SHIFTS IN MICROBIAL COMMUNITY STRUCTURE AS A RESULT OF A WILDFIRE IN THE NEW JERSEY PINELANDS

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ABSTRACT OF THE DISSERTATION

Shifts in Microbial Community Structure As a
Result of a Wildfire in the New Jersey Pinelands
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As the threat of wildfires in the United States increases due to global warming, understanding their effects on the soil biological community becomes central to recovery efforts. Therefore, it is important to study microbial community dynamics in forest soils impacted by fires from the view of elucidating how the new state compares with the original state of the microbial community. For this study, wildfires were hypothesized to cause a shift in the microbial community structure with dominant microbes being those best capable of responding to changes in their environment caused by the perturbation. The objectives of this research were to examine the recovery of the forest soil microbial communities after a wildfire and to investigate the state of the communities more than two years post-fire. After a wildfire occurred in the New Jersey Pinelands in 2007, soil samples were collected from the organic and mineral layers of two severely burned sites and an unburned control site over the span of two years following the fire. Microbial community composition was evaluated by principal component analysis and multivariate

analysis of variance of molecular fingerprint data for bacterial, archaeal, and fungal-specific amplicons from denaturing gradient gel electrophoresis. The bacterial communities in the samples collected from 2 and 5 months following the fire clustered separately from those collected 13 and 17 months post-fire in two-dimensional space, indicating that the soil bacterial community structure changed with time following the fire. Deeper evaluation of the bacterial, archaeal, and fungal community patterns revealed that even though there were common bands between the unburned and the severely burned samples, the community structure of the samples from the unburned site grouped separately from those of the severely burned sites collected 2, 13, and 25 months post-fire. Generally, the microbial community composition in the unburned samples did not change significantly over two years. These data support the hypothesis that the soil microbial community was selected by both the direct and indirect effects associated with the wildfire in the initial two years after the perturbation. Rather than return to the pre-disturbance state, the soil microbial communities may reflect an alternate state two years following the fire.

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INTRODUCTION

Wildfires are common disturbances in our environment. Their impact on humans and wildlife, in addition to their economic and ecological repercussions, are profound (Janzen and Tobin-Janzen, 2008). Additionally, the intensity and frequency of fires are influenced by humans; reports suggest that forest fires will become more intense and frequent as a result of conditions associated with the changing climate (IPCC, 2007; Westerling *et al.*, 2006). Generally, results in the literature do not suggest general trends to predict the state of a site following a fire; research in tracking the concentrations of these soil nutrients will provide a basis for better predictions and modeling of nutrient transformations.

Prescribed burns have been shown to reduce the frequency and intensity of subsequent wildfires and improve the resistance of soil microbial communities to future fires (Choromanska and DeLuca, 2001). In comparison, frequent, severe fires have uncertain impacts on soil biological and chemical processes because they may result in beneficial or deleterious effects on the forest system (Neary *et al.*, 1999). These impacts must be discerned in order to effectively manage forests after fire events. Recent burning events affect microorganisms in the soil organic layer where they are most abundant (Andersson *et al.*, 2004; Neary *et al.*, 1999). These microbes play integral roles in the cycling of essential nutrients. Focusing on how fire impacts microorganisms is important to better understand the ecology of a site. Like microbial biomass, microbial community composition may serve as an indicator of the effects of fire. More information regarding forest fires is needed to insure that essential microbial processes will not be reduced or eliminated by the occurrence of wildfires.

The New Jersey Pinelands served as a model system to study the impact of fire on the soil system. Soil microbial communities will evolve to a structure selected by the new soil properties existent after a wildfire. This community may or may not differ from what pre-existed. Members of the three domains of life were used in this study to gain a better understanding of microbial ecology and how different types of microbes were impacted by the fire. The main objectives of this research were to study the length of time it took for the soil microbial community to recover from the disturbance, the resultant community structure, and the types of microorganisms that were impacted by the severe wildfire.

With the aim of revealing the impact of the wildfire on important soil chemical and biological parameters, three main studies were conducted. Chapter 2 details two studies assessing the impact of the wildfire on the bacterial component of the soil system. First, selected physiochemical variables were measured to determine the effect of the wildfire on soil pH, gravimetric water content, organic matter, nitrogen, and micronutrients. In addition to the chemical analyses, soil microbial community patterns were analyzed through the DGGE molecular profiling technique in order to investigate the structure of the community with time following the fire. To determine the impact of the wildfire on the bacterial communities within the first year and a half following the fire, bacterial community composition was monitored in the samples collected 2, 5, 13, and 17 months post-fire. Because the community profiles changed with time following the fire, a more comprehensive study of the bacterial community was conducted. Specifically, to monitor the recovery of the bacterial community following the

disturbance, the bacterial patterns in the severely burned samples were compared to unburned control samples.

The effect of fire disturbance on the soil archaeal community is poorly understood even though archaea have been found to conduct meaningful nutrient cycling reactions (Aller and Kemp, 2008; Kirchman *et al.*, 2007; Könneke *et al.*, 2005; Wuchter *et al.*, 2003). Therefore, to further understand the role of archaea in disturbed soil systems, the archaeal community structure of the undisturbed and disturbed soils was compared in Chapter 3. Specifically, the shift in archaeal community composition as a result of the wildfire disturbance was revealed.

In an effort to understand how disturbance affects representatives from the three domains of life, Chapter 4 highlights the impact of the wildfire on soil fungal communities. Fungi are known decomposers that play a critical role in nutrient cycling in forest systems. Chapter 4 showed the impact of the fire on the fungal community structure, bringing insight into OTUs that dominated in certain treatments. Additionally, the environmental parameters that were driving the fungal community in the organic soil layer were revealed.

In nature, complex interactions between members of the three domains of life result in the environmental scenario we observe. Therefore, the scope of this project was to reveal the effects of wildfire on three separate types of microorganisms. When microorganisms are studied separately, only a small piece of the picture is demonstrated; however, taken together, these data provide strong evidence of the impact of wildfire on the complex soil biological system.

With an increase in frequency and intensity of forest fires in the future, the need for research concerning the stability of the ecosystems after the fire will increase. Policy that targets these issues of climate change conditions and forest fires can promote more sustainable and mutually beneficial relationships between humans and disturbed environments. Additionally, given the rapid pace of urbanization worldwide, it is important to recognize the significance of maintaining forest ecosystems such as the New Jersey Pinelands. As urban sprawl has increased in the United States, the challenge of integrating the needs of society with ecological system function has also increased. Therefore, the results from this project will provide corroboration that research on forest sites lends significant information on *in situ* processes that support plant growth and groundwater quality.

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CHAPTER 1

LITERATURE REVIEW

Managing and conserving natural forest systems is a tremendous challenge for humans. When disturbance strikes precious natural resources, it is critical to monitor the effects of the disturbance on all aspects of the ecosystem. An integral part of forest ecosystems is the soil microbial community. Indigenous soil microbes enhance the forest vegetation by mediating important chemical reactions that supply plants with the appropriate nutrients to grow and proliferate. Microbes, such as bacteria, also serve as substrates for larger predatory organisms such as nematodes and protists. Together, these different types of organisms make up the forest ecosystem. To study them collectively will enable better predictions regarding the effect of disturbance on the biological community.

A historical example of an environmental perturbation is wildfire, which is the unplanned ignition of plant biomass. Alternatively, prescribed burns are less intense controlled fires and are commonly used to reduce fuel loads (Certini, 2005). Unlike prescribed burns, the impact of wildfires on humans and wildlife, in addition to their economic and ecological repercussions, are profound (Janzen and Tobin-Janzen, 2008). For instance, habitats may be lost and homes may be leveled as a result of these environmental phenomena that occur world-wide due to anthropogenic or natural causes. The former include causes such as man-made fire or release of flares in forests, while the latter include lightning and volcanic eruptions (Bowman *et al.*, 2009). These wildfires across the globe only occur if there are optimal fire conditions such as sufficient oxygen concentration, dryness, and biomass.

Currently, detailed reports predict that wildfires will become more frequent and severe as a result of conditions associated with the changing climate (IPCC, 2007; Westerling *et al.*, 2006). Specifically, the increased concentration of carbon dioxide released to the atmosphere will result in a greater greenhouse effect, which in turn creates warmer, drier environments that foster more burns. Additionally, fires influence climatic conditions by increasing carbon and atmospheric aerosols, and changing the surface albedo (reflectivity) (Bowman *et al.*, 2009; Hart, *et al.*, 2005). By doing so, fires are proposed to contribute to the changing climate. As the threat of wildfires in the United States becomes greater each year, recognizing the importance of the effects on the soil biological community becomes more important, as it is central to recovery efforts.

When wildfires strike areas of primary production, they disrupt natural nutrient cycling, in addition to any human economic gains in that environment. Interestingly, there is a strong association between areas of frequent wildfires and areas of moderate net primary production (Bowman *et al.*, 2009). If these areas are more likely to burn, then there may be an increased release of carbon dioxide and other greenhouse gases into the atmosphere. These forests are immense sinks for carbon, and with an expected increase in fires, particularly in areas where net primary productivity is high, there is the potential for a large carbon release into the atmosphere. To offset this problem, biochar, the incomplete oxidation of woody residues, is being explored as a carbon sequestration method to store recalcitrant carbon in soils to reduce the flux of carbon to the atmosphere (Lehmann, 2007). However, biochar may contribute to ecosystem losses of carbon (Wardle *et al.*, 2008). While seemingly promising, more studies are needed to resolve the feasibility of this sequestration method.

A conspicuous result of wildfires is that they drastically reshape the landscape. First of all, burns result in the loss of vegetation; thus resulting in augmented soil erosion (Knoepp *et al.*, 2004). Subsequently, fewer nutrients are exuded out of plant roots, resulting in a reduced amount of bioavailable carbon for soil microbes. Additionally, when biomass has been ignited, the plant canopy is reduced; therefore, the increased solar penetration changes the soil microclimate both on daily and seasonal scales (Hart *et al.*, 2005). Specifically, there are warmer soil temperatures during the day and more rapid cooling of soil to progressively lower temperatures at night (Hart *et al.*, 2005). Such changes in the microclimate create a more hostile environment for microbes through increased temperature and reduced moisture. These physical factors may impact the types of dominant microorganisms residing in the soil matrix. Furthermore, moist soils will lead to greater mortality of soil microbes than dry soils due to increased temperature penetration to soil pores (Choromanska and DeLuca, 2001). Finally, the heat penetration of a fire directly influences soil organisms because various microorganisms thrive at a particular optimum temperature, mainly in the range of 30°C (Madigan *et al.*, 2003). Keeping this biological optimal temperature in mind, the maximum ground temperature of a typical forest fire is 200-300°C (Rundel, 1983; Neary *et al.*, 1999). The organic matter is completely consumed at approximately 450°C and above (Neary *et al.*, 1999). Granted, these incredibly hot temperatures only last while the aboveground fuel is being consumed and there is a buffering capacity of soil to reduce heat transfer; however, if high temperatures between 40-70°C penetrate, biological proteins may denature, resulting in cell mortality (as cited in Neary *et al.*, 1999).

Not only do forest fires alter the landscape, they change the chemistry in the soil matrix. For instance, abiotic factors such as soil pH, carbon, and temperature have been shown to strongly influence the recovery of the belowground microbial community (Lauber *et al.*, 2009; Hamman *et al.*, 2007; Fierer and Jackson, 2006). Despite some agreement with these trends, results from other studies investigating the changes in soil properties due to fire are conflicting. Soil pH post-fire is typically elevated due to the addition of base cation-rich ash into the soil (Mabuhay *et al.*, 2006b; Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999; Fernández *et al.*, 1997); however, soil pH became more acidic following a fire in a Ponderosa Pine forest in Colorado (Hamman *et al.*, 2007). Specifically, in the Hamman *et al.* (2007) study, the soil pH exhibited a minimal change in pH (from 6.7 to 6.5 pre-fire and post-fire respectively). These findings demonstrate that pH itself is not an adequate indicator of the effects of fire on the soil ecosystem.

