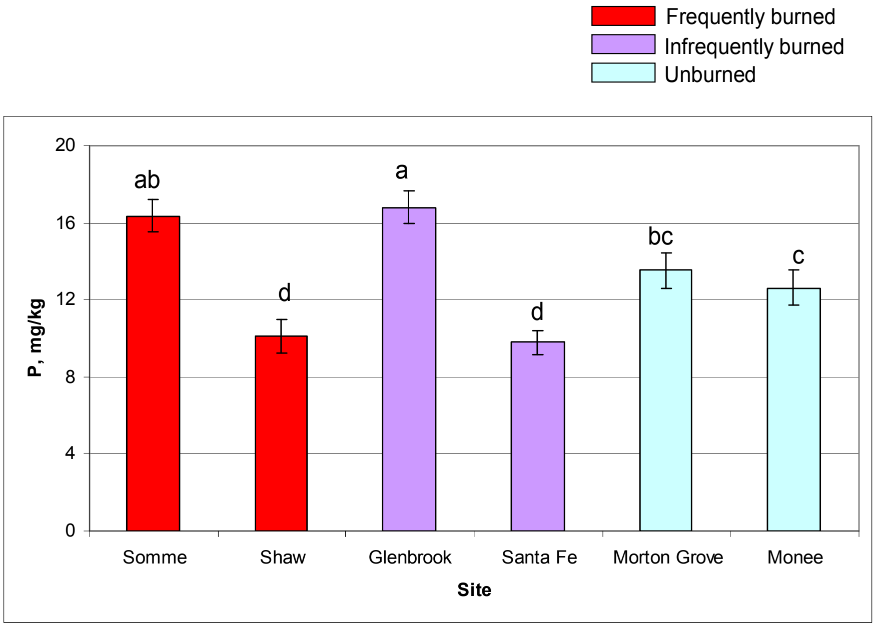


Figure 15. Available P at all sites (combined values from July and October). Letters indicate significant differences ($p<0.05$).

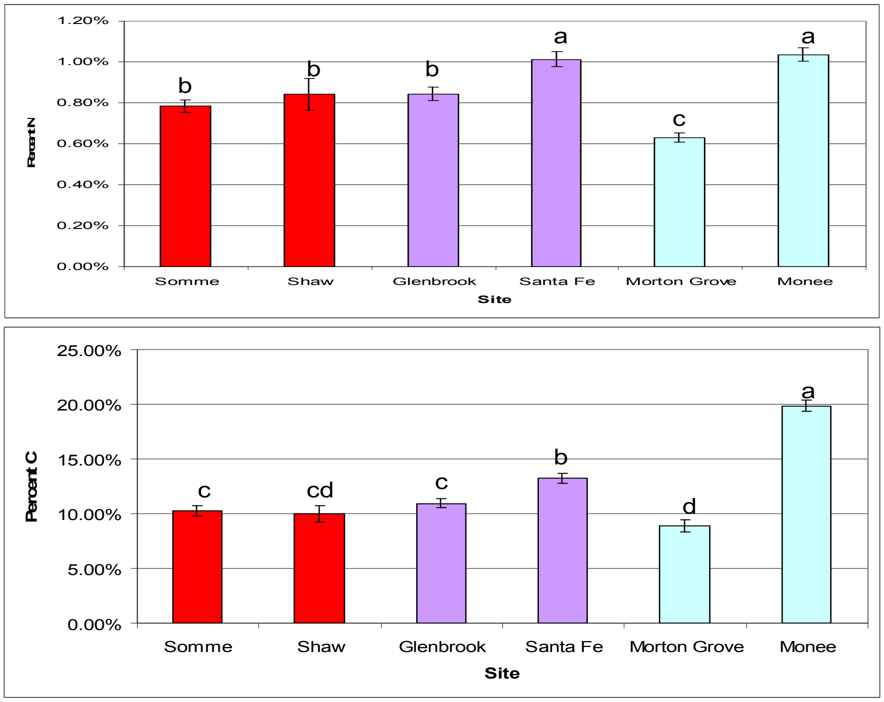


Figure 16. Total nitrogen (top) and carbon (bottom). Data reflect combined values from July and October. Letters indicate significant differences.

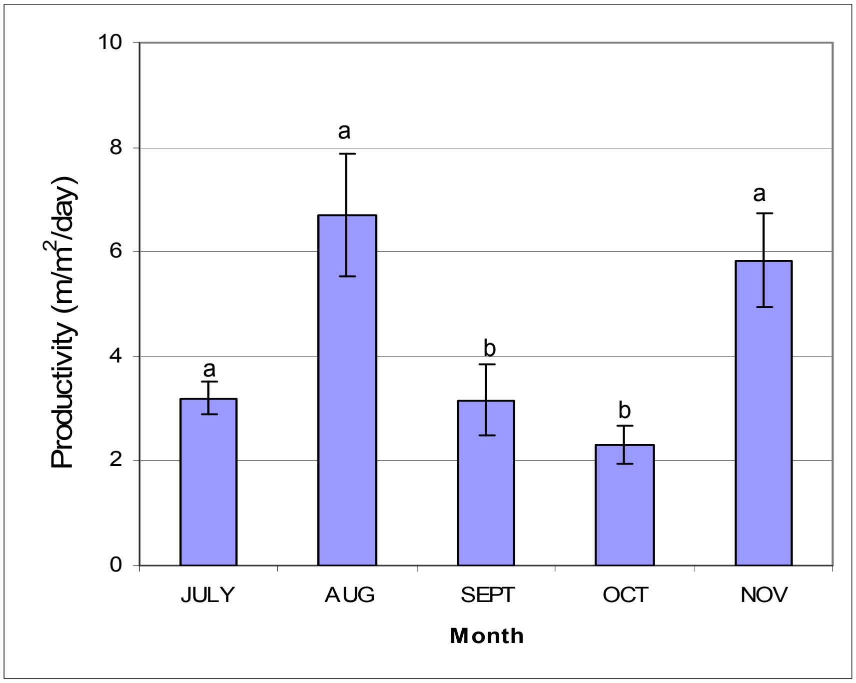


Figure 17. Mycorrhizal productivity (P_F) by month (top) and by site (bottom). Letters indicate significant differences.

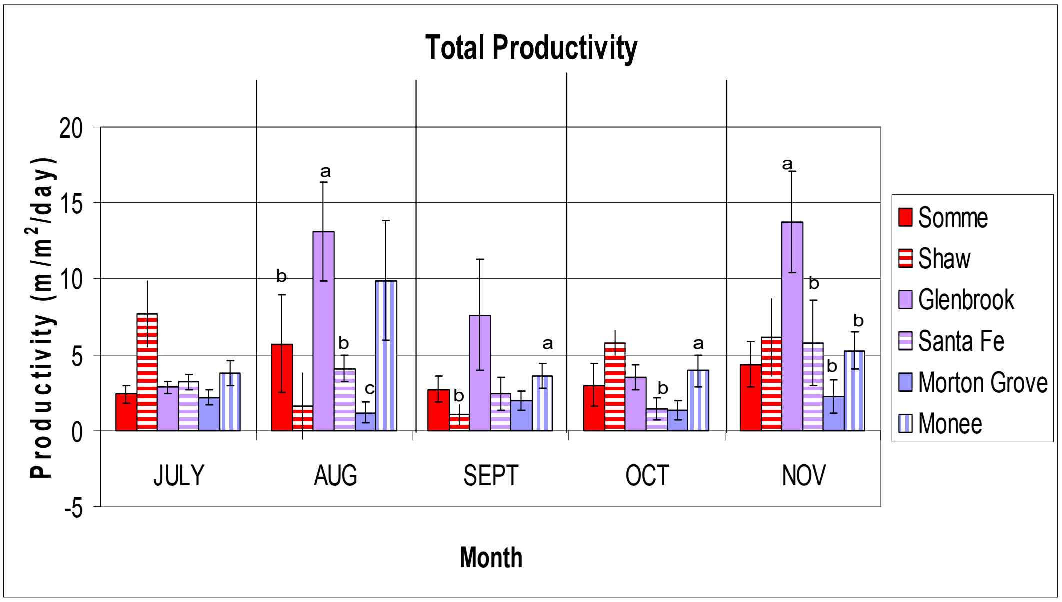


Figure 18. Mycorrhizal productivity (P_F) for each month. Letters refer to comparisons between sites for the same month. Between-month comparisons are not shown. Red=frequently burned sites; purple=infrequently burned sites; blue=unburned sites.

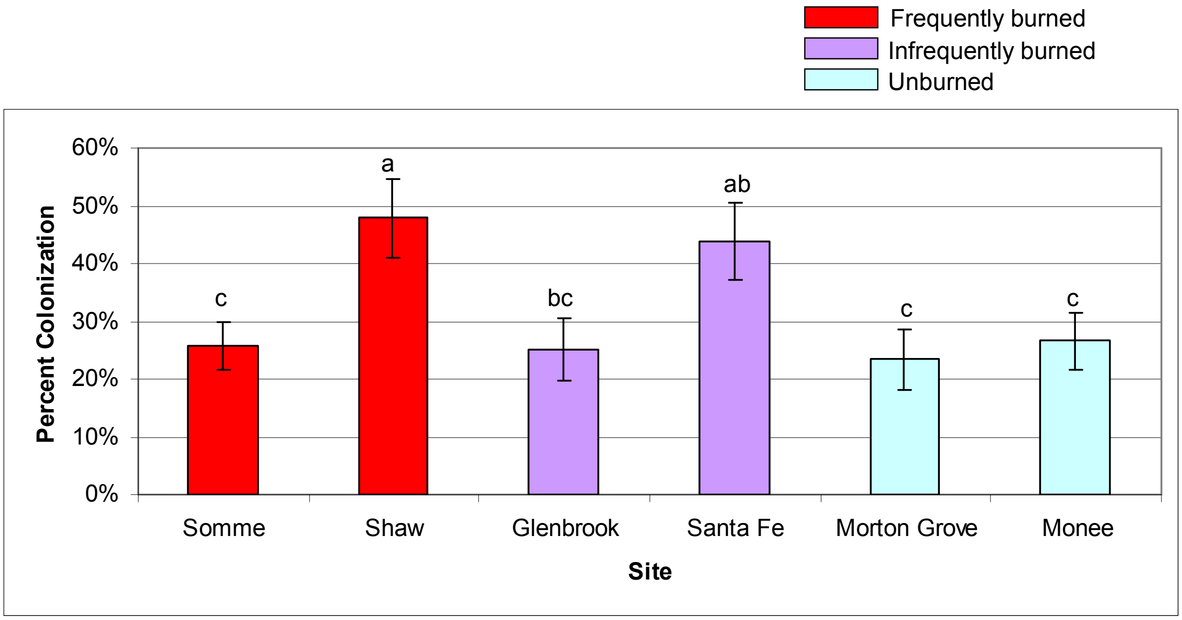
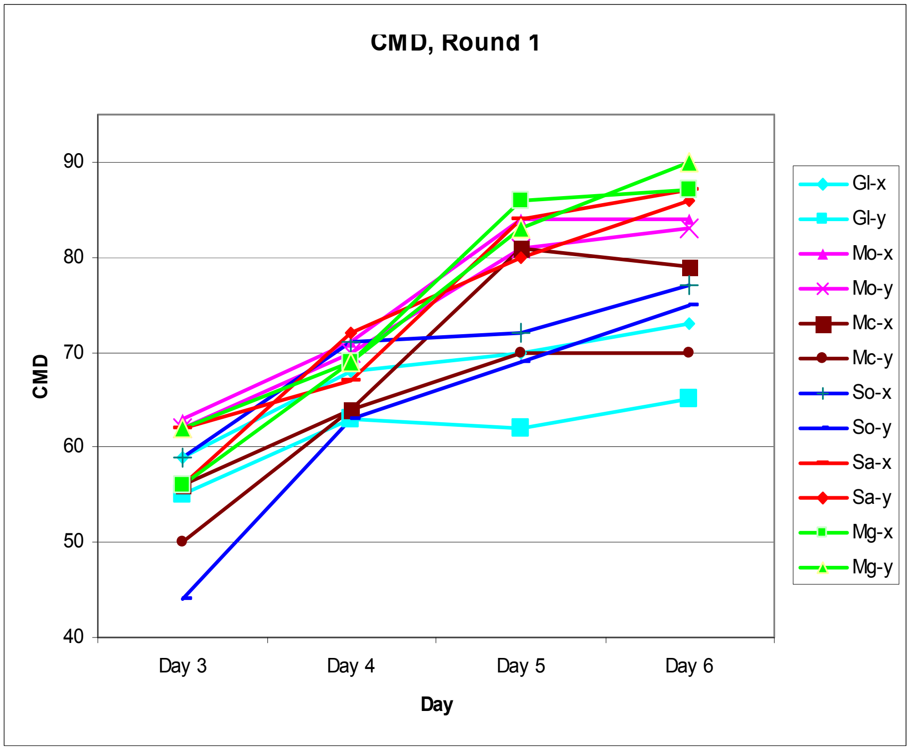