Additionally, the impact of burns on carbon and nitrogen cycling are variable. On one hand, both field studies and laboratory simulations report losses of carbon (Mabuhay *et al.*, 2006b; Fernandez *et al.*, 1997) and nitrogen (Castro *et al.*, 2006; Mabuhay *et al.*, 2006b; Murphy *et al.*, 2006b; Fernandez *et al.*, 1997) from the soil. Since the forest system is generally nitrogen limited, the loss of nitrogen due to its low volatilization temperature at approximately 200°C may delay the recovery of the plant community, which affects the overall stability of the ecosystem. Therefore, nitrogen fixation is a significant process following a forest fire, as is exemplified by data from both a prescribed burn and a wildfire (Murphy *et al.*, 2006a; Murphy *et al.*, 2006b; Neary *et al.*, 1999). If nitrogen is lost to the atmosphere during a fire, soil bacteria capable of fixing

nitrogen become key players in converting dinitrogen to bioavailable forms. In contrast to the report of loss of nitrogen, Knicker *et al.* (2005) demonstrated that the concentrations of organic carbon and nitrogen were doubled in the A horizon of a burned soil. This was probably due to the incorporation of humic material and charred plant matter into that soil layer. They demonstrated that humic material gained aromaticity and more heterocyclic nitrogen components as a result of a fire event (Knicker *et al.*, 2005). The formation of these compounds in the temperature range of 250-500°C may provide a recalcitrant carbon pool in the soil matrix (Baldock and Smernik, 2002). Additionally, a prescribed burn in a oak-pine forest in the southern Appalacians (Knoepp *et al.*, 2004) and a wildfire in a Sierra Nevada mixed conifer forest consisting mainly of pine and fir trees (Johnson *et al.* 2007), reported no significant loss of soil carbon or nitrogen in the mineral soil fraction as a result of a fire event. Overall, results in the literature do not suggest general trends to predict soil pH, and carbon and nitrogen concentrations following a fire; research in tracking the concentrations of these nutrients in a site-specific manner is necessary to provide a basis for better predictions and modeling of nutrient transformations.

In addition to carbon and nitrogen, soil micronutrients are essential elements available to soil microbiota. After the fire event, nutrients are expected to be liberated and made bioavailable to the soil community. Generally, the concentrations of calcium, potassium, and magnesium have been shown to increase following a fire (Murphy *et al.*, 2006b; Knoepp *et al.*, 2004). Since calcium has a high volatilization temperature, it is not lost during the fire and the input from ash may increase the surface soil calcium concentrations many years after the initial burn (Neary *et al.*, 1999). Calcium is an

important soil nutrient because it is essential to all plants and aids in stabilizing bacterial cell walls and endospores (Madigan *et al.*, 2003; Brady and Weil, 2002). Magnesium is another important soil nutrient because it is a component of the chlorophyll molecule, as well as required for many enzymes (Madigan *et al.*, 2003; Brady and Weil, 2002). The potential fates of potassium and magnesium include being washed into the soil from ash, held by the soil, or leached into the groundwater (Murphy *et al.*, 2006b; Neary *et al.*, 1999). If these cations are leached, then they are effectively lost to the soil microbial community. In a study by Murphy *et al.* (2006b), there were no significant differences between the micronutrient concentrations of the unburned and burned samples one year after a wildfire. In some sites, the soil system recovery may occur within the first year post-fire. Similarly, following a wildfire in Sierra Nevada mixed conifer forest, soil carbon, nitrogen, phosphorus, and potassium were not significantly different in the mineral layer soil following a wildfire while calcium, magnesium, and sulfur were significantly different post-fire (Johnson *et al.*, 2007). The unpredictable nature of the soil chemical properties contributes the need for multi-dimensional studies regarding forest disturbance.

Changes in soil chemical properties may result in modifications in soil microbial biomass and community composition. For example, not only have prescribed burns been shown to reduce the frequency and intensity of subsequent wildfires, they also improved the resistance of soil microbial communities to future fires through increased soil respiration rates (Choromanska and DeLuca, 2001). In comparison, frequent, severe fires have uncertain impacts on soil biological and chemical processes because they may result in beneficial or deleterious effects on the forest system (Neary *et al.*, 1999). Therefore,

until a trend is established, each wildfire study requires a complete set of chemical and biological data to effectively manage forests after fire events.

Recent burning events affect microorganisms in the soil organic layer where microorganisms are most abundant (Andersson *et al.*, 2004; Neary *et al.*, 1999). These microbes mediate chemical reactions and play integral roles in the cycling of essential plant and microbe nutrients. If the general microbial biomass decreased following a fire, then nutrient cycling may also be affected since a smaller number of microorganisms mediate important chemical reactions.

In general, reports of changes in the community's response to fire perturbation are conflicting. Microbial biomass has been used as an indicator of the status of the microbial community following disturbance events. While one study reported no significant difference in the microbial biomass between unburned and burned soils (Hamman *et al.*, 2007), others reported a decline in the total soil microbial biomass after a fire (Smith *et al.*, 2008; Mabuhay *et al.*, 2006b; Yeager *et al.*, 2005; Choromanska and DeLuca, 2001). In these studies, microbial biomass has been reported by the fumigation extraction method (Smith *et al.*, 2008; Mabuhay *et al.*, 2006b; Choromanska and DeLuca, 2001), total concentration of ester-linked fatty acid methyl esters (Hamman *et al.*, 2007), or the less sensitive technique of measuring DNA yields (Yeager *et al.*, 2005). More specifically, in the Choromanska and De Luca (2001) study of a Ponderosa Pine forest, soil microbial biomass was elevated in the soil samples that experienced both a prescribed burn and a wildfire, when specifically compared to the wildfire-impacted soils. Therefore, the authors argued that exposure to prescribed burning allowed for the community to exhibit a better recovery when exposed to a subsequent wildfire.

Furthermore, a limitation to the Hamman *et al.* (2007) study was that the first soil samples were collected approximately 14 months following the fire, and in that time period, the microbial community dynamics could have changed dramatically. Microbial biomass provides information on the amount of cellular material; however, it has been found to be an insufficient tool to determine the impact of fires on soil microbial community composition (Bárcenas-Moreno and Bååth, 2009).

In addition to soil microbial biomass, soil microbial community composition has been demonstrated to change as a result of fire events; however, no clear predictable pattern has been established, especially in long term chronosequence studies. The microbial community composition indicates the types of microorganisms that were dominant in the samples. In a study of a fire-impacted boreal forest in Canada dominated by spruce, the bacterial community composition between unburned and burned soil samples collected one year after the fire was significantly different (Smith *et al.*, 2008). For instance, DNA sequences related to *Bacillus* were found only in the burned samples (Smith *et al.*, 2008). The presence of *Bacillus* is expected since it is a known spore-forming bacterium that has protection mechanisms to survive extreme events such as fires. In addition, members of the *Betaproteobacteria* dominated in the burned soil samples (Smith *et al.*, 2008). This study only sampled the mineral soil layer and did not collect the first sample until a year post-fire (Smith *et al.*, 2008). In another study of a Ponderosa pine forest in Colorado, there was a decrease in fungal biomarkers in the burned soils even though bacterial abundance did not change (Hamman *et al.*, 2007). The study noted a shift in bacterial and fungal community structure, but did not investigate the discrete operational taxonomic units (OTUs) associated with each

treatment (Hamman *et al.*, 2007). In two studies by Mabuhay *et al.* (2006a; 2006b), they observed a similar trend of contrasting bacterial profiles in burned soils following wildfire in western Japan, and similar to the previous study, they did not investigate the OTUs impacted by the fire. In the former Mabuhay *et al.* (2006a) study, five study areas, each experiencing a different wildfire during different seasons was compared; therefore, it is difficult to study the true progression of microbial community dynamics for completely different burn events. Controlled laboratory studies discerned that bacteria were favored by heating perturbation (Bárcenas-Moreno and Bååth, 2009). Further analysis of bacterial communities following a stand-replacing fire in a pine-forest fire in New Mexico indicated that the abundance and composition of nitrogen-fixing and ammonia-oxidizing bacteria shifted as a result of fire (Yeager *et al.*, 2005). Specifically, the diversity of nitrogen-fixing sequence types was greater in the burned samples and certain nitrifying *Nitrosospira* species responded favorably to the disturbance (Yeager *et al.*, 2005). The role of nitrogen cycling organisms and the maintenance of a diverse soil microbial community are important factors in the recovery of the ecosystem following the disturbance. It is critical to manage the ecosystem in such a way as to promote essential microbial processes such that they are not reduced or eliminated by the growing incidence of wildfires.

Following an ecosystem perturbation, the state of the system is widely unknown. One hypothesis is that the types of dominant organisms in the disturbed area change as a result of the perturbation, and then with time, the original community members present before the disturbance once again become dominant members of the community. The alternate hypothesis is that different community members become dominant and persist

based on the new, sustained conditions in the site. The latter scenario is termed the alternate state hypothesis, as investigated by Schröder *et al.* (2005) and Bertness *et al.* (2002).

Furthermore, the roles of macroorganisms and large microorganisms in pristine ecosystems are well-documented (Price and Morin, 2009; Bertness *et al.*, 2002); however, little is known regarding the alternate states of microorganisms such as bacteria, archaea, and fungi in disturbed ecosystems. This hypothesis is most commonly applied to rocky intertidal communities because of the selective nature of the extreme conditions in this environment in addition to the rather quick proliferation rates of the organisms (Bertness *et al.*, 2002). In general, the alternate state hypothesis suggests that biological communities can exist in more than one state (Bertness *et al.*, 2002), and the community shifts to an alternate stable point when a disturbance pushes one or more species above or below a critical threshold (Price and Morin, 2009; Schröder *et al.*, 2005). More than one type of community can be present in a given habitat and the dominant community is a result of any cell or seed able to proliferate and continue to proliferate when space was made available (Bertness *et al.*, 2002). Therefore, before the disturbance, a particular set of microorganisms dominate; however, after the disturbance, a new set of dominant microorganisms persist in the soil matrix.

The progression of microbial community dynamics in forest soils impacted by disturbance is largely unknown. Many studies have investigated the impact of a disturbance on the biological community; however, most do not reveal how the new state compares with the original state of the system. An overarching issue in ecological

studies is if the community returns to the state prior to the disturbance, or progresses to an alternate state.

While results on short term time-series experiments or comparisons between sites may support the alternate state hypothesis, there is still the possibility of abrupt, but still continuous responses to environmental parameters or long term cycles (Schröder *et al.*, 2005). Therefore, an important objective of these types of studies is to reveal the long term perspective of the system so that the transient states of the system are not inadvertently considered as the final stable condition (Schröder *et al.*, 2005).

Most studies regarding alternate state regard the environmental effects of larger organisms. In these studies, ecological stability is determined over one generation time (Schröder *et al.*, 2005). Because the concept of alternate state is mostly applied to macro-ecology, this criterion becomes challenging with respect to micro-ecology due to the relatively short generation times of microorganisms. However, this can be overcome by monitoring the state of the biological community in the undisturbed samples.

Alternate states may result in unexpected consequences with regard to the overall system function (Schröder *et al.*, 2005). For instance, the emerging microbial community may not support the same plant species as the previous community. Consequently, the ecosystem as a whole would dramatically change and it becomes paramount to determine if the soil microbial structure in the fire impacted soils resembles that of the unburned soils. If alternate stable states exist, accompanied by functional dissimilarity, then the microbial functions of the system may change and perhaps result in varied interactions with the plant community.

The model system we used to monitor the impact of change on the soil biological community, in addition to relating the state of the system to the alternate state hypothesis is the New Jersey Pinelands. In this non-glaciated region located in the outer coastal plain in New Jersey, wildfires and prescribed burns are common occurrences (Forman, 1979). Forest systems similar to the Pinelands are located in Pennsylvania, southwestern Ohio, eastern shore of Maryland, Rhode Island, Massachusetts to southern Maine, Staten Island, Long Island, and north of the Champlain Valley in New York (Forman, 1979). Wildfires have shaped the Pinelands throughout history and have influenced the forest ecosystem for over the past three hundred years (Collins and Anderson, 1994; Forman, 1979). The first record of fire was in 1632 whereby Native Americans were setting fires to drive out deer and encourage foot travel (Forman, 1979).

Today, the New Jersey Pinelands are an ecological island of about 1.4 million acres (approximately 30% of the state) in the southern portion of the state, surrounded by urban and suburban populations. The Pinelands are approximately 15 to 46 meters above sea level and all Pinelands streams originate in the region (Forman, 1979). This forest is relatively homogenous compared to other forest systems; trees and shrubs common to the Pinelands include *Pinus rigida*, *Quercus alba*, *Quercus marilandica*, *Quercus ilicifolia*, *Gaylussacia bata*, and *Vaccinium angustifolium* (Collins and Anderson, 1994). Typical soil properties include a one to two centimeter organic layer in the uplands, soil pH of 3.5 to 4.0, and predominantly sandy composition (Ehrenfeld *et al.*, 1997; Zhu and Ehrenfeld, 1996). The sandy soil is dominated by quartz (SiO_2), which contributes to the low cation exchange capacity, low pH, and poor nutrient status in the soils of this region (Forman, 1979). Due to the low nutrient status of the Pinelands, the cycling of minerals is very

important (Collins and Anderson, 1994). Thus, the nutrient cycling patterns post-fire are predicted to have a large impact on the composition of the soil microbial community.

The purpose of this project was to demonstrate the impact of a wildfire on the belowground soil system, specifically the soil chemical and biological parameters. The resilience of soil bacterial, archaeal, and fungal communities was directly assessed, revealing which types of microorganisms were impacted by the fire event. Soil microorganisms were predicted to be impacted by the direct effects of wildfire such as temperature and immediate loss of nutrients, in addition to the indirect effects such as reduced vegetation and long term nutrient concentration changes. Therefore, these physical and chemical changes were hypothesized to select for a particular microbial community that dominated in the severely burned samples. The resilience of these organisms is important because they provide ecosystem services by mediating essential nutrient cycling reactions that support water purification and soil fertility.

The bacterial, archaeal, and fungal communities were hypothesized to respond differently to the wildfire disturbance. Because bacteria and archaea are in high abundance, widely dispersed, evolve rapidly, and are metabolically versatile, they were expected to be rather resilient to change (Allison and Martiny, 2008; Finlay *et al.*, 1997). For instance, bacterial communities commonly exhibit functional redundancy in that many different species utilize similar substrates and conduct similar nutrient cycling processes. The greater the number of taxa that perform a process, the more buffered the process is to environmental perturbations (as cited in Allison and Martiny, 2008). Furthermore, biogeochemical cycling is not limited by genetic diversity (Finlay *et al.*, 1997). Thus, even if many bacteria initially perish as a result of the extreme burn, the

functions provided by the biological community may remain the same. Alternatively, the functions could change, which would dramatically influence the concentration of nutrients in the soil that are bioavailable to the flora. There is redundancy of metabolic functions between species, and therefore, it is reasonable to claim that the capacity of the system does not always decline when species diversity declines (Bardgett *et al.*, 2005; Madigan *et al.*, 2003; Nannipieri *et al.*, 2003). Thus, the functional redundancy of soil bacteria may help to maintain the capacity of the system to function despite a decline in species diversity.

Soil organisms in general may not be directly influenced by the temperature of the fire because they are protected in the soil micropores, especially in the mineral layer. Thus, prominent bacteria were expected to survive the wildfire perturbation. There are many indirect effects of the wildfire that were expected to influence the soil bacterial community. These include but are not limited to increased soil temperature due to decreased soil canopy, changes in nutrient concentrations, and elevated concentrations of root exudates as young plants grow.

Using current techniques and knowledge, only one to ten percent of soil microorganisms can be cultured in the laboratory. As a result of this limitation, isolation experiments have been unable to reveal important soil community organisms and processes (Kowalchuk *et al.*, 2007; Nannipieri *et al.*, 2003). Therefore, molecular tools for assessing soil microbial DNA, such as polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), were used in this study because they are valuable methods that allow for identification of soil microorganisms in the environment (Muyzer, 1999; Muyzer *et al.*, 1993). Due to the incredibly low biomass in the

Pinelands, the study focused on community compositional changes. Additionally, due to PCR biases, the intensity of the DGGE bands do not necessarily correlate with the number of DNA strands extracted from the original number of microorganisms in each sample. Therefore, the intensity of the bands, or relative abundance, was not considered, but rather the presence or absence of the particular band.

When comparing DGGE to other molecular fingerprinting techniques, it was found that when four soils were subjected to DGGE, single strand conformation polymorphism (SSCP), and terminal restriction length polymorphism (T-RFLP), all three techniques yielded similar community compositions and served as appropriate tools to assess changes in soil community composition (Smalla *et al.*, 2007; Lynch *et al.*, 2004). An advantage of DGGE and SSCP is that selected bands can be sequenced, and therefore, the presence of a particular phenotype can be monitored in environmental samples (Diez *et al.*, 2001; Lee *et al.*, 1996). While DGGE has been shown to underestimate diversity relative to clone libraries, it is still one of the most useful tools for identifying community shifts (Bardgett *et al.*, 2005). In a survey of literature conducted to test if microbial composition is sensitive to disturbance, the authors found that no patterns suggesting that methodology (i.e. phospholipid fatty acid analysis, clone libraries, T-RFLP, and DGGE) influenced whether a compositional change was detected (Allison and Martiny, 2008). Therefore, the PCR-DGGE technique was a suitable tool to assess the impact of the wildfire on the soil microbial community.

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CHAPTER 2

VARIATIONS IN BACTERIAL COMMUNITY STRUCTURE IN A WILDFIRE IMPACTED FOREST SOIL

ABSTRACT

Wildfires are common disturbances that are expected to increase in frequency and intensity as a result of conditions associated with the changing climate. In turn, forest fires can exacerbate climate conditions by increasing carbon and atmospheric aerosols, and changing the surface albedo. Fires have substantial economic, environmental, and ecological repercussions; however, there is a limited understanding of the effect of severe wildfires on the composition, diversity, and function of belowground microorganisms. The objective of this research was to examine the shift of the forest soil bacterial community as a result of a severe wildfire in the New Jersey Pinelands. Over the span of two years following the fire, soil samples from the organic and mineral layers of the severely burned sites were collected six times. Samples were also collected twice from an unburned control site. It was hypothesized that soil bacterial communities in the severely burned soils would change with time following the fire. Additionally, soil bacterial communities from severely burned soils collected shortly after the fire would be significantly different from (1) the unburned soils that served as controls and (2) the severely burned soils collected more than a year after the fire. Bacterial community composition was analyzed by principal component analysis and multivariate analysis of variance of molecular fingerprint data from denaturing gradient gel electrophoresis of bacterial-specific amplicons. Bacterial community composition was significantly

different among all the organic and mineral layer samples collected 2, 5, 13, and 17 months following the fire. This indicated a shift in the bacterial communities with time following the fire. Common operational taxonomic units (OTUs) from the burned organic layer samples collected 2 months after the fire related closely to members of the phyla Cyanobacteria and Acidobacteria, whereas those from later samples (5, 13, and 17 months following the fire) were closely related to members of the genus *Mycobacteria*. Additionally, molecular analysis of the soil bacterial communities in both the unburned and severely burned soils revealed that while the unburned and burned soils share common OTUs, the bacterial communities from the unburned site clustered separately from those of the burned site collected 2, 13, and 25 months after the fire. The identities of the dominant OTUs were evaluated. The results bring insight into how disturbances associated with the changing climate affect the dominant bacteria residing in the soil organic and mineral layers.

1. Introduction

Wildfires, common perturbations in our environment, have devastating economic and ecological repercussions (Janzen and Tobin-Janzen, 2008). Areas of moderate to high net primary production have been found to experience frequent wildfires, which impact both economy and ecology (Bowman *et al.*, 2009). Wildfires occur world-wide due to both anthropogenic and natural (i.e., lightning and volcanic eruptions) sources. Thus, with an expected increase in fires due to predictions associated with climate change (IPCC, 2007), especially where net primary productivity is high, studies focused on the post-fire recovery of forest ecosystems are critical. The frequency of fire events is important because the repeated disturbance may shape the soil microbiological community in meaningful ways. Prescribed burns have been shown to reduce the frequency and intensity of subsequent wildfires and improve the resistance of soil microbial communities to future fires (Choromanska and DeLuca, 2001). In comparison, frequent, severe fires have uncertain impacts on soil biological and chemical processes because they may result in beneficial or deleterious effects on the forest system (Neary *et al.*, 1999). These impacts must be discerned in order to effectively manage forests after fire events.

Abiotic factors such as soil pH, carbon, and temperature strongly influence the recovery of the belowground microbial community as a result of forest fires (Hamman *et al.*, 2007). Soil pH is a strong contributor to the structure in shaping the soil bacterial community (Lauber *et al.*, 2009; Fierer and Jackson, 2006). Soil pH is typically elevated following a fire due to the addition of base-cations into the soil matrix (Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999); however, Hamman *et al.* (2007)

observed a decrease in soil pH following a fire in Colorado. By transforming important soil elements such as carbon and nitrogen, bacteria promote plant growth and groundwater quality. Losses of carbon and nitrogen from the soil were reported in both field studies and laboratory simulations (Castro *et al.*, 2006; Murphy *et al.*, 2006b; Knoepp *et al.*, 2004). Since forest systems are generally nitrogen limited, this loss of nitrogen due to its low volatilization temperature is anticipated to delay the recovery of the plant community, which affects the overall stability of the ecosystem. Therefore, nitrogen fixation is an important process following a forest fire, as is exemplified by data from both a prescribed burn and a wildfire (Murphy *et al.*, 2006a; Murphy *et al.*, 2006b; Neary *et al.*, 1999). Results in the literature do not suggest general trends to predict soil chemistry following a fire (Knicker *et al.*, 2005); research in tracking the concentrations of elements such as carbon and nitrogen will provide a basis for better predictions and modeling of nutrient transformations.

Studies monitoring the fate of other soil chemical properties after a fire suggest that nutrients are liberated and may be recycled to the soil biological community. The concentrations of calcium, potassium, and magnesium generally increase following a fire (Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999); however, Murphy *et al.* (2006b) determined that there were no significant differences between the unburned and burned samples for these micronutrient concentrations one year after a wildfire. Therefore, soil physical and chemical recovery may occur rapidly in some sites; however, the status of the system may be better predicted by the recovery of the soil bacterial community.

Reports of changes in the soil microbial community response to fire induced chemical and physical changes in soil properties are conflicting. One study observed no significant difference in the microbial biomass between unburned and burned soils (Hamman *et al.*, 2007), but others have reported a decline in the total soil microbial biomass after a fire (Smith *et al.*, 2008; Yeager *et al.*, 2005; Choromanska and DeLuca, 2001). Recent burning events affect microorganisms in the soil organic layer where microorganisms are most abundant (Andersson *et al.*, 2004; Neary *et al.*, 1999). These surface-layer microbes mediate chemical reactions and play integral roles in the cycling of essential plant and microbial nutrients. In a study of a fire-impacted site, the bacterial community composition between unburned and burned soil samples collected one year after the fire was significantly different, with members related to *Betaproteobacteria* detected in the burned samples (Smith *et al.*, 2008). In another study, analysis of bacterial communities following a wildfire indicated that the abundance and composition of nitrogen-fixing and ammonia-oxidizing bacteria shifted as a result of fire (Yeager *et al.*, 2005). Common limitations to such studies are that they are frequently based on single sampling events (typically a year or more following the fire) and some were conducted on prescribed burns, which are less intense than wildfires. More research on the impact of wildfires on forest ecosystems is needed to improve our understanding of the microbial community response to insure that essential microbial processes will not be reduced or eliminated by the growing incidence of wildfires.