Figure 19. Mycorrhizal colonization (T) for each site (combined values from July and October). Letters indicate significant differences.



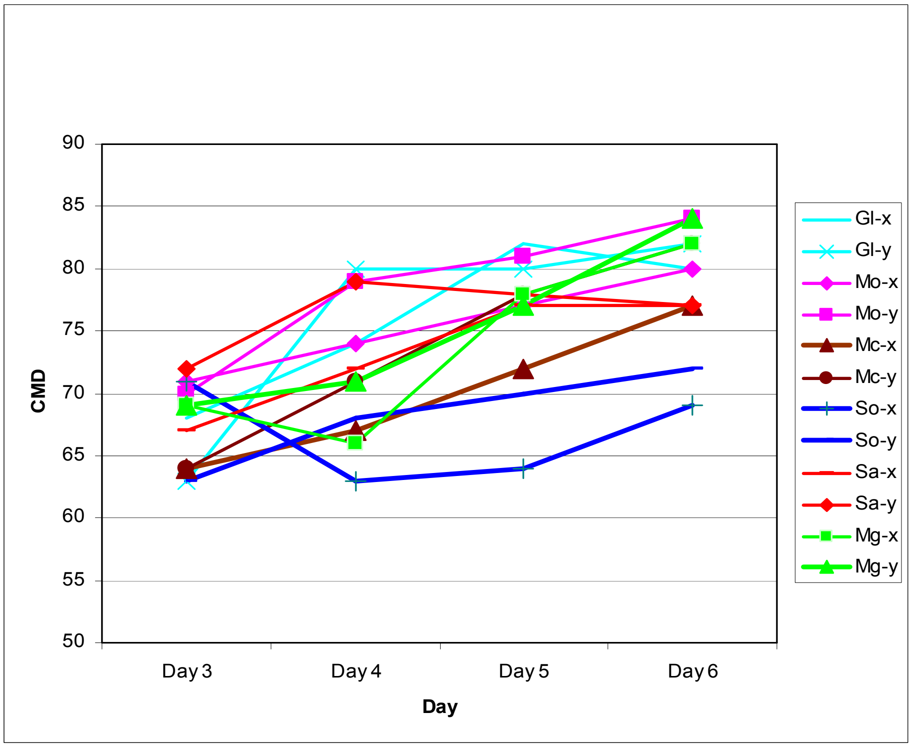


Figure 20. Community Metabolic Diversity for Biolog runs 1 (top) and 2 (bottom). GL=Glenbrook, MO=Monee, MC=McLaughlin, SO=Somme, SA=Santa Fe, MG=Morton Grove. x and y are duplicates of the same sample.

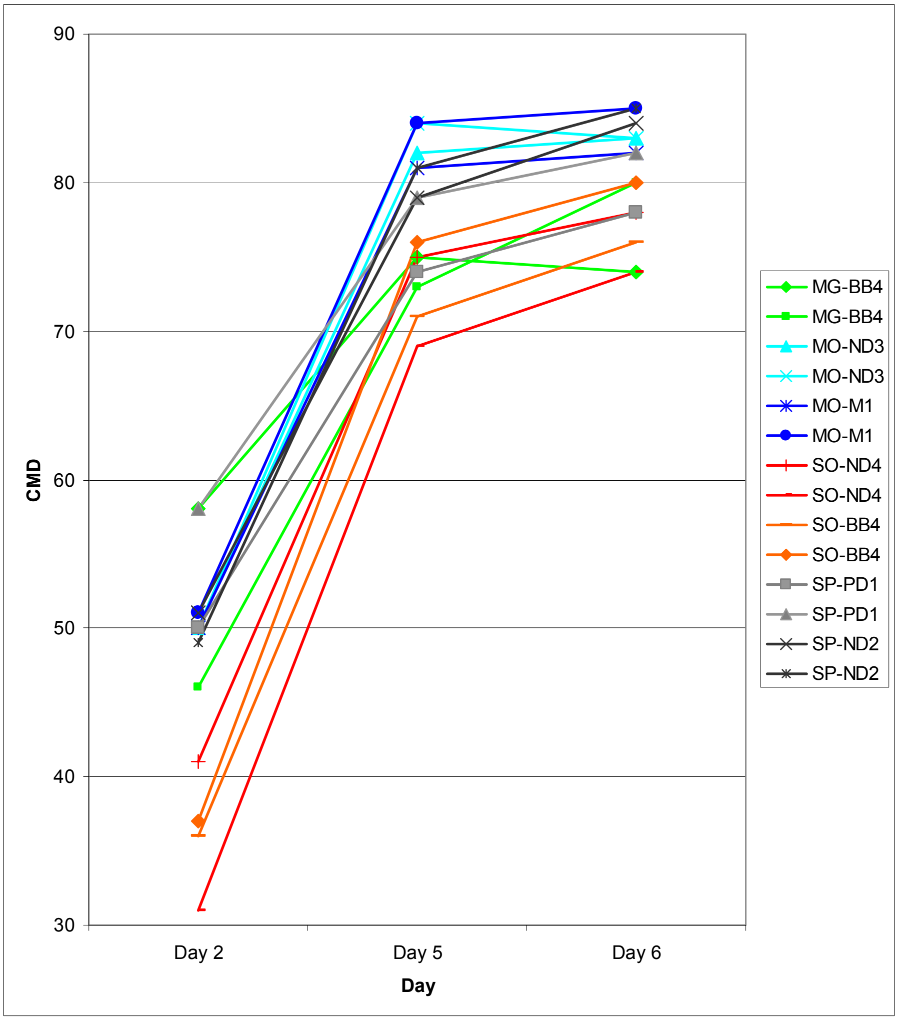


Figure 21. Community Metabolic Diversity for Biolog run 3. MG=Morton Grove, MO=Monee, SO=Somme, SP=Shaw. BB4, ND3, ND2, ND4, M1, and PD1 are sample names. The two lines for each sample correspond to the two duplicate plates of each sample.

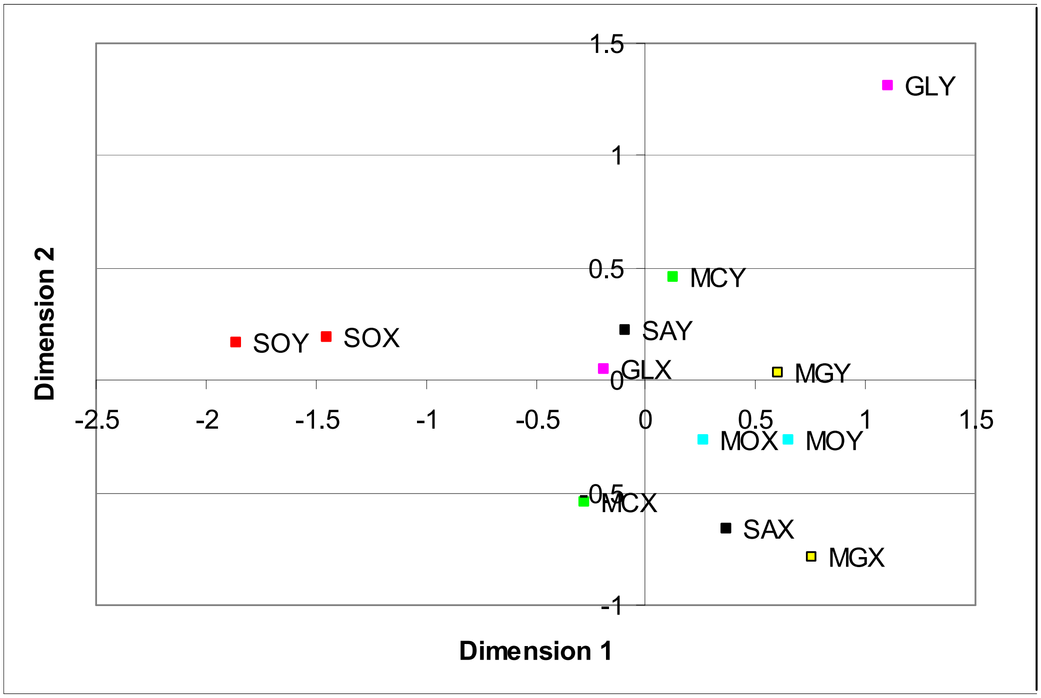


Figure 22. Multidimensional Scaling, Run 2. The distribution of six samples (x and y are duplicate plates of the same sample), Day 3. SO=Somme, GL=Glenbrook, MC=McLaughlin, SA=Santa Fe, MG=Morton Grove, MO=Monee.