In the New Jersey Pinelands, a non-glaciated region located in the outer coastal plain, wildfires and prescribed burns are common occurrences (Forman, 1979). Forest systems similar to the Pinelands are located in Pennsylvania, southwestern Ohio, eastern

shore of Maryland, Rhode Island, Massachusetts to southern Maine, Staten Island, Long Island, and north of the Champlain Valley in New York (Forman, 1979). Today, the New Jersey Pinelands are an ecological island of about 1.4 million acres (approximately 30% of the state) in the southern portion of the state, surrounded by urban and suburban populations. The Pinelands are approximately 15 to 46 meters above sea level and all Pinelands streams originate in the region (Forman, 1979). This forest is relatively homogenous compared to other forest systems (Collins and Anderson, 1994). Typical soil properties include a one to two centimeter organic layer in the uplands, soil pH of 3.5 to 4.0, and predominantly sandy composition (Ehrenfeld *et al.*, 1997; Zhu and Ehrenfeld, 1996). The sandy soil is dominated by quartz (SiO_2), which contributes to the low cation exchange capacity, low pH, and poor nutrient status in the soils of this region (Forman, 1979).

The objectives of this study were to 1) determine if the bacterial community was impacted by the fire event, 2) determine the length of time it took for the soil bacterial community to recover, 3) reveal the types of bacteria that were impacted by the wildfire, and 4) reveal soil nutrients that impacted the soil bacterial community. We addressed these objectives by studying the structure of the bacterial community within the first year and a half of a New Jersey Pinelands wildfire, and by comparing the bacterial communities dominant in the severely burned soil samples collected shortly after the fire to the unburned samples that serve as controls and the severely burned samples collected over a year after the fire. Within the first year, the sites that experienced the wildfire were expected to undergo substantial changes in soil bacterial community structure.

However, with time, the soil microbial communities were hypothesized to return to a non-transient state.

2. Materials and Methods

2.1 Study site

In May of 2007, a wildfire burned approximately 18,000 acres of forest in the Warren Grove section of the New Jersey Pinelands (correspondence with Nicholas Skowronski). The wildfire was a crown fire; therefore, all impacted sites were identified as severely burned. Site selection was based on soil type and the intensity of the fire. All sites had a similar plant community, climate, topography (0% slope), and a loamy sand to a sand soil type (>89% sand). More specifically, trees and understory associated with the sample sites included *Pinus rigida*, *Quercus alba*, *Quercus marilandica*, *Quercus ilicifolia*, *Gaylussacia baccata*, and *Vaccinium angustifolium*.

2.2 Soil sampling

Soil samples were collected in a 36 m^2 area from an unburned site and two severely burned sites. The approximate coordinates of the unburned site, first severely burned site, and second severely burned site were N39° 44’ 25.3” and W74° 22’ 8.6”, N39° 43’ 39.3” and W74° 22’ 14.3”, and N39° 44’ 33.3” and W74° 20’ 44.0”, respectively (Figure 1). The adjacent, unburned site served as a basis for comparison. It did not have any visible indications of fire. Some characteristics of the severely burned sites included severely burned trees and a visible ash layer.

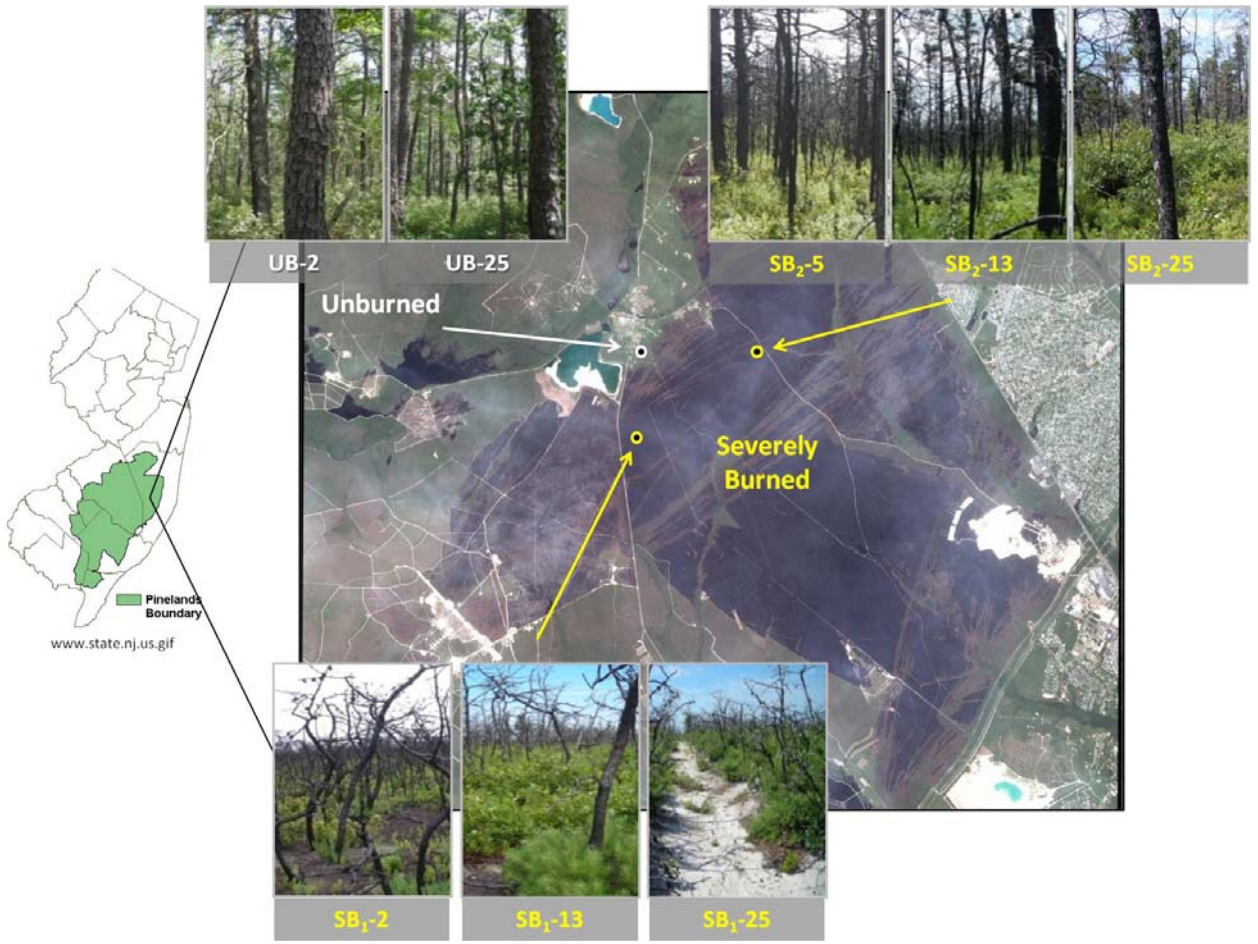

Figure 1. Outline of the New Jersey Pinelands in southern NJ; Aerial image of wildfire that occurred in May, 2007; Aerial image credit: Nicholas Skowronski.

To monitor how the biological community changed over the course of about two years, soil samples were collected 2, 5, 13, 17, and 25 months after the fire from one severely burned site (SB_1) and 5, 13, 17, and 25 months post-fire from the second severely burned site (SB_2). Soil samples from the unburned site (UB) were collected 2 and 25 months after the fire. The samples were collected during early-summer and mid-fall. At each sampling time, nine soil samples were collected per site to a depth of 15 cm, and the organic and mineral layers were separated. All soil samples were well mixed and analyzed by the Soil Testing Lab at Rutgers University, New Brunswick, NJ for pH, as well as concentrations of phosphorus, potassium, magnesium, calcium, copper, manganese, zinc, boron, and iron (measured as soil extractable cations in ammonium-acetate extract solutions) using standard procedures (Sims and Eckert, 2009; Wolf and Beegle, 2009). Additionally, the Soil Testing Lab measured the Total Kjeldahl Nitrogen (TKN), ammonium-N (NH_4^+-N), and nitrate-N (NO_3^--N) only on the organic layer samples (Griffin *et al.*, 2009). For these analyses, only three samples per site were measured. Organic matter analysis was conducted on the soil organic layer with the loss on ignition method and on the mineral layer with the dichromate-oxidation (Walkley-Black) method by the Soil Testing Lab (Rutgers University, New Brunswick, NJ) (Schulte and Hoskins, 2009). Nine samples per site were analyzed. The samples were then stored at -20°C until molecular analyses were conducted.

2.3 DNA extraction and PCR amplification

Total genomic DNA was extracted with the PowerSoil™ DNA Kit (MoBio, Carlsbad, CA) according to the manufacturer's instructions in addition to eluting the genomic DNA with 10mM Tris buffer (pH of 8). DNA was analyzed by electrophoresis

on a 1% agarose gel. The gel was stained with ethidium bromide and visualized under UV light. DNA from three of nine samples per site was randomly combined prior to polymerase chain reaction (PCR); in total, three observations per site were obtained. Primers 27 forward and 519 reverse were used to target the conserved 16S rRNA region of bacteria (Lane, 1991). To yield 50 µl reaction mixtures, 2 µl of DNA template, 1 µl of each 20 µM primer, 1 µl of 20 µg/µl Bovine Serum Albumen (Roche, Mannhein, Germany), and 45 µl Supermix (Invitrogen, Carlsbad, CA) were added to each reaction. The PCR cycling parameters for the bacterial 16S rRNA genes included denaturation at 95°C for 5 min., followed by 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min. 30 s, followed by 10 min. extension at 72°C. The amplified products were separated on a 1% agarose gel to determine the concentrations based on the lambda HindIII standard (Invitrogen, Carlsbad, CA). The concentrations of the amplified products were calculated with the Kodak Gel Logic 100 gel imaging system (Eastman Kodak Company, Rochester, NY) software and the amplified products were diluted to yield the same concentration per sample. In each experiment, positive and negative controls were used, along with sequence analysis of the amplicons, to limit the instance of the primers targeting other genes.

2.4 DGGE

The PCR products from the bacterial primers were further analyzed through denaturing gradient gel electrophoresis (DGGE). Particular bands of interest were excised for further phylogenetic analysis (Muyzer *et al.*, 1993). The same concentration of PCR products were run on various DGGE gels using the DCode universal mutation system (Bio-Rad, Hercules, CA). The amplicon concentrations on different gels ranged

from 350 to 1,200 ng of PCR product per DGGE gel. The various denaturing gradients ranged from 55-75%; the 100% denaturant contained 40% formamide and 7 mM urea. Electrophoresis was performed at 60°C at 55 volts for 17.5 hours in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). The DGGE gels were stained with SYBR green I (Sigma-Aldrich, St. Louis, MO) and photographed under UV transillumination using the Kodak Gel Logic 100 gel imaging system. Excised bands of interest were eluted in approximately 15 µl of autoclaved QDI water for 24 hours and reamplified with the same bacterial primer sets and purified with the ExoSAP-IT® Kit (Affymetrix, Santa Clara, CA). The purified products were sent to GeneWiz, Inc. (South Plainfield, NJ) for sequence analysis. Sequences were analyzed with DNASTAR Lasergene™ (Madison, WI) software and compared to sequences found in GenBank by using the BlastN algorithm (Altschul *et al.*, 1990).

2.5 Statistical analyses

To analyze the DGGE gels conservatively, each set of statistical analyses were limited to one gel at a time due to the variability between DGGE gels. Therefore, multiple DGGE gels were not compared, but rather each set of samples were run on individual gels and subsequently analyzed. The banding patterns of the DGGE gel were converted to a binary matrix as presence and absence of bands (van Hannen *et al.*, 1998). Principal component analysis (PCA) was conducted to condense the information contained in the large number of original variables (banding patterns) into a smaller set of new composite dimensions (principle component [PC] scores) with SAS® (Cary, NC) software (McGarigal *et al.*, 2000). The PC scores were graphed to visualize the data and determine the degree of similarity between samples (Rudi *et al.*, 2007; McGarigal *et al.*,

2000). A limitation to some ordination techniques, such as PCA, is that the PC scores may be distributed in the shape of a horse-shoe or arch. The horse-shoe shape may be due to the fact that the species response to the physical gradient is non-linear (most ordination techniques assume a linear gradient) (Podani and Miklos, 2002). Some groups argue that the horse-shoe is an artifact (Austin, 1985), whereas others argue that it is a real result of the variation in the data set (Wartenberg *et al.*, 1987). By detrending, the data can be manipulated to flatten the horse-shoe shape; however, this technique may omit real trends in the data. Many groups use PCA to elucidate data from molecular microbial analyses (Krumins *et al.*, 2009; Girvan *et al.*, 2003; Boon *et al.*, 2002).

To confirm that the PCA results were not due to artifacts, cluster analysis was performed on the same data set to determine if the trends visualized from PCA could be supported by another statistical technique. Ordination techniques share the same goal of cluster analysis in that they both represent how similar observations lie near each other and dissimilar observations fall far apart in an ordination diagram (Ramette, 2007). Thus, minimum variance clustering was conducted on the DGGE banding pattern data because the data were neat, compact clusters. In addition, proc cluster was used because the data were not continuous measures. Multivariate analysis of variance (MANOVA) was employed on the PC scores to determine if samples were significantly different (McGarigal *et al.*, 2000).

Canonical correlation analysis (CANOCO) was conducted on two sets of groups (the chemical and biological parameters measures) to maximize the correlation between the variables in those groups (Cooper *et al.*, 2006; McGarigal *et al.*, 2000). CANOCO was used to determine which physical variables correlated with the bacterial communities

in the unburned or severely burned soils. The PC1 and PC2 values from PCA were used to represent the bacterial communities while the physical variables included pH, gravimetric water content, and organic matter content, in addition to the concentrations of phosphorus, potassium, magnesium, calcium, copper, boron, and iron (in micrograms per gram). To obtain the same number of observations for the physical data as the biological data, the concentrations from three samples (the same composite as the DNA samples) were averaged; three observations per site were obtained.

3. Results

3.1 Effects of fire on soil physical and chemical properties

The unburned samples had an average organic layer depth of 6 cm and a mineral layer depth of 9 cm. A 4 cm organic layer and 11 cm organic layer was observed in the burned samples collected from the first severely burned site. The second severely burned site included samples with an organic layer depth of 5 cm and a mineral layer depth of 10 cm. The mineral layer soil from the three sites was approximately 88 ± 4.67% sand, 6.67 ± 4.31% silt, and 5.08 ± 1.51% clay (n=12), which is between the sand and loamy sand soil textures.

The pH of the soil organic and mineral layers in the severely burned samples collected two and 25 months after the fire was significantly more alkaline ($F_{7,64}$=15.68; P<0.0001; $F_{7,64}$=11.38, P<0.0001, respectively) than the unburned control samples (Tables 1 and 2). While some of the soil organic layer calcium and magnesium concentrations differed significantly from site to site, there was no clear trend. A similar pattern was observed for the soil mineral layer calcium, potassium, and iron

Table 1. Chemical variables associated with the organic layer samples. n=9, ± standard deviation. Letter indicates significant difference; samples with the same letters in the same column were not significantly different.

Site	pH	Ca (µg g^{-1} soil)	Mg (µg g^{-1} soil)	K (µg g^{-1} soil)	P (µg g^{-1} soil)	Fe (µg g^{-1} soil)
UB-2	3.37 ± 0.08^{cd}	369.44 ± 68.32^{b}	83.33 ± 19.85^{ab}	227.67 ± 26.19^{a}	24.56 ± 5.22^{a}	75.11 ± 18.99^{a}
UB-25	3.35 ± 0.15^{d}	425.11 ± 65.60^{ab}	63.44 ± 6.82^{ab}	150.78 ± 28.55^{b}	9.22 ± 2.22^{c}	60.33 ± 17.87^{a}
SB_1-2	3.81 ± 0.24^{ab}	353.67 ± 111.22^{b}	51.22 ± 27.20^{b}	129.44 ± 36.20^{b}	22.22 ± 11.95^{a}	83.73 ± 20.59^{a}
SB_2-5	3.69 ± 0.17^{bc}	382.33 ± 74.89^{ab}	95.78 ± 41.03^{ab}	158.33 ± 37.93^{b}	20.56 ± 7.26^{ab}	65.39 ± 23.48^{a}
SB_1-13	3.53 ± 0.28^{bcd}	436.78 ± 170.15^{ab}	93.89 ± 30.34^{ab}	58.33 ± 11.43^{c}	6.67 ± 3.39^{c}	74.22 ± 30.28^{a}
SB_2-13	3.57 ± 0.17^{bcd}	455.00 ± 74.89^{ab}	95.78 ± 42.27a	158.33 ± 13.32^{c}	12.44 ± 7.26^{bc}	95.78 ± 28.44^{a}
SB_1-25	4.07 ± 0.30^{a}	424.89 ± 101.04^{ab}	50.44 ± 27.02^{b}	42.00 ± 9.03^{c}	5.22 ± 4.02^{c}	70.67 ± 50.52^{a}
SB_2-25	4.07 ± 0.23^{a}	560.78 ± 141.12^{a}	58.44 ± 28.27^{ab}	65.00 ± 10.82^{c}	8.11 ± 1.69^{c}	58.67 ± 10.72^{a}
	$F_{7,64}$=15.68 P<0.0001	$F_{7,64}$=2.60 P=0.0199	$F_{7,64}$=4.25 P=0.0007	$F_{7,64}$=63.41 P<0.0001	$F_{7,64}$=15.13 P<0.0001	$F_{7,64}$=1.82 P=0.0989

Site	Cu (µg g^{-1} soil)	Mn (µg g^{-1} soil)	Zn (µg g^{-1} soil)	B (µg g^{-1} soil)	GWC (%)
UB-2	0.77 ± 0.62^{a}	4.54 ± 1.38^{ab}	5.83 ± 1.54^{a}	2.62 ± 0.15^{d}	62.21 ± 25.50^{a}
UB-25	0.97 ± 1.19^{a}	4.50 ± 2.00^{ab}	5.21 ± 1.50^{ab}	3.00 ± 0.18^{bcd}	45.95 ± 12.94^{a}
SB_1-2	0.83 ± 0.31^{a}	4.56 ± 3.47^{ab}	4.40 ± 1.64^{abc}	3.20 ± 0.30^{bc}	14.21 ±7.06^{b}
SB_2-5	0.61 ± 0.24^{a}	4.37 ± 1.87^{b}	4.94 ± 1.27^{ab}	2.93 ± 0.40^{cd}	19.85 ± 12.01^{b}
SB_1-13	0.63 ± 0.23^{a}	5.30 ± 2.40^{ab}	3.36 ± 0.83^{bc}	1.43 ± 0.17^{e}	5.54 ± 2.54^{b}
SB_2-13	0.81 ± 0.15^{a}	6.08 ± 3.10^{ab}	2.82 ± 0.77^{c}	1.41 ± 0.34^{e}	8.80 ± 6.05^{b}
SB_1-25	0.21 ± 0.11^{a}	5.61 ± 1.83^{ab}	3.01 ± 0.65^{c}	3.72 ± 0.32^{a}	15.68 ± 8.94^{b}
SB_2-25	0.22 ± 0.13^{a}	8.28 ± 3.05^{a}	3.56 ± 1.38^{bc}	3.39 ± 0.23^{ab}	7.84 ± 4.28^{b}
	$F_{7,64}$=2.74 P=0.0148	$F_{7,64}$=2.46 P=0.0266	$F_{7,64}$=7.13 P<0.0001	$F_{7,64}$=87.97 P<0.0001	$F_{7,64}$=26.22 P<0.0001

Table 2. Chemical variables of the mineral layer soil samples. n=9 ± standard deviation. Letter indicates significant difference. Samples with the same letters in the same column were not significantly different.

Site	pH	Ca (µg g^{-1} soil)	Mg (µg g^{-1} soil)	K (µg g^{-1} soil)	P (µg g^{-1} soil)	Fe (µg g^{-1} soil)
UB-2	3.44 ± 0.19^{d}	143.33 ± 30.19^{c}	45.44 ± 10.41^{ab}	49.56 ± 18.93^{b}	21.56 ± 2.51^{a}	135.34 ± 88.86^{a}
UB-25	3.58 ± 0.15^{cd}	219.78 ± 35.19^{a}	20.44 ± 3.91^{c}	74.67 ± 26.52^{a}	11.56 ± 4.53^{b}	101.56 ± 25.18^{ab}
SB_1-2	3.81 ± 0.30^{bc}	150.78 ± 57.73^{bc}	36.00 ± 12.12^{b}	35.56 ± 13.87^{bc}	22.00 ±3.87^{a}	52.99 ± 20.34^{b}
SB_2-5	3.72 ± 0.22^{bcd}	139.56 ± 23.09^{c}	42.11 ± 6.97^{ab}	27.22 ± 6.00^{c}	2.67 ± 1.80^{d}	74.41 ± 21.04^{ab}
SB_1-13	3.61 ± 0.16^{cd}	181.78 ± 34.55^{abc}	42.22 ± 5.21^{ab}	28.78 ± 4.76^{bc}	6.00 ± 3.54^{cd}	73.44 ± 44.06^{ab}
SB_2-13	3.63 ± 0.14^{cd}	210.22 ± 72.57^{ab}	52.78 ± 20.39^{a}	38.44 ± 10.47^{bc}	9.44 ± 4.69^{bc}	69.33 ± 31.15^{b}
SB_1-25	4.13 ± 0.21^{a}	153.00 ± 19.62^{bc}	15.22 ± 3.63^{c}	26.11 ± 4.76^{c}	4.67 ± 0.71^{cd}	47.56 ± 26.76^{b}
SB_2-25	3.99 ± 0.20^{ab}	210.56 ± 45.85^{ab}	16.00 ± 5.59^{c}	39.78 ± 10.24^{bc}	6.22 ± 1.86^{cd}	74.89 ± 15.54^{ab}
	$F_{7,64}$=11.38 P<0.0001	$F_{7,64}$=5.41 P<0.0001	$F_{7,64}$=18.88 P<0.0001	$F_{7,64}$=11.92 P<0.0001	$F_{7,64}$=48.10 P<0.0001	$F_{7,64}$=4.28 P=0.0006

Site	Cu (µg g^{-1} soil)	Mn (µg g^{-1} soil)	Zn (µg g^{-1} soil)	B (µg g^{-1} soil)	GWC (%)
UB-2	0.74 ± 0.12^{ab}	0.94 ± 0.59^{a}	1.18 ± 0.33^{b}	1.93 ± 0.32^{c}	10.33 ± 4.04^{a}
UB-25	0.40 ± 0.23^{cd}	1.21 ± 0.59^{a}	2.20 ± 0.86^{a}	3.27 ± 0.93^{b}	6.02 ± 1.61^{bc}
SB_1-2	0.62 ± 0.23^{bc}	1.27 ± 1.15^{a}	1.13 ± 0.49^{b}	1.87 ± 0.89^{c}	6.26 ± 2.16^{b}
SB_2-5	1.04 ± 0.28^{a}	1.54 ± 0.54^{a}	2.21 ± 0.46^{a}	6.83 ± 1.35^{a}	2.43 ± 1.43^{c}
SB_1-13	0.38 ± 0.17^{cd}	0.74 ± 0.51^{a}	1.14 ± 0.46^{b}	1.78 ± 0.43^{c}	3.47 ± 1.11^{bc}
SB_2-13	0.66 ± 0.22^{bc}	1.34 ± 1.24^{a}	1.53 ± 0.62^{ab}	1.71 ± 0.34^{c}	4.26 ± 1.76^{bc}
SB_1-25	0.16 ± 0.11^{d}	1.12 ± 0.50^{a}	1.42 ± 0.44^{ab}	4.00 ± 0.21^{b}	5.77 ± 4.35^{bc}
SB_2-25	0.21 ± 0.13^{d}	1.26 ± 0.84^{a}	1.50 ± 0.63^{ab}	3.44 ± 0.25^{b}	2.30 ± 0.51^{c}
	$F_{7,64}$=20.39 P<0.0001	$F_{7,64}$=0.86 P=0.5461	$F_{7,64}$=5.60 P<0.0001	$F_{7,64}$=67.16 P<0.0001	$F_{7,64}$=10.13 P<0.0001

concentrations. The soil organic layer potassium concentrations of the samples collected approximately a year and two years post-fire were significantly different ($F_{7,64}$=63.41, P<0.0001) from the unburned control samples, in addition to the severely burned samples collected two and five months after the fire (Table 1). The organic layer phosphorus data indicated that the severely burned samples collected 25 months after the fire were statistically different ($F_{7,64}$=15.13, P<0.0001) from the unburned control samples (Table 1). The mineral layer phosphorus concentrations were significantly reduced ($F_{7,64}$=48.10, P<0.0001) in the severely burned samples collected over two years after the fire when compared to the unburned control samples (Table 2). In addition, the mineral layer magnesium samples exhibited statistically ($F_{7,64}$=18.88, P<0.0001) decreased magnesium concentrations in the samples collected over two years post-fire in comparison to the unburned samples (Table 2).