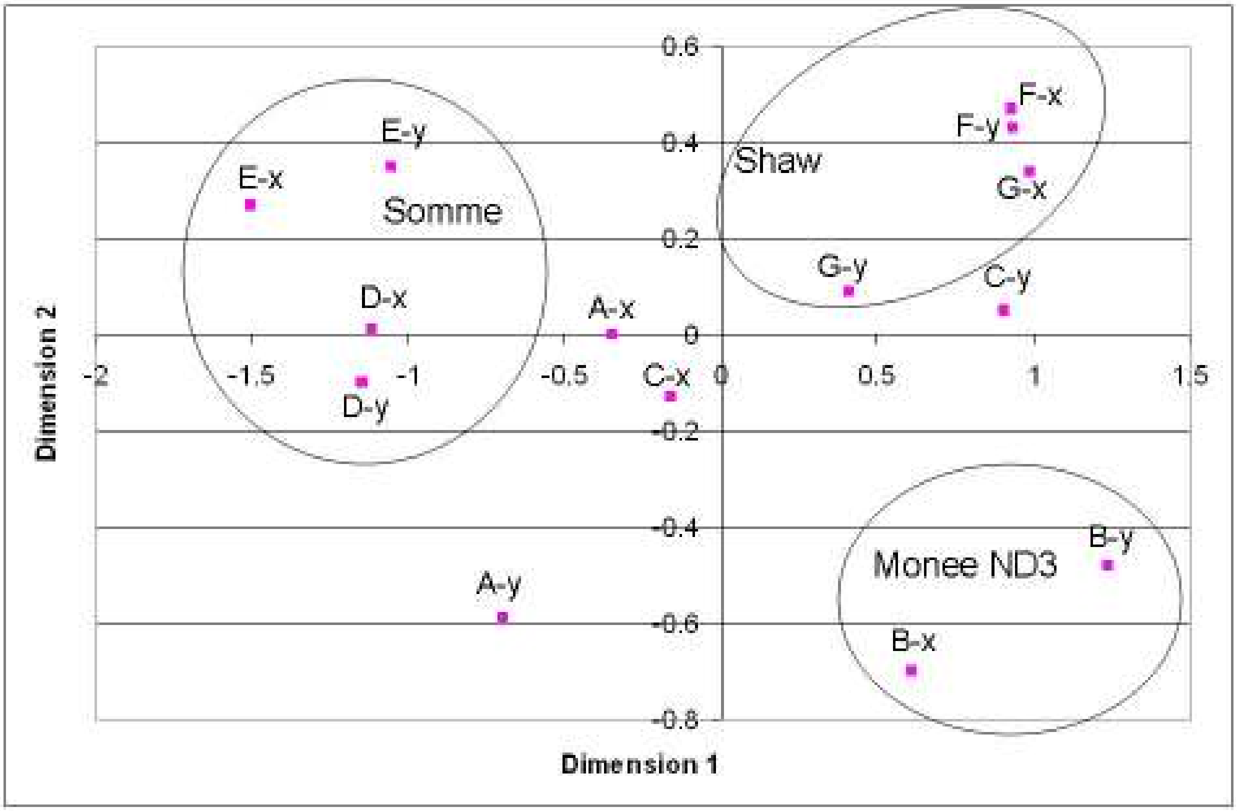


Figure 23. Multidimensional Scaling, Run 3. The distribution of seven samples (x and y are duplicates of the same sample), Day 2. A=Morton Grove BB4, B=Monee ND3, C=Monee M1, D=Somme ND4, E=Somme BB4, F=Shaw PD1, G=Shaw ND2.

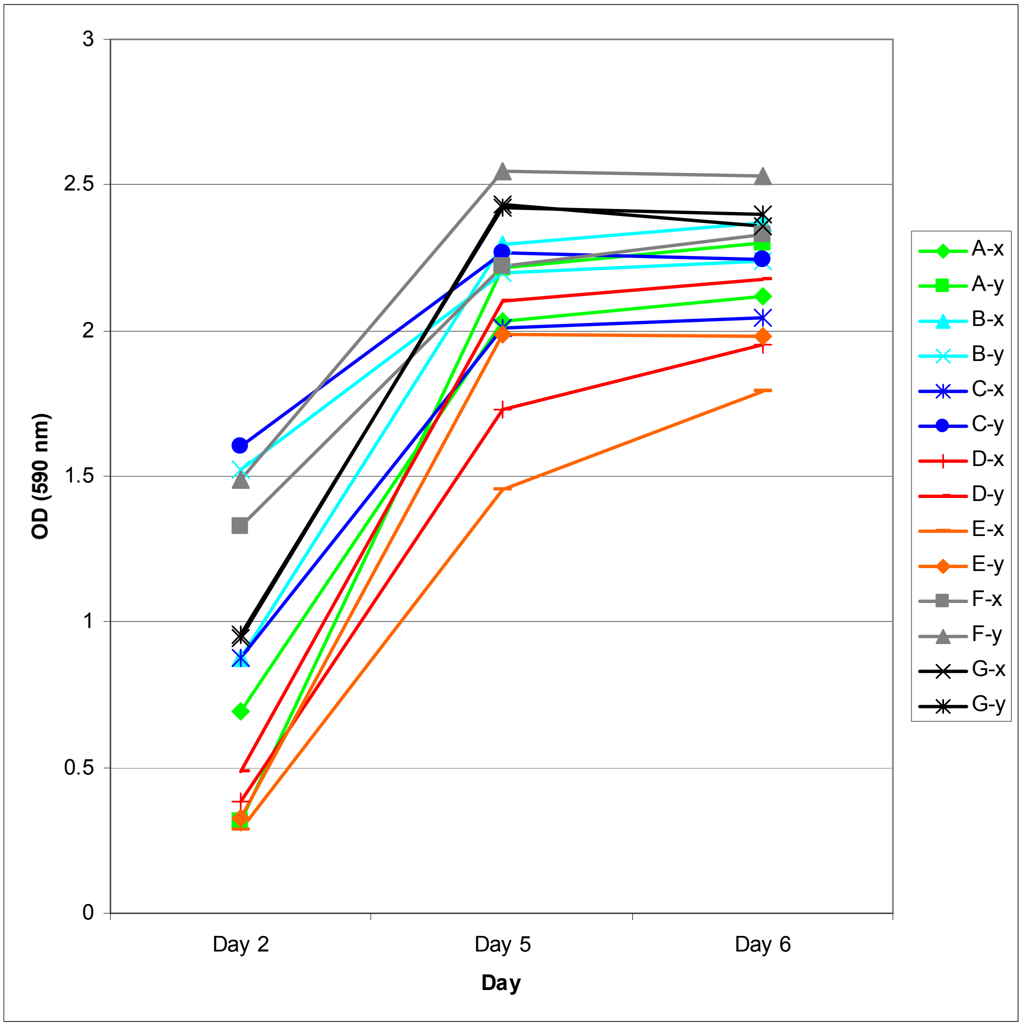


Figure 24. Activity levels in the sucrose well for 14 plates during Biolog run 3 (x and y are duplicate plates of the same sample). A=Morton Grove BB4, B=Monee ND3, C=Monee M1, D=Somme ND4, E=Somme BB4, F=Shaw PD1, G=Shaw ND2.

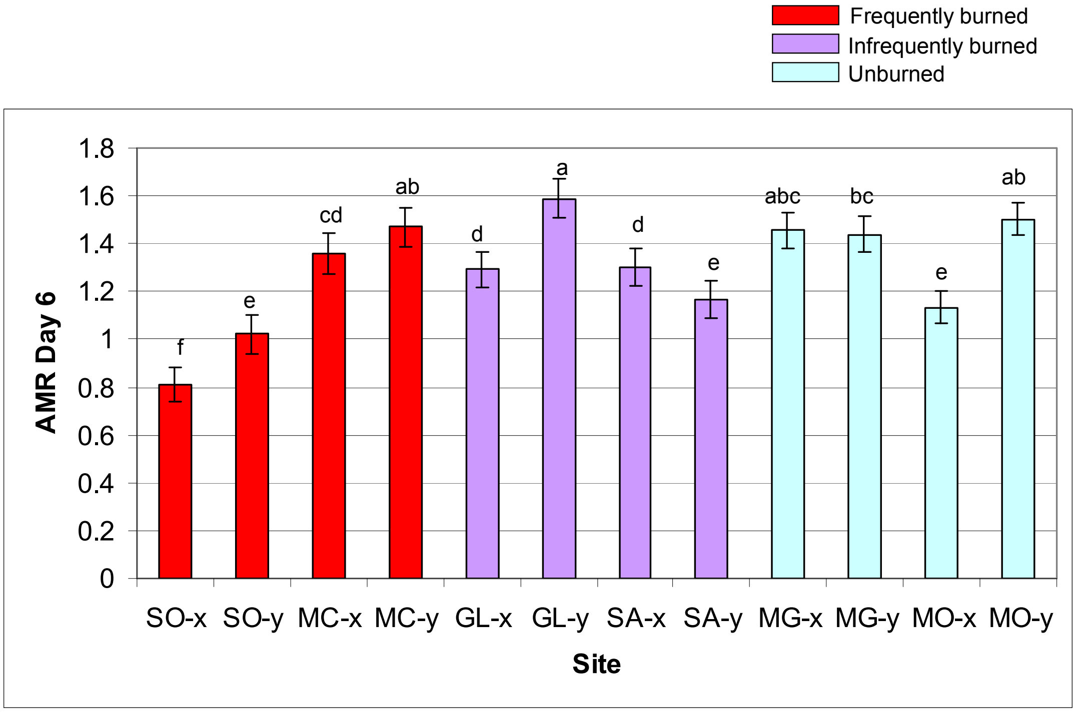


Figure 25. Average Metabolic Rate for the 12 plates used in Biolog Run 2, using readings from Day 6 after inoculation. SO=Somme, GL=Glenbrook, MC=McLaughlin, SA=Santa Fe, MG=Morton Grove, MO=Monee (x and y are duplicate plates of the same sample). Letters indicate significant differences.

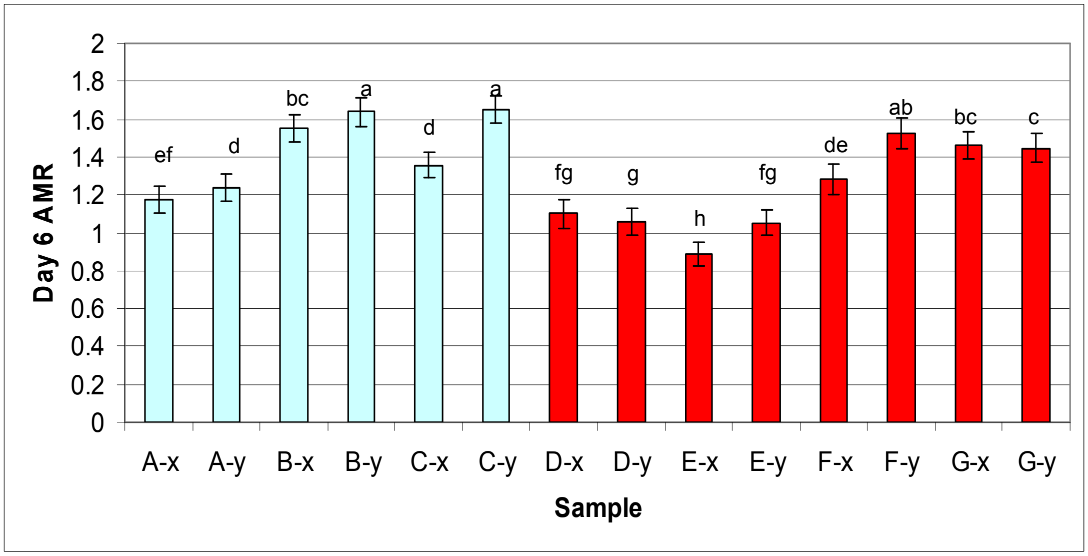


Figure 26. Average Metabolic Rate for the 14 plates used in Biolog Run 3, using readings from Day 6 after inoculation. A=Morton Grove BB4, B=Monee ND3, C=Monee M1, D=Somme ND4, E=Somme BB4, F=Shaw PD1, G=Shaw ND2 (x and y are duplicate plates of the same sample). Letters indicate significant differences.