Total Kjeldahl Nitrogen (TKN) was measured to determine the sum of the organic nitrogen, ammonia, and ammonium in the organic layer of the samples. Two years following the fire, the TKN was significantly ($F_{7,16}$=10.48, P<0.0001) reduced in the severely burned samples (Table 3). The NO_3^--N concentrations were not affected by the fire treatment, nor were they affected by time (Table 3). While NH_4^+-N was significantly ($F_{7,16}$=3.84, P=0.0122) different among some samples, no clear trend was established between fire treatment and NH_4^+-N concentrations in the organic layer samples (Table 3).

Additionally, soil organic matter was measured in both the organic and mineral layer soil samples to reveal carbon transformations as a result of the wildfire. The percent of organic carbon in the organic layer of the unburned samples was significantly ($F_{7,64}$=36.32, P<0.0001) elevated when compared to the severely burned

Table 3. Soil extractable nitrogen levels in the organic layer soil samples. n=3 ± standard deviation. Letter indicates significant difference. Samples with the same letters in the same column were not significantly different. bdl indicates below detection limit.

Site	TKN (%)	NH_4^+-N (µg g^{-1} soil)	NO_3^--N (µg g^{-1} soil)
UB-2	0.63 ± 0.11^{a}	5.00 ± 0.00^{ab}	1.00 ± 0.00^{a}
UB-25	0.48 ± 0.15^{ab}	3.33 ± 1.15^{b}	1.67 ± 1.15^{a}
SB_1-2	0.17 ± 0.07^{bc}	11.33 ± 6.11^{a}	3.67 ± 2.08^{a}
SB_2-5	0.25 ± 0.17^{bc}	4.67 ± 2.08^{ab}	1.00 ± 0^{a}
SB_1-13	0.17 ± 0.05^{bc}	3.67 ± 1.53^{b}	2.00 ± 1.00^{a}
SB_2-13	0.17 ± 0.06^{bc}	4.33 ± 1.53^{ab}	1.33 ± 0.58^{a}
SB_1-25	0.07 ± 0.03^{c}	1.67 ± 0.58^{b}	bdl
SB_2-25	0.15 ± 0.09^{c}	4.33 ± 1.53^{ab}	3.33 ± 2.83^{a}
	$F_{7,16}$=10.48	$F_{7,16}$=3.84	$F_{7,16}$=2.95
	P<0.0001	P=0.0122	P=0.0347

samples (Table 4). In contrast, the mineral layer soils did not exhibit as clear of a trend. Specifically, the severely burned samples collected 13 months after the fire had a significantly ($F_{7,64}$=5.59, P<0.0001) lower percentage of organic carbon when compared to the unburned samples (Table 4).

3.2 Variations in bacterial community composition on a monthly scale within the first year and a half

Using molecular techniques to determine how the severely burned samples were changing with time following the fire, the bacterial community profiles in the severely burned samples collected 2, 5, 13, and 17 months after the fire were compared. These included samples from the organic and mineral layers of both severely burned sites. The assessment of the emergence of a new set of dominant bacteria is important to understand how the bacteria respond to the changing physical and chemical properties of the soil.

The first comparison highlighted the differences in bacterial community structure in the organic layer samples in the first severely burned site 2, 5, 13, and 17 months following the fire (Figure 2a). Cluster analysis demonstrated the pattern of separation between treatments and the similarity between the molecular profiles in the soils collected over a year following the fire (Figure 2b). Additionally, in the PCA graph, each point on the graph represents the bacterial community composition of all nine soil samples at one sampling time (averages of the three composite samples). The structures of the bacterial communities represented in each set of samples were spatially distinct on the ordination graph; therefore, the bacterial community in the organic layer samples from SB_1 changed within the first year and a half following the fire. PC1 accounted for a

Table 4. Percentage of soil organic matter (loss on ignition and dichromate-oxidation method; Walkley-Black methods, respectively) in the organic and mineral layer soil samples. n=9 ± standard deviation. Letter indicates significant difference. Samples with the same letters in the same column were not significantly different.

	OM (%)	
Site	**Organic Layer**	**Mineral Layer**
UB-2	44.74 ± 5.15^{a}	3.53 ± 1.25^{ab}
UB-25	58.36 ± 12.40^{a}	4.07 ± 0.92^{a}
SB_1-2	10.01 ± 4.76^{b}	2.09 ± 1.02^{bc}
SB_2-5	23.76 ± 13.14^{b}	2.48 ± 0.71^{abc}
SB_1-13	12.20 ± 4.45^{b}	1.64 ± 0.50^{c}
SB_2-13	11.56 ± 6.92^{b}	1.70 ± 0.71^{c}
SB_1-25	10.99 ± 5.29^{b}	1.83 ± 2.08^{bc}
SB_2-25	18.08 ± 13.46^{b}	2.18 ± 0.73^{bc}
	$F_{7,64}$=36.32	$F_{7,64}$=5.59
	P<0.0001	P<0.0001

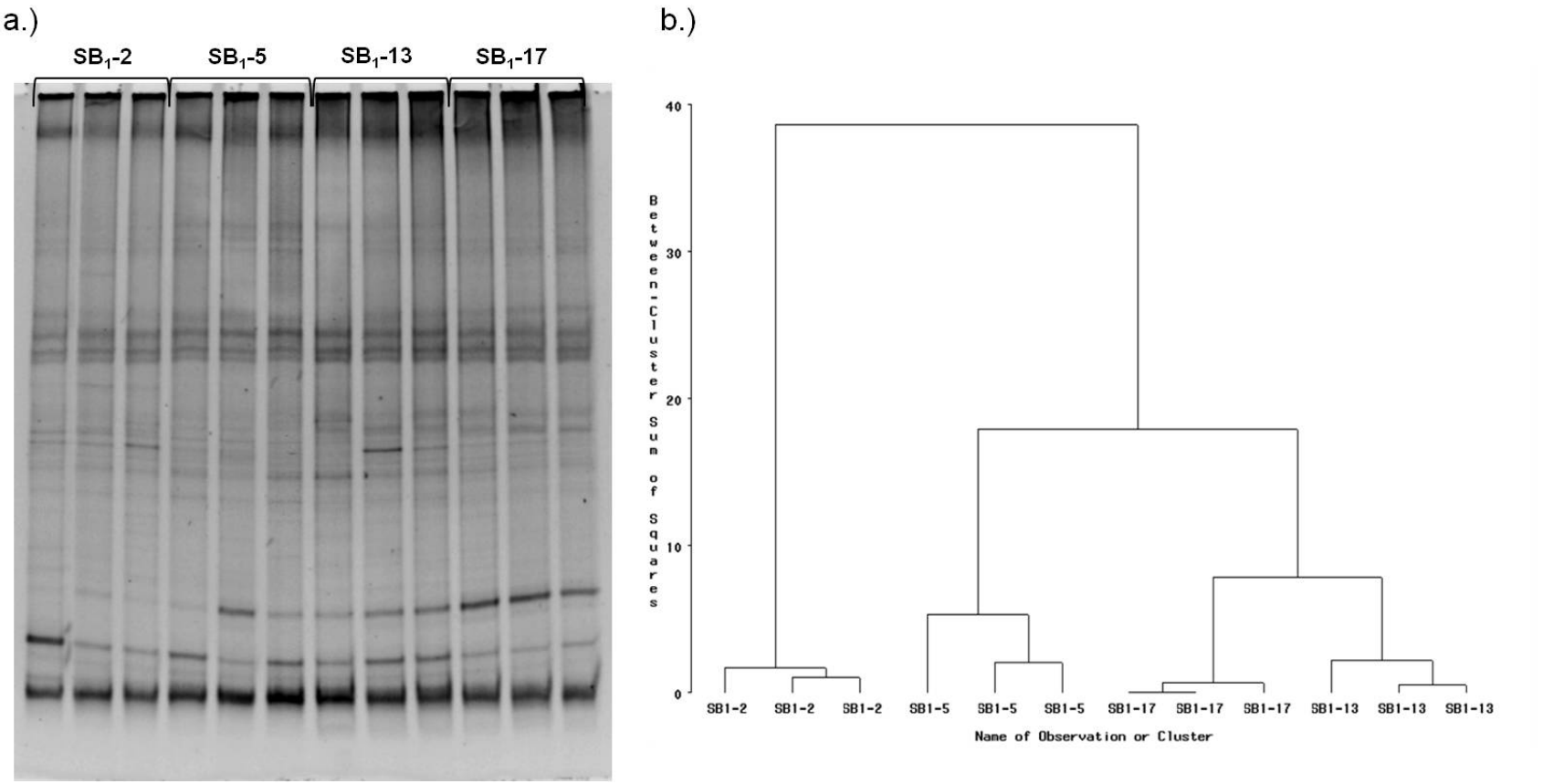

Figure 2. a. DGGE gel of the bacterial community profiles in the organic layer from the SB_1 site. 1,000 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Organic layer samples from SB_1 were collected 2, 5, 13, and 17 months following the wildfire. For each branch, *n*=3.

large (56.1%) portion of variation in the dataset (Figure 3). The MANOVA data revealed that the communities in the samples collected 2 and 5 months after the fire were significantly different in both PC1 and PC2 ($F_{3,8}$=73.70, P<0.0001; $F_{3,8}$=83.06, P<0.0001, respectively) (Table 5). However, the bacterial profiles in the soil samples collected approximately one and two years post-wildfire were not significantly different from one another in PC1 (Table 5). Other, perhaps seasonal factors may be contributing to the variations in community composition in PC2 and PC3.

Similar to the organic layer samples, the bacterial communities in the mineral layer samples from the first severely burned site changed with time following the wildfire. In the cluster analysis, while the bacterial profiles in the samples were separated by time point following the fire, there are two meaningful points worth noting (Figure 4). One is that the molecular profiles of the samples collected two months post-fire branched separately from the other samples (Figure 4b). The second is that the samples collected 5 and 13 months after the fire had the most similar molecular profiles (Figure 4b). PCA showed that the communities in the samples from the four time points grouped separately along PC1, which accounted for 40.9% of the variation in the analysis (Figure 5). MANOVA resolved that the bacterial community structure in the samples collected 2 and 5 months after the fire were significantly different ($F_{3,8}$=87.54, P<0.0001), in addition to the communities in the samples collected more than a year post-wildfire (Table 6). Similar to the organic layer samples, the trend in PC2 may also be attributed to seasonal or temporal factors that influenced the bacterial community structure.