Carbon Source	Highest R^2	Day of highest R^2	Most active samples	Least active samples
Putrescine	.981	Day 6	So-y, MG-x, Mc-x, Mc-y	Sa-y, Mo-x
N-Acetyl-D-Glucosamine	.957	Days 3, 4	Gl-y	So-x, So-y
L-Arabinose	.954	Days 3, 4	Mo-x, Gl-y	So-x, So-y
D-Trehalose	.953	Day 3	Mo-y	Mc-x, Sa-x, Gl-y, Gl-x
Gentiobiose	.949	Days 3, 4	Gl-y	So-x, So-y
L-Rhamnose	.946	Day 6	Gl-y	So-x, So-y
Sucrose	.931	Day 5	Gl-y	So-x, So-y
α-D-Glucose-1-Phosphate	.917	Day 6	Mo-y, Mg-y	So-x, So-y
p-Hydroxy-phenylacetic acid	.912	Day 5	Sa-x	So-x, So-y
D-Mannose	.901	Day 5	Gl-y, Sa-x	So-x

Table 2. Most important carbon sources in second Biolog analysis. “Most active” and “Least active” refer to the samples that had the greatest and the least activity on the “Day of highest R^2.”

Carbon Source	Highest R^2	Day of highest R^2	Most active samples	Least active samples
D-Cellobiose	0.962	Day 2	B2, G1	D1, D2, E1, E2
D-Mannose	0.953	Day 2	G1	A2, D1, D2, E1, E2
Hydroxy-L-Proline	0.943	Day 2	F1, G1	A2, D1, D2, E1
Inosine	0.94	Day 6	B1, F1, F2, G1	E1
L-Alanine	0.925	Day 5	B2, C2	E1
Sucrose	0.917	Day 2	B2, C2, F2	A2, D1, D2, E1, E2
N-Acetyl-D-Glucosamine	.916	Day 2	B2, G1	D1, E2
L-Pyroglutamic Acid	0.899	Day 2	F1, F2	A1, A2, D1, D2, E1, E2
D-Glucosaminic Acid	0.897	Day 6	C2	A2, D1, D2, E1, E2
Maltose	0.895	Day 5	F1, F2, G2	E1

Table 3. Most important carbon sources in third Biolog analysis. A=Morton Grove BB4, B=Monee ND3, C=Monee M1, D=Somme ND4, E=Somme BB4, F=Shaw PD1, G=Shaw ND2. “Most active” and “Least active” refer to the samples that had the greatest and the least activity on the “Day of highest R^2.”

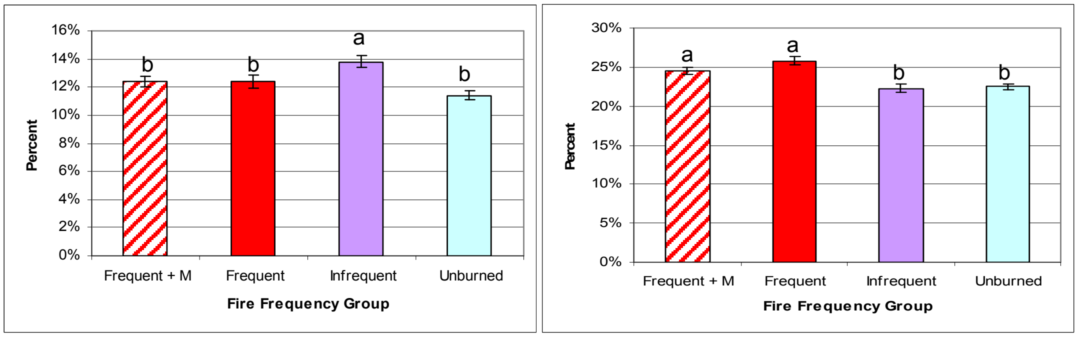


Figure 27. Average soil moisture as determined by gravimetric analysis for July (left) and October (right). Letters indicate significant differences ($p<0.05$).

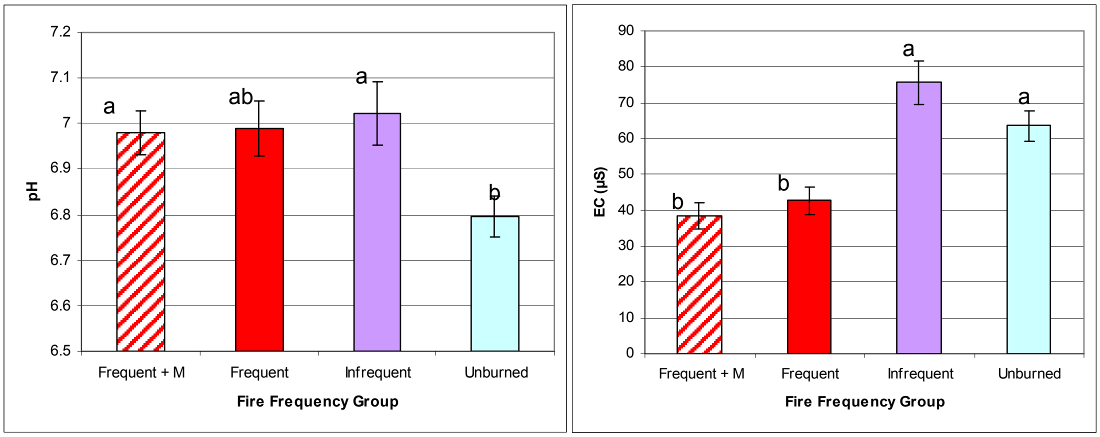


Figure 28. Average pH (left) and Electrical Conductivity (right) of soil collected in July. Letters indicate significant differences ($p<0.05$).

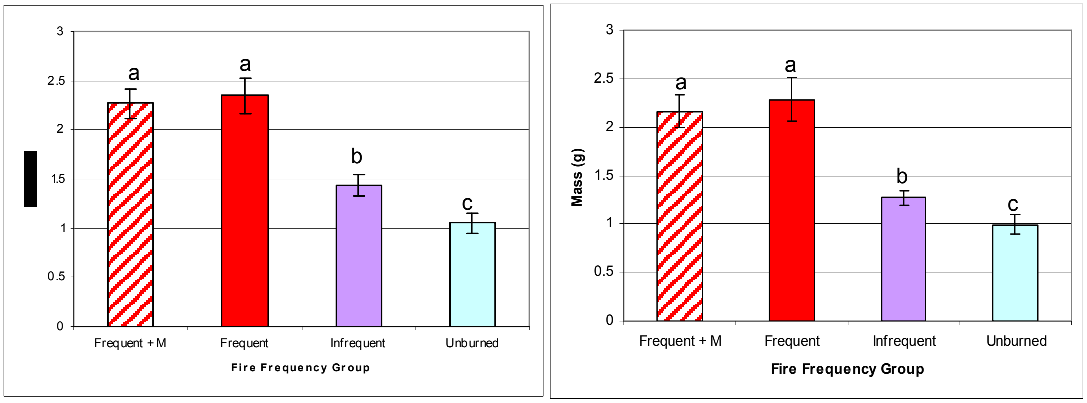


Figure 29. Average dry root mass in July (left) and October (right). Letters indicate significant differences ($p<0.05$).

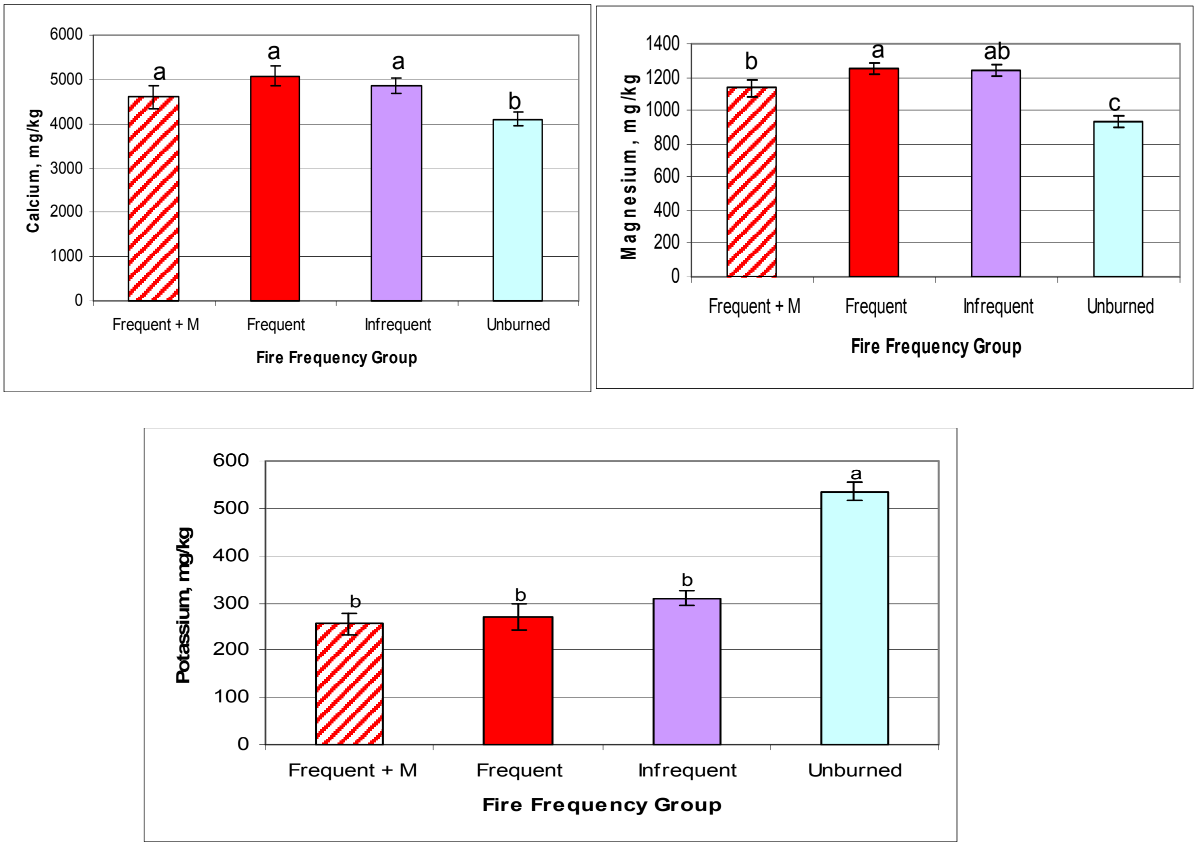


Figure 30. Average calcium (top left), magnesium (top right), and potassium (bottom) levels. Letters indicate significant differences between sites ($p<0.05$).

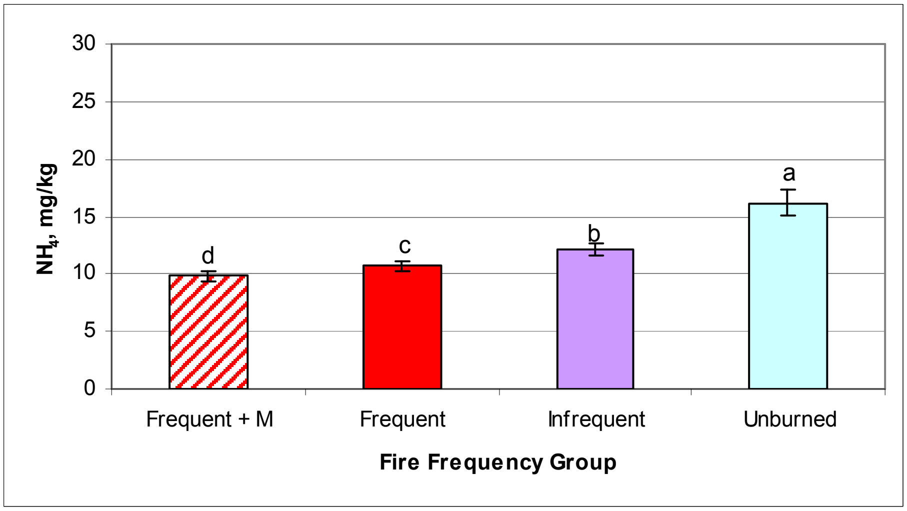


Figure 31. Average NH_4 levels. Data reflect combined values from July and October. Letters indicate significant differences ($p<0.05$).

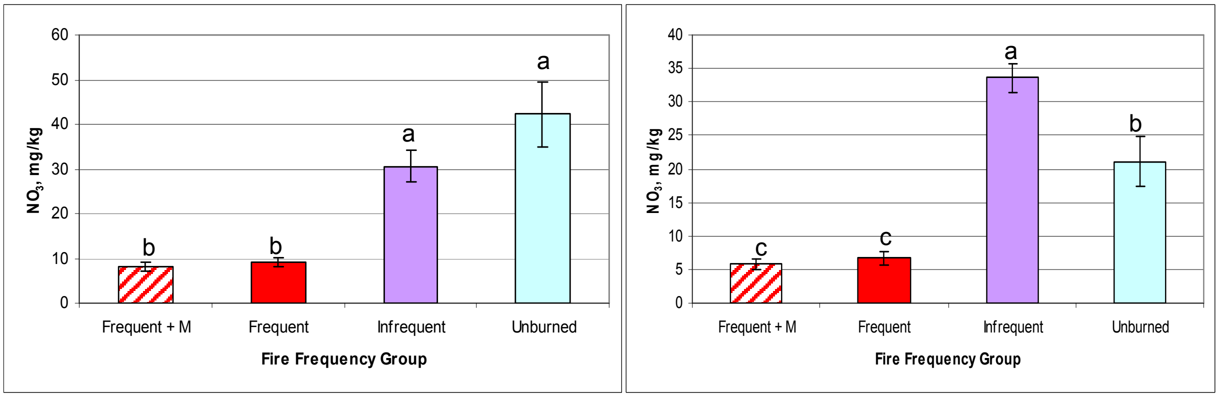


Figure 32. Average NO_3 levels in July (left) and October (right). Letters indicate significant differences ($p<0.05$).

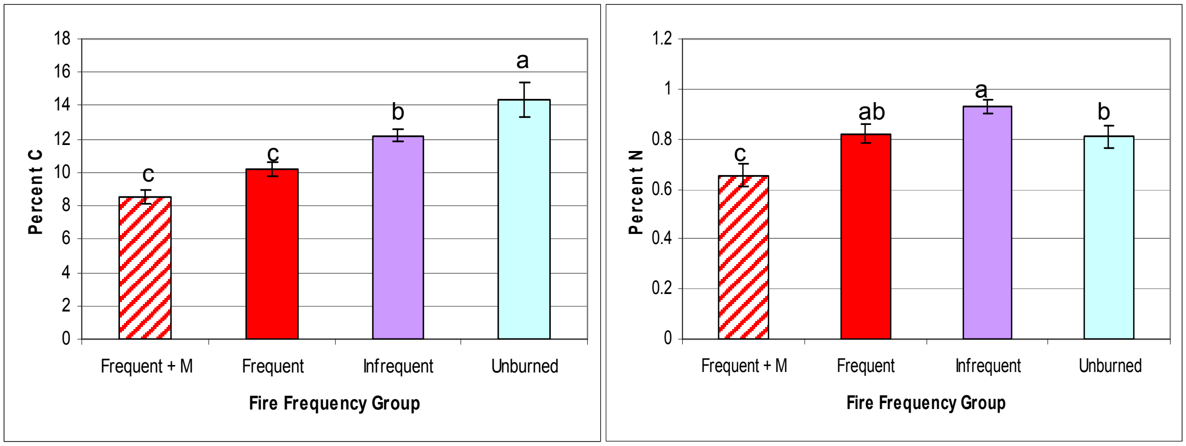


Figure 33. Average total carbon (left) and total nitrogen (right). Data reflect combined values from July and October. Letters indicate significant differences ($p<0.05$).

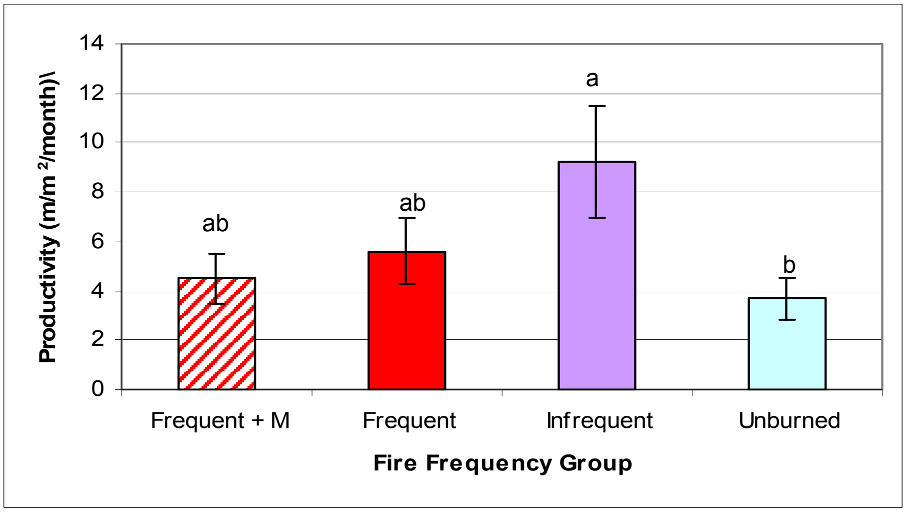


Figure 34. . Mycorrhizal productivity (P_F) for November. Letters indicate significant differences ($p<0.05$).

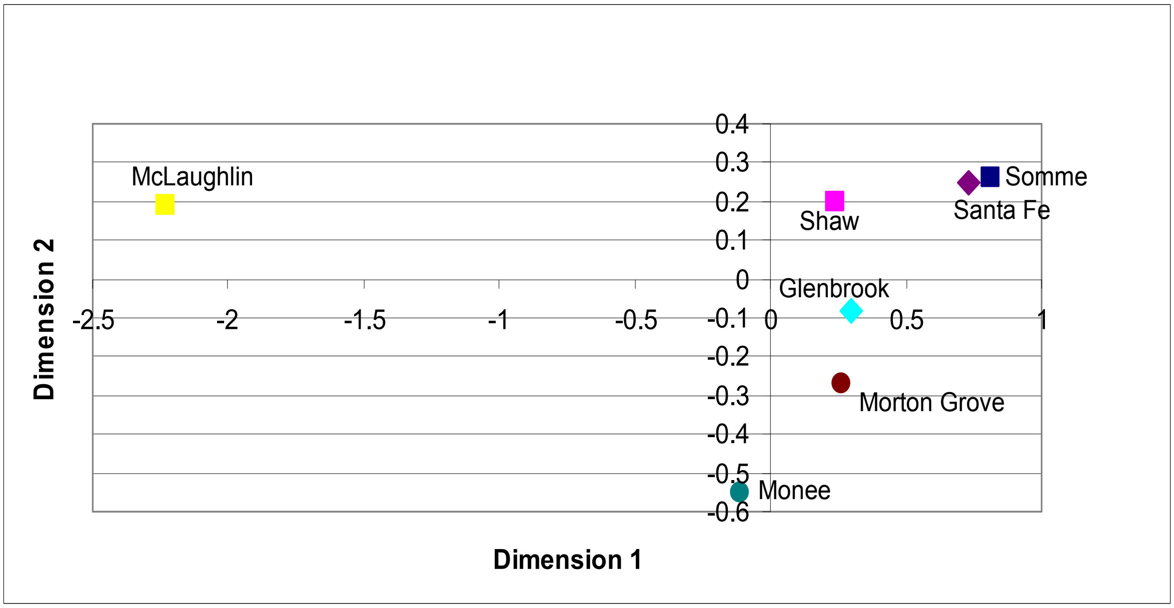


Figure 35. Nutrient MDS. Overall site arrangement as placed by MDS analysis combining all nutrient variables.

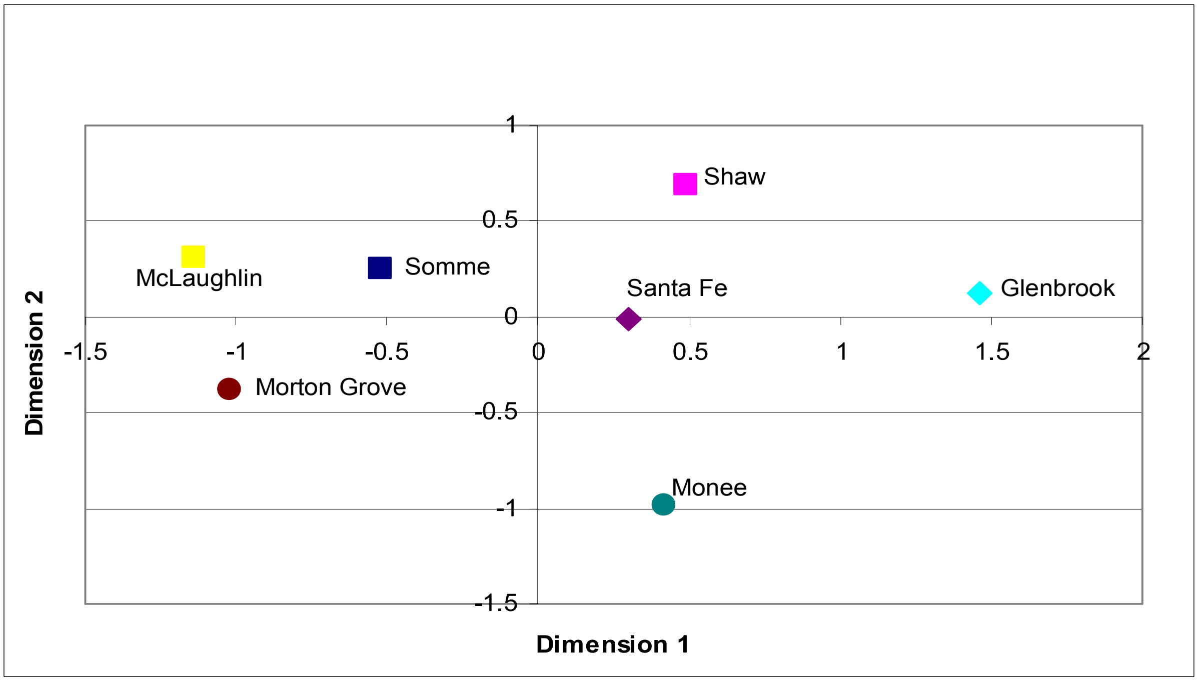


Figure 36. Mycorrhizal MDS. Overall site arrangement as placed by combining the mycorrhizal variables T (colonization) and P_F (productivity)