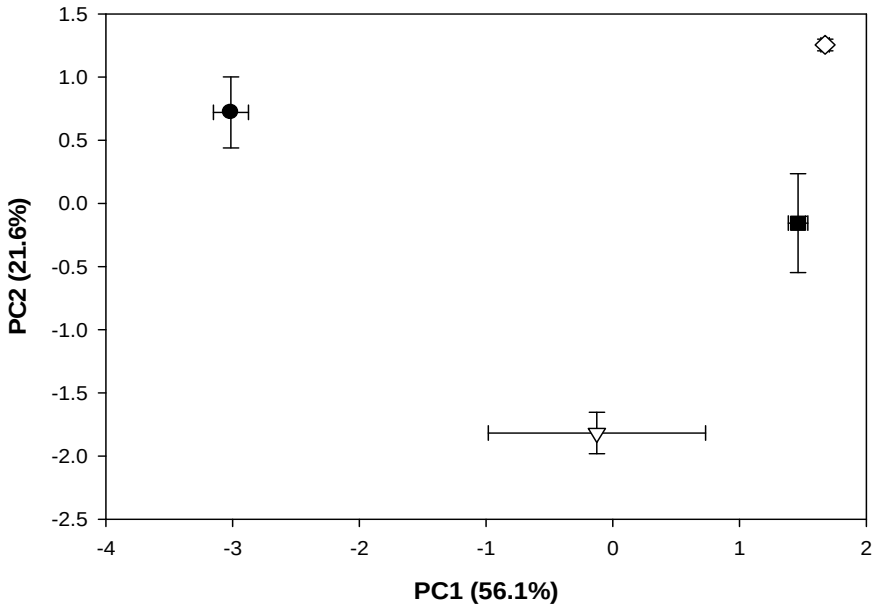

Figure 3. PCA of the DGGE banding patterns. Organic layer samples from the SB_1 site were collected 2 (closed circle), 5 (open triangle), 13 (closed square), and 17 (open diamond) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 5. MANOVA of the DGGE banding patterns. Organic layer samples from the SB_1 site were collected 2, 5, 13, and 17 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Time after Wildfire	PC1 (56.1%)	PC2 (21.6%)	PC3 (9.3%)
2 mo.	a	a	ab
5 mo.	b	b	b
13 mo.	c	c	a
17 mo.	c	a	b
	$F_{3,8}$=73.70	$F_{3,8}$=83.06	$F_{3,8}$=5.89
	P<0.0001	P<0.0001	P=0.0201

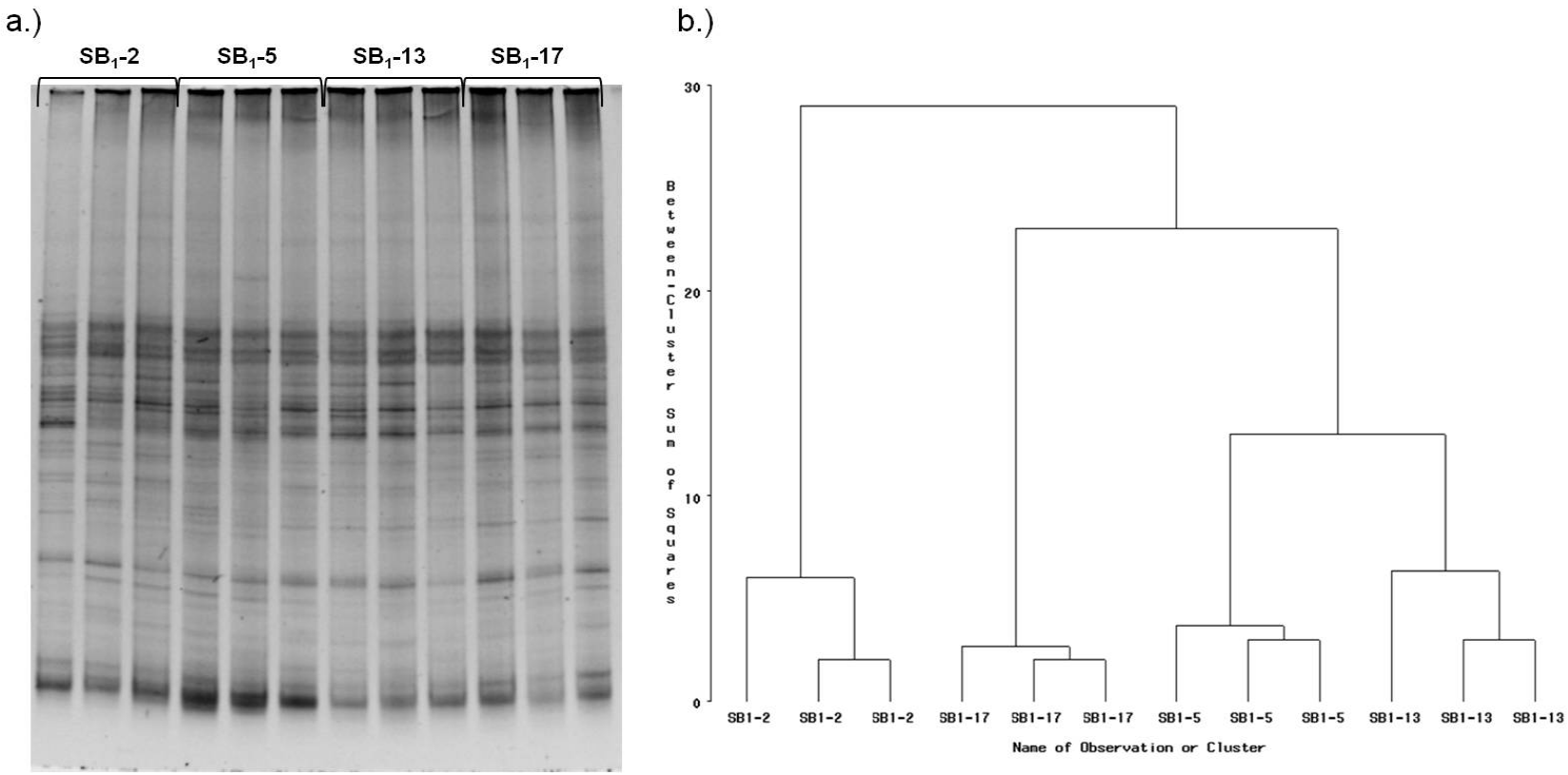

Figure 4. a. DGGE gel of the bacterial community profiles in the mineral layer from the SB_1 site. 1,000 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Mineral layer samples from SB_1 were collected 2, 5, 13, and 17 months following the wildfire. For each branch, *n*=3.

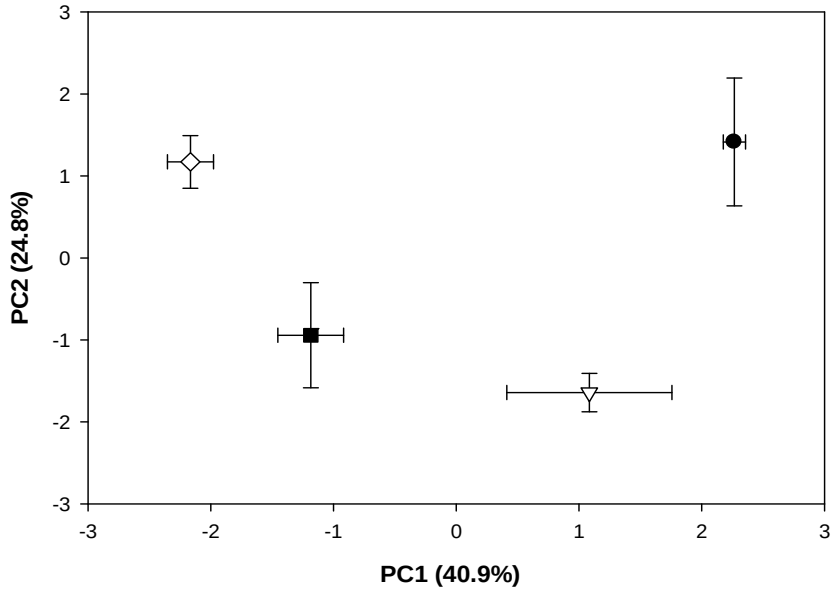

Figure 5. PCA of the DGGE banding patterns. Mineral layer samples from the SB_1 site were collected 2 (closed circle), 5 (open triangle), 13 (closed square), and 17 (open diamond) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 6. MANOVA of the DGGE banding patterns. Mineral layer samples from the SB_1 site were collected 2, 5, 13, and 17 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Time after Wildfire	PC1 (40.9%)	PC2 (24.8%)
2 mo.	a	a
5 mo.	b	b
13 mo.	c	c
17 mo.	c	a
	$F_{3,8}$=87.54	$F_{3,8}$=23.67
	P<0.0001	P=0.0002

Contributing to the established pattern from the first severely burned site, the bacterial communities in both the organic and mineral layer soils from the second severely burned site clustered according to sample times in the cluster analysis (Figures 6 and 8). The cluster analysis data demonstrated the pattern whereby the samples collected 13 and 17 months post-fire had more similar molecular profiles compared to the samples collected 2 months after the fire (Figures 6 and 8). Additionally, the results were corroborated by both PCA graphs (Figures 7 and 9). Specifically, the communities in the samples collected 5 months post-fire were distal to those in the samples collected 13 and 17 months after the fire in both the organic and mineral layer soils. MANOVA indicated that the bacterial profiles in the organic and mineral layer samples collected 5 months after the fire were significantly ($F_{2,6}$=851.75, P<0.0001; $F_{2,6}$=264.60, P<0.0001, respectively) different from the profiles in the samples collected 13 and 17 months after the fire in PC1 (Tables 7 and 8). In PC2, the bacterial communities in the samples were all statistically ($F_{2,6}$=91.78, P<0.0002=1; $F_{2,6}$=128.43, P<0.0001, respectively) different from one another in both the organic and mineral soil layers (Tables 7 and 8).

3.3 Bacterial OTUs associated with the burned soils

Sequence analysis from bands excised from the various DGGE gels resolved the related organisms present in particular soil samples. Members from genera *Acidobacterium* and *Mycobacterium* were detected in almost all of the samples in both the organic and mineral layer soils (Tables 9 and 10). Microbes related to these genera were expected due to the acidic pH of the forest soil. Bacteria related to members of the