Parameter	McLaughlin average value	Standard error	Shaw value	P-value
Moisture, July	12%	*0.8%*	15%	**0.025†**
Moisture, October	21%	*0.6%*	27%	**0.00***
pH	6.96	*0.08*	7.18	0.499†
Electrical Conductivity	20.27 µS	*3.98 µS*	46.91 µS	0.128†
Dry root mass, July	2.079 g	*0.251 g*	2.451 g	0.177†
Dry root mass, October	1.833 g	*0.218 g*	3.122 g	**0.011***
Calcium	2227 mg/kg	*203 mg/kg*	4747 mg/kg	**0.00***
Magnesium	544 mg/kg	*66.9 mg/kg*	1198 mg/kg	**0.00***
Potassium	184 mg/kg	*14.0 mg/kg*	118 mg/kg	0.282*
NH_4	7.23 mg/kg	*0.53 mg/kg*	10.28 mg/kg	0.245†
NO_3, July	4.1 mg/kg	*1.546 mg/kg*	11.62 mg/kg	**0.028†**
NO_3, October	3.78 mg/kg	*0.986 mg/kg*	10.3 mg/kg	**0.017†**
Available Phosphorus	9 mg/kg	*0.458 mg/kg*	10.12 mg/kg	0.887*
Total Nitrogen	0.30%	*0.03%*	0.84%	**0.00***
Total Carbon	5.12%	*0.32%*	9.97%	**0.00***
Mycorrhizal Productivity (P_F), July	2.901 m/ m^2/day	*0.763m/ m^2/day*	6.123 m/ m^2/day	0.116†
P_F, August	8.221 m/m^2/day	*7.478 m/m^2/day*	5.799 m/m^2/day	0.753†
P_F, September	0.7 m/m^2/day	*0.248 m/m^2/day*	1.034 m/m^2/day	0.893†
P_F, October	0.844 m/m^2/day	*0.352 m/m^2/day*	1.644 m/m^2/day	0.484†
P_F, November	1.959 m/m^2/day	*0.745 m/m^2/day*	7.679 m/m^2/day	0.128†
Root colonization (T)	16%	*4.6%*	47%	**0.036†**

Table 4. Soil parameters at McLaughlin compared to Shaw. “Standard error” (middle column) refers to McLaughlin average values. “P-value” (far right column) refers to statistical similarity between Shaw and McLaughlin, according to either the Fisher’s LSD test or the Wilcoxon signed-rank test. Significant p values ($p<0.05$) are in bold.

*Fisher’s LSD

†Wilcoxon

DISCUSSION

The overall MDS tests illustrated that when many soil variables were taken into account at once, a given site was not generally most similar to the other site(s) sharing the same fire frequency. However, there were substantial differences between the seven sites for all measured soil characteristics. These soil characteristics are a combined result and reflection of many aspects of the sites, including management history, disturbance history, elevation, topography, preserve size, and neighboring land characteristics.

Management history has a considerable influence on soil characteristics, and in tallgrass prairie, fire is a sizeable component of the management, but fire management is by no means the only important influence on ecosystem characteristics. Other important influences were kept as constant as was feasible in this study. McLaughlin, in particular, is a special case as discussed below. Thus, the differences observed between the sites ultimately reflect a combination of fire history and other factors.

Treatment differences and site differences

McLaughlin

McLaughlin Meadow is unique in that it is not a remnant prairie. It has an Illinois Natural Areas Inventory grade of B, designating a previously disturbed, late-successional habitat. Any differences between McLaughlin and the other sites may be due in part to this quality difference, in addition to a fire history difference. The main reason that McLaughlin was included in the study was to follow up on a soil study conducted at Skokie River Nature Preserve the preceding year. Because they are located in the same preserve and receive exactly the same management, a direct comparison of Shaw and McLaughlin can reveal differences between restored and remnant prairie.

McLaughlin had significantly higher average root mass than most of the sites, while it had the lowest Ca levels, the lowest Mg levels, the lowest NO_3 and NH_4 levels, the lowest total N and the lowest total C levels. In addition, available P levels were significantly lower at McLaughlin than at most other sites, and mycorrhizal activity (in terms of both productivity and colonization) was lower than at most other sites. In terms of bacterial activity, McLaughlin had a high Average Metabolic Rate (AMR). Low levels of so many nutrients at McLaughlin may be a result of long-term depletion during the period of disturbance.

Somme

Somme Prairie Nature Preserve had an average root mass that was significantly higher, and Ca levels that were significantly higher, than most other sites. Its NO_3 and NH_4 levels were among the lowest, and its mycorrhizal colonization (T) levels were lower than most other sites. In the bacterial analysis, Somme had low Community Metabolic Diversity (CMD) for two of the three runs, the lowest Average Metabolic Rate (AMR), and the lowest level of activity for almost all of the carbon sources that were identified as contributing most significantly to the results.

Shaw

Shaw Prairie had the highest average root mass and it had Ca levels that were significantly higher than most sites. Shaw had significantly lower available P levels than most sites, and the lowest K levels. Shaw also had the lowest NH_4 levels of all the sites except McLaughlin. As far as mycorrhizal activity, Shaw had mostly high activity with the highest colonization (T) levels and among the highest productivity (P_F) levels. In the bacterial analysis, Shaw had high CMD, high AMR in Round 3, and a high level of activity for some of the most significant carbon sources.

The frequently burned sites

The most striking commonalities among the frequently burned sites were high root mass and low available N. In addition, McLaughlin and Somme had relatively low levels of mycorrhizal activity, while Shaw had relatively high levels. Both Shaw and McLaughlin had among the lowest P levels, while Somme had among the highest P levels.

The values for most parameters at Shaw and McLaughlin were comparable to those found by the previous study (Umek 2007). These include higher levels of available N, total N, and total C at Shaw than at McLaughlin, and statistically equivalent P and K values. One difference is that while Umek (2007) found no difference between Shaw and McLaughlin in either mycorrhizal colonization (T) or productivity (P_F), I found both mycorrhizal parameters to be significantly greater at Shaw. This difference likely reflects inter-annual variability in mycorrhizal growth in response to plant, soil, and climatic factors.

Glenbrook

Glenbrook High School Prairie had significantly higher available P levels, and in October, NO_3 levels, than most other sites. For mycorrhizal activity, it had significantly higher productivity (P_F) than most sites. For bacterial activity, Glenbrook had a low CMD in round 1, but a high CMD and AMR in round 2.

Santa Fe

Santa Fe Prairie had higher Ca, Mg, and total N levels, and in October, higher NO_3 levels, than most other sites. It also had lower available P levels than most other sites. In terms of microbial activity, Santa Fe had higher mycorrhizal colonization (T) and higher CMD than most other sites.

The infrequently burned sites

The clearest similarities between Glenbrook and Santa Fe were their high levels of NO_3 (in October) and their high levels of bacterial activity, as quantified by the CMD values. For several other variables, the two sites did differ significantly from each other, but were relatively similar in comparison to the other five sites. These include intermediate K levels, high Ca levels, intermediate NH_4 levels, high total N levels, and intermediate total C levels.

Glenbrook and Santa Fe were classed as infrequently burned because they both had three burns during the 20 years preceding the time of sample collection in 2007. But it is important to note that the two sites are also similar in that both recently started receiving more regular burning. At Santa Fe, all three burns occurred after 1999, and at Glenbrook, two of the three burns occurred after 2000. Since at the time of the study, both sites had most recently burned in 2003, it was still safe to classify them as less frequently burned than Somme and Skokie River, but the recent management shifts are worth noting.

The relatively high amounts of soil nitrogen and other similarities may not only reflect the effect of an infrequent fire interval, but also the effect of fire returning to an area that has been unburned for an extended period. Prescribed fire after a period of no burning can cause an especially large pulse in nitrogen levels as a result of the release from detritus and soil organic matter (Fenn et al. 1998).

Morton Grove

Morton Grove Prairie had significantly higher K levels than all other sites, while it had significantly lower total C and total N levels than all sites except for McLaughlin. In terms of mycorrhizal activity, Morton Grove had significantly lower colonization (T) and productivity

(P_F) than most other sites for most months. Bacterial activity results were mixed. In the first two Biolog runs, Morton Grove had a higher CMD than most other sites, but in Round 3, the site had a low CMD value and low activity for many of the significant carbon sources.

Monee

The site in Monee was unique in that it was the only un-managed site. It had the lowest average root mass, among the lowest Ca and Mg levels, and significantly higher K values than most sites. The Monee site also had significantly higher NO_3, NH_4, and total N levels than most other sites, and the highest total C levels of all the sites. In terms of microbial activity, the site had significantly higher fungal productivity (P_F), higher CMD (Rounds 2 and 3), and higher AMR (Rounds 2 and 3) than most other sites. It also had high activity for many of the most significant carbon sources.

The unburned sites

The clearest similarities between Monee and Morton Grove were high potassium levels and high CMD values in Round 2. Although there were significant differences between them for all nutrients, Monee and Morton Grove were also similar, relative to the other sites, in that they both had intermediate calcium levels, low magnesium levels, and intermediate available P levels. However, the fact that the nitrogen and carbon levels of the two sites differ so greatly suggests that either there was an important difference in their fire histories, or that there are other more influential factors at one or both of these sites.

Monee's fire history cannot be confirmed. Vegetational clues, such as large mounds under *Sporobolus heterolepis*, and the fact that it is not managed, led me to assume that the site has not been burned in a long while. However, it is possible that its transect was last burned more recently than the Morton Grove transect was, leading to differences between them. However, this explanation seems unlikely given that although Monee's overall suite of soil characteristics is quite distinct from that of Morton Grove, it is also quite distinct from the rest of the sites, which have had confirmed burns in the last 20 years. Another possibility is that the Monee site's last burn was much longer than 20 years ago, and some of the differences between the two sites are due to residual effects of burn management that Morton Grove received from 1979-1987.

A third possibility is that many of the differences are due to factors other than fire. For example, although the sites are similar in size, they have different surroundings. The Morton Grove prairie is located in a somewhat urban area and is fenced off from athletic fields that completely surround it and are likely subject to intensive management. On the other hand, the Monee site is in a less densely developed area and is bordered by some brush and some lower-quality prairie. It is very close to a major road (< 20 m) but is separated from the road by two sets of raised railroad tracks. A final major difference is that Morton Grove receives human management activities such as herbicide treatment (for invasive plants), while the Monee site receives no management. One of these differences may make Morton Grove more prone to carbon and nitrogen losses, or Monee more prone to nitrogen and carbon storage.

Soil characteristics

There are two main reasons that the set of soil characteristics measured in this study was chosen. The first was to characterize the degree of similarity between the sites based on the combination of multiple abiotic and biotic soil parameters. The second was to make a judgment about the total benefit of each fire frequency – frequent, infrequent, and absent – by comparing the results with an independent "optimal value" range for each of the measured parameters.

For many of the measured characteristics, though, especially abiotic nutrients, "optimal" values are unknown or are so subjective that they are meaningless. Therefore, for all nutrients except available N and electrical conductivity (for reasons explained below), the sites with the highest levels are highlighted. This is because while it is possible for an ecosystem to have an excess of any of these nutrients, they are all important plant nutrients and more likely to be limiting in tallgrass prairie.

Physical soil characteristics

Despite significant differences between sites, all measured pH values were well within the range that would be expected based on levels that have been found at Konza Prairie in Kansas. Konza is a reasonable baseline due to its function as the tallgrass prairie Long-Term Ecological Research (LTER) site (Ransom et al. 1998). Electrical Conductivity (EC) of a soil sample represents the salinity level of the site. Despite significant differences in EC between sites in this study, all measured EC values were very low in comparison with published Konza levels (Ransom et al. 1998). Thus, it appears that salinity is a very minor issue at all of the sites. But the presence of fire may cause decreased conductivity, as the lowest EC levels were found at McLaughlin, Glenbrook, and Somme, and the "frequent" group had a lower average EC than the "infrequent" and the "unburned" groups.

Moisture has been found to be affected by fire, but the effect is usually not prolonged beyond one growing season (Ojima et al. 1994). This variable was measured in the study to determine whether the sites had comparable soil moisture characteristics. Moisture levels were found to be significantly different between sites in both July and October. Consistently, Shaw had the highest moisture and Monee had the lowest moisture in both months. Monee's dryness can be explained in large part by its elevation: at 242 m, it is higher than the others, which range from 195-207 m in elevation.

However, because most of the sites fluctuate in their relationships to each other between July and October (e.g., Somme soil collected in July had the lowest moisture, and Somme soil collected in October had the highest moisture), the two sets of measurements probably do not adequately capture the range of moistures possible at these sites. The reflectometer data (not statistically analyzed because only single measurements were taken each month) further underscore the degree of variation. Moisture is a soil quality that can vary over the course of a day, and it was not possible to collect all the soil samples at the same time of day. Thus, the moisture data indicate differences in moisture at the time of soil collection, giving a suggestion of the extent of the range for these sites, but does not prove that there would be any difference in average moisture between the sites if measured continuously over the course of a week or a month.

Roots

Root mass was examined primarily as a proxy for belowground productivity. Ecosystem productivity, whether it is measured aboveground or belowground, is a common measure used to quantify ecosystem function. High productivity is equated with a well-functioning ecosystem (Baer et al. 2002). Greatest average root mass was consistently found at Somme, Shaw, and McLaughlin, and in the “frequent” group, in both July and October. Overall, there were significant differences between average root masses in July and average root masses in October, but it is not surprising that they would be lower in October, close to the end of the growing season, when plant roots have already begun to senesce.

Soil nutrients

Cation levels reflected the concentration of potassium, calcium, and magnesium ions bound to soil exchange sites. These exchangeable cations levels reflect the cation exchange capacity of the soil, a property which is affected by the mineral and organic matter content in the soil (Coleman et al. 2004). The highest potassium levels were found at Morton Grove and Monee. The highest calcium levels were found at Somme, Shaw, and Santa Fe. The highest magnesium values were found at Santa Fe and Somme. In addition, the “unburned” group exhibited the highest potassium levels, and the lowest calcium and magnesium levels. Thus, although strong patterns related to fire treatment were not expected for the cations, potassium levels were highest at the unburned sites, and calcium and magnesium values were highest at sites that have had fire recently, excluding McLaughlin and Glenbrook. This could be because magnesium and calcium are more easily re-deposited in the ash following a fire, while potassium is more easily leached out of the ash.

As far as available N levels, the sites with the lowest NH_4 values were Shaw and McLaughlin. The groups with the lowest NH_4 values were the “frequent” and the “frequent + M” groups. The sites with the lowest NO_3 levels were Morton Grove and McLaughlin in July, and Somme and McLaughlin in October. In both months, the “frequent” and “frequent + M” groups had the lowest NO_3 levels. NO_3 varied from July to October. A main feature of this difference was that Glenbrook and Santa Fe went from having among the lowest NO_3 values in July to having among the highest NO_3 values in October. This indicates that the organisms involved in nitrogen cycling at Glenbrook and Santa Fe may vary seasonally in their activity more than those at the other sites.

Available P is often a limiting nutrient for plant growth (Bever et al. 2001, Paul 2007). The effect of fire frequency on available P was expected to be inconclusive, and it was. Increased soil levels of available P in an ecosystem can be caused by increased P mineralization or decreased P immobilization by microbes, or decreased P uptake by plants. In response to fire, increased available P can also be explained by re-deposition of P released from volatilized vegetation. The sites that had the highest levels of available P were Somme and Glenbrook. This may be due to some combination of these reasons, with the effect mediated by fire more at Somme than at Glenbrook. However, if fire was a primary influence on this nutrient, fire had an opposite effect on Shaw, McLaughlin, and Santa Fe, which had the lowest levels of available P.

Total soil carbon pools may also have been affected by fire in opposing ways. Because fire generally leads to increased net primary productivity and increased C:N ratios in plant tissue, total soil C may be expected to increase with increasing fire frequency (Ansley et al. 2006). At the same time, organic matter decomposition rates can also increase with fire, which would cause the net effect to be a lack of increase, or even a decrease, in total soil C (Ansley et al. 2006). Total C was highest at Monee, followed by Santa Fe, and was highest in the "unburned" group, suggesting the latter effect. Total soil nitrogen pools are affected by fire through fire's effect on C:N ratios in plant tissue that eventually decompose in soil, microbial mineralization rates, and the loss of volatilized nitrogen in plant tissue at the time of fire (Blair et al. 1998). Thus, similarly to available N, total N was expected to decrease with increasing fire frequency. This expectation was partly met because total N was highest at Monee and Santa Fe, and in the "infrequent" group. However, excluding the "frequent + M" group, total N was actually lowest in the "unburned" group.

Mycorrhizal activity and diversity

Mycorrhizal colonization (T) and productivity (P_F) quantify the amount of active mycorrhizal fungi in the ecosystem. Fungal productivity (P_F) had some seasonal variability, with

P_F values in September and October being significantly different from P_F values in July, August, and November. This is probably a reflection of seasonal plant activity. Mycorrhizal activity began to wind down toward the end of the growing season, in September and October, but went back up again in November when many plants were senescing. The spurt of activity may be a response of the mycorrhizal fungi to the end of the growing season.

Mycorrhizal activity was expected to increase with increasing fire frequency due to increased plant productivity and increased abundance of host plants, as has been observed in other studies (Torpy et al. 1999). Yet, some studies have observed decreased mycorrhizal activity after fire and explained this as a result of soil heating or soil chemical changes (Torpy et al. 1999). Mycorrhizal colonization (T) was highest at Shaw and Santa Fe while productivity (P_F) was highest at Glenbrook. Thus, mycorrhizal activity showed no clear response to fire frequency, suggesting that perhaps frequent fire creates opposing conditions that both stimulate and slow mycorrhizal fungal activity.

Mycorrhizal fungal diversity was not extensively explored by this study. However, this analysis did confirm the presence of known AM fungal genera, and made available PCR and RFLP products that can be identified by a future analysis. Any differences in mycorrhizal fungal diversity were due to the presence or absence of fire. Difference in fire frequency treatments did not appear to produce significant differences in AM community structure.

Bacterial activity

The purpose of the Biolog test was to quantify bacterial activity and diversity by observing the number of carbon sources utilized by each sample, and the degree of use of each sample (the level of the color change). The purpose of the MDS analysis was to examine how different or similar the overall assemblages of each site were. Also, because two Biolog plates were inoculated for each sample, the arrangement of each pair of duplicates in the MDS analysis gave information about the heterogeneity of the sites.

CMD (Community Metabolic Diversity) was calculated in order to define the bacterial metabolic diversity in each sample. In the first run (which did not include Shaw), the samples from Morton Grove, Santa Fe, and Monee had the highest CMD. In the second run (which did not include Shaw), the samples from Monee, Glenbrook, and Santa Fe had the highest CMD for most days. In the third run (which did not include Glenbrook or Santa Fe), the samples from Monee and Shaw had the highest CMD values.

AMR (Average Metabolic Response) was calculated in order to characterize the bacterial activity level in each sample. In the second run, samples from Glenbrook, Monee, and McLaughlin had the highest AMR. In the third run (which did not include Glenbrook or McLaughlin), two samples from Monee had the highest AMR. CMD and AMR showed which sites had the most diverse (Morton Grove, Santa Fe, Monee, Glenbrook) and most active (Glenbrook, Monee, McLaughlin) bacterial communities.

Meanwhile, the MDS arrangements show how much fire frequency impacts the bacterial assemblage (i.e., whether sites with the same fire frequencies were clustered most closely on the MDS grid.). For the most part, samples did not cluster based on fire frequency of the study site of origin, or even based on the study site alone. In both Rounds 2 and 3, the Somme plates clustered relatively near each other, suggesting that the bacterial communities of the Somme samples are more homogeneous than most of the other sites. In Round 3, the two plates from one of the two Monee samples clustered relatively near each other, suggesting relative homogeneity for this sample as well. The most consistent result from the Biolog analysis, across all measures and all runs (to a slightly lesser extent in Round 1), was the low activity level at Somme. This suggests that Somme is the site with the most different bacterial assemblage.

Variable relationships

Mycorrhizal colonization (T) and productivity (P_F) were expected to yield parallel results, where the site with the greatest amount of mycorrhizal activity would have the highest value for both of these measures, and the site with the least active mycorrhizal fungi would have the lowest value for both measures. However, some sites had T and P_F values that did not seem to match. The difference between the two measures is that T, colonization, examines mycorrhizal activity that was actively occurring in the field between a fungus and a plant root at the time of soil collection. On the other hand, P_F, productivity, is a measure that reflects the amount of actively growing fungal hyphae in the soil, searching for a plant partner or nutrients. For sites where these two measures conflicted somewhat, such as Glenbrook and Monee, the difference may be viewed as the difference between actual fungal colonization and potential fungal colonization.

Although many nutrients affect mycorrhizal fungi to some degree, mycorrhizal activity and phosphorus levels were expected to have a particularly close relationship because mycorrhizal associations are known to decrease when P is abundant (Paul 2007). This potential relationship is somewhat confirmed by the mycorrhizal data. The three sites that had relatively high T and P_F levels were Shaw, Santa Fe, and Glenbrook. Shaw and Santa Fe were two of the three sites (along with McLaughlin) that had the lowest available P levels. However, Glenbrook had the highest available P levels, so mycorrhizal activity there is less well explained in connection with phosphorus.

Aboveground Diversity

The study of Bowles and Jones (2004) investigated floristic characteristics of many prairies in the Chicago region, including the seven sites in this study. The distribution of one of the variables, plant species richness, represented as average number of species per plot, is shown in Figure 37 for the seven sites in the current study. Although the overall conclusion of Bowles and Jones (2004), taking into account many more sites, was that plant species richness and species diversity decline with declining fire frequency, this is not entirely evident in the figure.

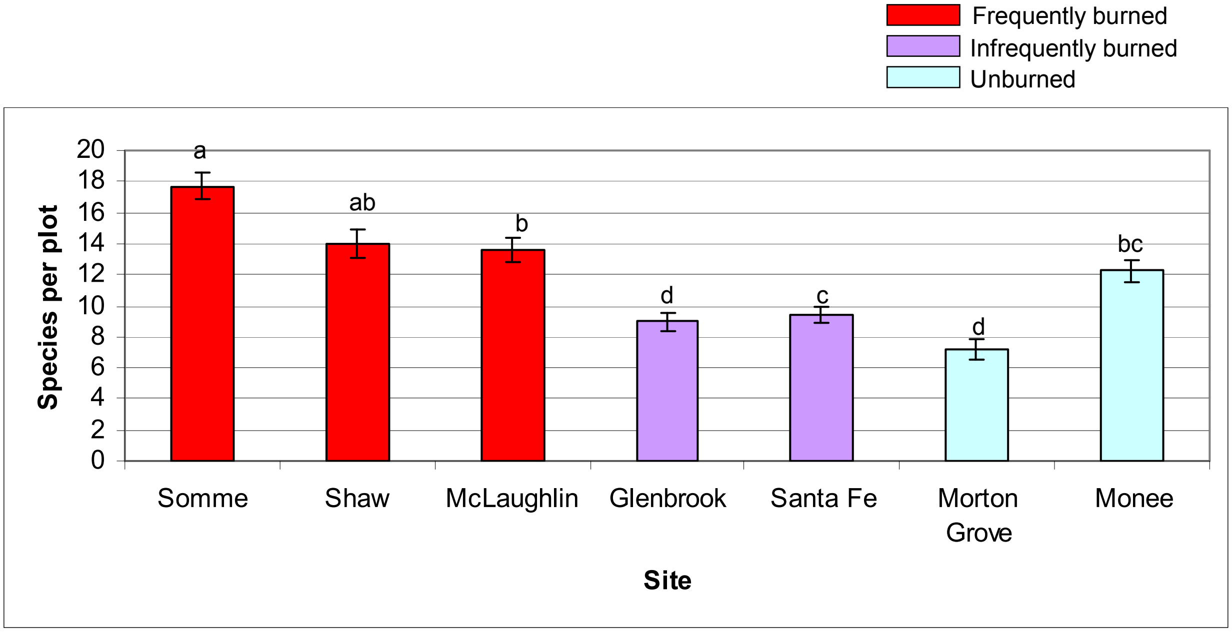


Figure 37. Average plant species richness at the seven sites, based on vegetation data collected in 2001 (Bowles and Jones 2004). Letters indicate significant differences ($p<0.05$).

Despite the fire frequencies of the sites, the relationships shown in the figure are that Somme and Shaw had the highest plant species richness, followed by McLaughlin and Monee, followed by Santa Fe, with Glenbrook and Morton Grove having the lowest species richness. This sorting (with Somme and Shaw at the top and Glenbrook and Morton Grove at the bottom) does not closely resemble the sorting that was observed for any of the soil nutrients. This implies that soil ecosystem functioning cannot necessarily be inferred by aboveground observations.

Implications

A primary goal of this study was to assess how the tallgrass prairie soil community is affected by different prescribed fire regimes, by identifying any soil characteristics that correlate strongly with fire frequency. Most abiotic nutrients were not expected to correlate strongly with fire frequency due to a lack of established information, except for nitrogen, which was expected to decrease with increasing frequency. The biotic characteristics of mycorrhizal activity, mycorrhizal diversity, bacterial activity, and root biomass were expected to increase with increasing fire frequency.

For the most part, clear patterns did not emerge based on fire frequency. This may be because fire frequency does not have a strong effect on many of the soil characteristics, but it is possible that some variables were confounded by uncontrolled differences. Soil characteristics that seemed to have the strongest relationship to fire frequency were root mass, potassium levels, and available nitrogen levels.

The frequently burned sites, Somme, Shaw, and McLaughlin, had the highest root masses in both July and October. The unburned sites, Monee and Morton Grove, had the lowest root masses in October, and among the lowest root masses (along with Santa Fe) in July. The infrequently burned sites, Glenbrook and Santa Fe, had intermediate root masses in October, and Glenbrook had intermediate root masses in July as well. This is consistent with other studies that have found increased root biomass, increased root growth, and increased belowground productivity in frequently burned prairie (Rice et al. 1998, Johnson and Matchett 2001). This result implies that frequent burning is best for high belowground productivity.

For the most part, measures of available N indicated that frequently burned sites had the lowest available N. The only exception was that Morton Grove also essentially had equally low levels of NO_3. The low NO_3 at Morton Grove may be due to some un-measured factor, such as low microbial N mineralization rates. Decreased nitrogen with increasing fire frequency was expected based on other studies (Ojima et al. 1994), and the results for total N levels followed a similar, but less strong, pattern. The results mostly confirm, though, that increased fire frequency reduces soil nitrogen levels. Because many tallgrass prairie plant species are particularly adapted to low nitrogen (Reich et al. 2001) and because of widespread pollution problems with excess nitrogen deposition (Gilliam 2006), the lowest nitrogen may be considered the most high-functioning state for tallgrass prairie. This result implies that frequent burning is also the best for keeping nitrogen levels from becoming excessive.

Potassium levels appeared to be linked to fire frequency because the two sites with the highest K levels were the two unburned sites, Morton Grove and Monee. Although there was no clear distinction between the frequently burned and the infrequently burned sites, the result implies that burning causes reduced soil K. This was not expected, since K is more likely to be re-deposited in the ash after a fire (Blair et al. 1998). Because potassium is an important plant nutrient that does not tend to cause problems of excess in tallgrass prairie, this result implies that very infrequent burning is best for maintaining potassium levels.

A final soil characteristic from which fire frequency patterns may be extracted, although with less certainty, is the structure of the bacterial community. On one hand, there is no strong correlation because almost every site had a “high” value of at least one of the Biolog measures (CMD or AMR for at least one of the rounds). However, a notable exception was Somme, a frequently burned site which had an intermediate to low value every time. Also, the site that most consistently had high values was Monee, an unburned site. A comparison of this pair of sites suggests that increased fire frequency causes a decrease in the bacterial metabolic diversity of the soil community.

The fact that Skokie River and Morton Grove do not as clearly fall into this pattern may be due to other, unknown characteristics of these two sites. Skokie River Nature Preserve may have a particularly robust microbial community. Along the same lines, Morton Grove Nature Preserve may have a particularly poor microbial community, as already suggested by the nitrogen data. On the other hand, the microbial activity differences between Somme and Monee may be simply due to unknown unique characteristics of the two sites.

In sum, the data set makes it difficult to recommend decisively a particular fire frequency for all tallgrass prairies. The fire frequency does have a noticeable influence on at least some soil characteristics, but the overall picture is less clear. Is it more important to have greater belowground root productivity, or a more diverse bacterial assemblage? The answer to this kind of question may differ depending on the site. In addition, for some variables the individual site effects may have been stronger than the fire treatment effect. However, these results do not overtly contradict the standing recommendation of Bowles and Jones (2004), which is currently accepted by many Chicago-area land managers as the ideal, of burning every other year. Therefore, based on the soil characteristics investigated, this study is compatible with the recommendation of biennial burning for tallgrass prairie.

It was difficult to produce a definitive recommendation because of the lack of baseline information, the difficulty in creating a totally controlled experiment, and limitations in the number of samples that could be collected (due to permit and funding limitations). In addition, soil attributes that were left out of the study may have provided a more coherent overall picture.

For example, although soil levels of many key nutrients were measured, this only tells one part of the nutrient cycle. In the future, measures of plant nutrient concentrations, and microbial mineralization and immobilization rates, may help explain some observations.

Despite these limitations, it is clear that the fire management regime in tallgrass prairie impacts the soil community. If there is an ideal fire frequency that optimizes the health of every component of the tallgrass prairie ecosystem, it remains unknown. In the meantime, fire is well-acknowledged as essential for maintaining tallgrass prairie as prairie (Collins et al. 1998, Evans 1984, Copeland et al. 2002). Therefore, land managers will be wise to continue to practice fire management as more precise ecological relationships continue to be studied.

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APPENDIX 1

Carbon Sources in Biolog GN2 MicroPlate

1. α-Cyclodextrin
2. Dextrin
3. Glycogen
4. Tween 40
5. Tween 80
6. N-Acetyl-D-Galactosamine
7. N-Acetyl-D-Glucosamine
8. Adonitol
9. L-Arabinose
10. D-Arabitol
11. D-Cellobiose
12. i-Erythritol
13. D-Fructose
14. L-Fucose
15. D-Galactose
16. Gentiobiose
17. α -D-Glucose
18. m-Inositol
19. α -D-Lactose
20. Lactulose
21. Maltose
22. D-Mannitol
23. D-Mannose
24. D-Melibiose
25. ß-Methyl-D-Glucoside
26. D-Psicose
27. D-Raffinose
28. L-Rhamnose
29. D-Sorbitol
30. Sucrose
31. D-Trehalose
32. Turanose
33. Xylitol
34. Pyruvic Acid Methyl Ester
35. Succinic Acid Mono-Methyl-Ester
36. Acetic Acid
37. Cis-Aconitic Acid
38. Citric Acid
39. Formic Acid
40. D-Galactonic Acid Lactone
41. D-Galacturonic Acid
42. D-Gluconic Acid
43. D-Glucosaminic Acid
44. D-Glucuronic Acid
45. α-Hydroxybutyric Acid
46. ß-Hydroxybutyric Acid
46. γ-Hydroxybutyric Acid
47. p-Hydroxy Phenylacetic Acid
48. Itaconic Acid
49. α-Keto ButyricAcid
50. α-Keto Glutaric Acid
51. α-Keto Valeric Acid
52. D,L-Lactic Acid
53. Malonic Acid
54. Propionic Acid
55.Quinic Acid
56. D-Saccharic Acid
57. Sebacic Acid
58. Succinic Acid
59. Bromosuccinic Acid
60. Succinamic Acid
61. Glucuronamide
62. L-Alaninamide
63. D-Alanine
64. L-Alanine
65. L-Alanyl-glycine
66. L-Asparagine
67. L-Aspartic Acid
68. L-Glutamic Acid
69. Glycyl-L-Aspartic Acid
70. Glycyl-L-Glutamic Acid
71. L-Histidine
72. Hydroxy-L-Proline
73. L-Leucine
74. L-Ornithine
75. L-Phenylalanine
76. L-Proline
77. L-Pyroglutamic Acid
78. D-Serine
79. L-Serine
80. L-Threonine
81. D,L-Carnitine
82. γ-Amino Butyric Acid
83. Urocanic Acid
84. Inosine
85. Uridine
86. Thymidine
87. Phenyethylamine
88. Putrescine
89. 2-Aminoethanol
90. 2,3-Butanediol
91. Glycerol
92. D,L-α-Glycerol
93. Phosphate
94. α-D-Glucose-1-Phosphate
95. D-Glucose-6-Phosphate

APPENDIX 2 Arbuscular mycorrhizal community composition

AM family	Genus and species	**Unburned**	**Infrequently Burned**	**Frequently Burned**
Acaulosporaceae	*Acaulospora colosicca*	1	1	1
	Ac. delicata	1		
	Ac. denticulata		1	
	Ac. mellea	1		
	Ac. spinosa	1		
	Acaulospora 893*		1	
	Acaulospora 1415		1	
Archaeosporaceae	Archaeospora gerdemannii	1		1
	Ar. species (SM2)	1		
	Ar. species (SM1)		1	
	Ar. leptoticha	1	1	1
	Ar. trappeii			1
	Archaeospora 717			1
Glomaceae	*Glomus aggregatum*	1		
	Gl. etunicatum	1		
	Gl. claroideum	1	1	
	Gl. clarum	1		
	Gl. luteum	1		1
	Gl. mosseae	1		1
	Gl. versiforme	1		
	Gl. intraradices	1	1	1
	Gl. geosporum		1	1
	Glomus sp. 6	1		
	Gl.aff monosporum	1		
	Glomus 1159			1
	Glomus 519	1		
	Glomus 625	1		
	Glomus 700		1	
	Glomus 717		1	
	Glomus 745		1	
	Glomus 774	1		
	Glomus 787		1	
	Glomus 833			1
	Glomus 857		1	
	Glomus 884	1		
Paraglomaceae	*Paraglomus occultum*	1		1
	P. brasilianum	1	1	

AM family	Genus and species	**Unburned**	**Infrequently Burned**	**Frequently Burned**
Gigasporaceae	*Gigaspora margarita*			1
	Gi. rosea	1		
	Gi. gigantea	1		
	Scutellospora castanea	1	1	
	Sc. persica	1		
	Gigaspora 644	1		
	Gigaspora 721	1		
	Gigaspora 689	1		
	Total species richness	30	16	13

* AM genera appended with a number indicate taxa for which there were no known matches at the species level. These numbers have no systematic value and simply denote the sample identification scheme used in the study.