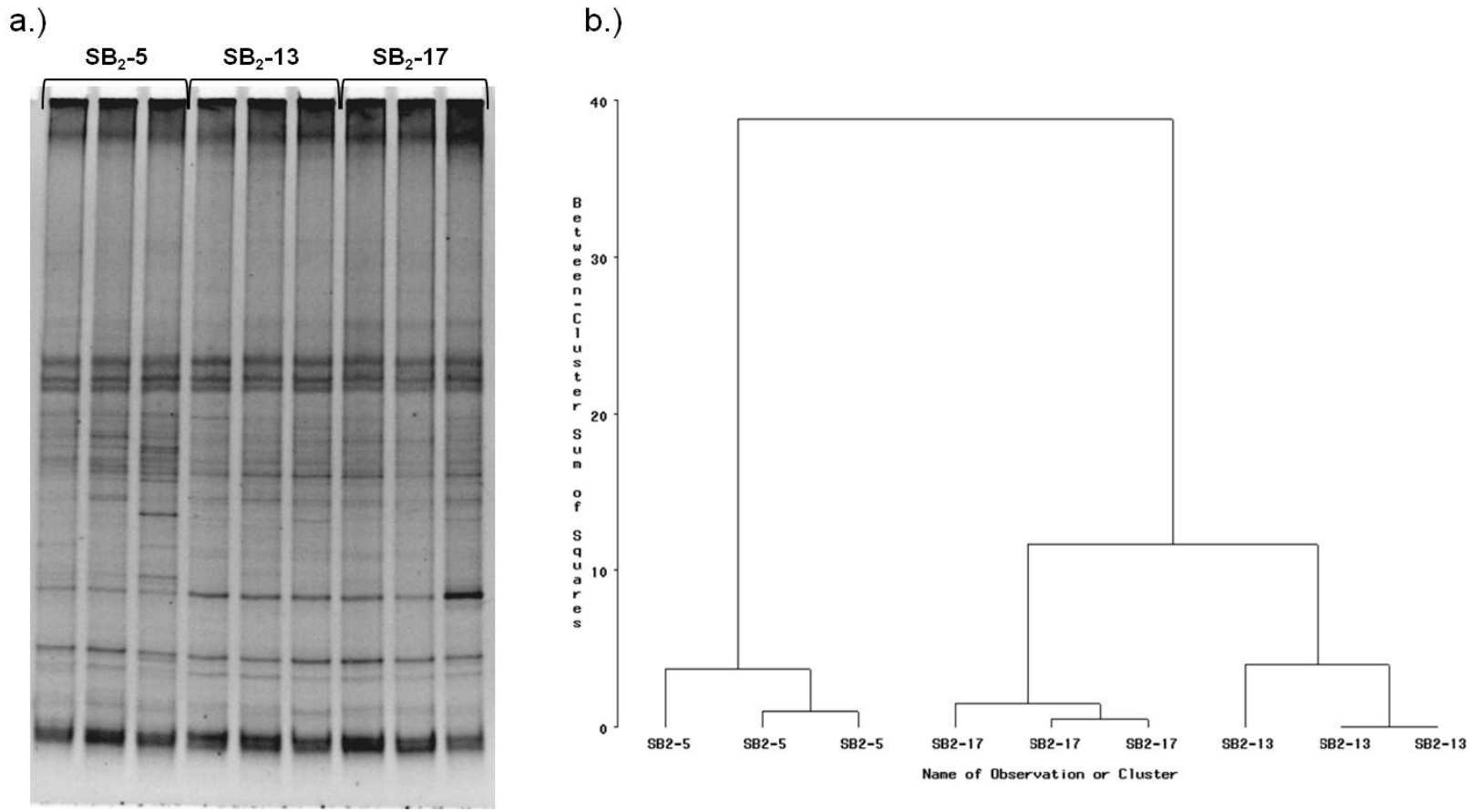

Figure 6. a. DGGE gel of the bacterial community profiles in the organic layer from the SB_2 site. 1,000 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Organic layer samples from SB_2 were collected 5, 13, and 17 months following the wildfire. For each branch, *n*=3.

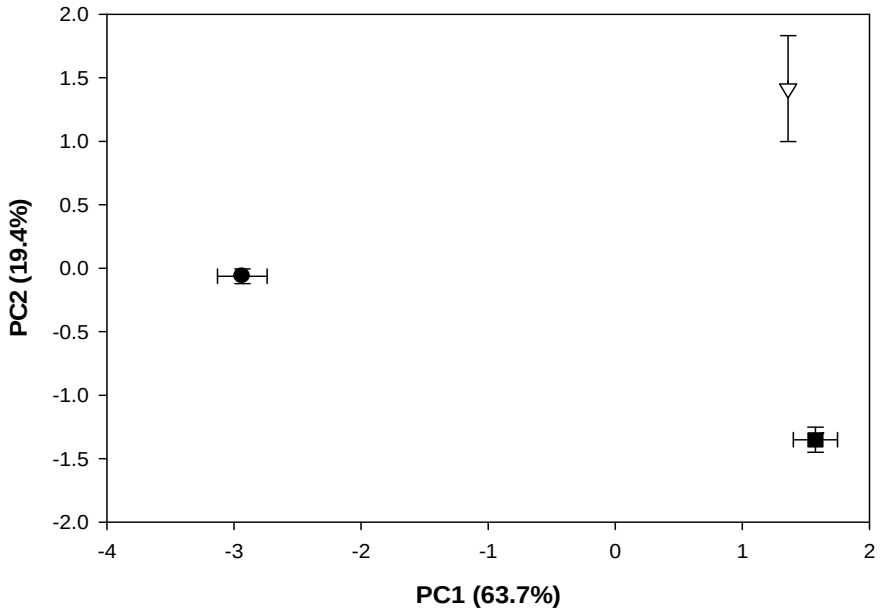

Figure 7. PCA of the DGGE banding patterns. Organic layer samples from the SB_2 site were collected 5 (closed circle), 13 (open triangle), and 17 (closed square) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 7. MANOVA of the DGGE banding patterns. Organic layer samples from the SB_2 site were collected 5, 13, and 17 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Time after Wildfire	PC1 (63.7%)	PC2 (19.4%)
5 mo.	a	a
13 mo.	b	b
17 mo.	b	c
	$F_{2,6}$=851.75	$F_{2,6}$=91.78
	P<0.0001	P<0.0001

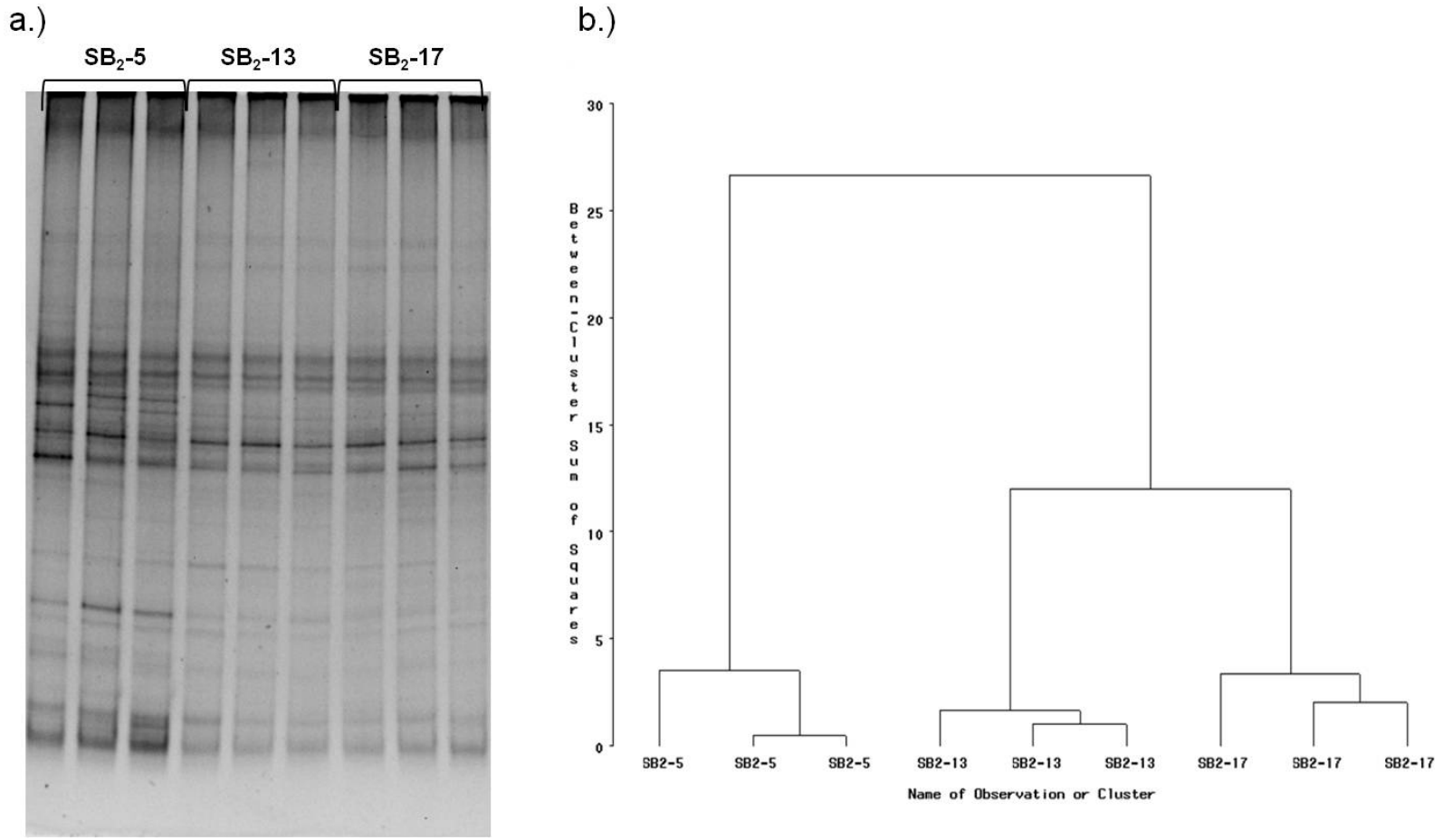

Figure 8. a. DGGE gel of the bacterial community profiles in the mineral layer from the SB_2 site. 1,000 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Mineral layer samples from SB_2 were collected 5, 13, and 17 months following the wildfire. For each branch, *n*=3.

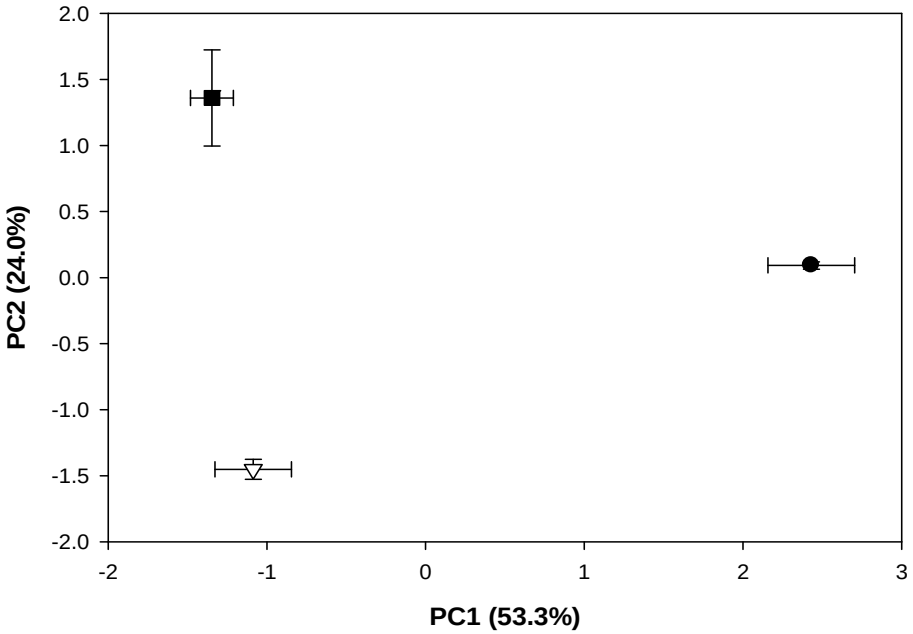

Figure 9. PCA of the DGGE banding patterns. Mineral layer samples from the SB_2 site were collected 5 (closed circle), 13 (open triangle), and 17 (closed square) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 8. MANOVA of the DGGE banding patterns. Mineral layer samples from the SB_2 site were collected 5, 13, and 17 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Time after Wildfire	PC1 (53.3%)	PC2 (24.0%)
5 mo.	a	a
13 mo.	b	b
17 mo.	b	c
	$F_{2,6}$=264.60	$F_{2,6}$=128.43
	P<0.0001	P<0.0001

Table 9. Sequence analysis of bacterial bands excised from the DGGE gel of the organic samples.

Closest Relative in GenBank (Identities) GenBank Accession Number	**SB-2**	**SB-5**	**SB-13**	**SB-17**
u. Cyanobacterium clone (366/395 [92%])* gb|EF662970.1	+			
u. *Clostridum* clone (224/231 [96%])* gb|FJ036998.1	+			
Edaphobacter sp. (427/470 [90%])* gb|DQ528760.1		+		
Acidobacteria sp. (246/271 [90%])* gb|FJ870383.1		+		
Acidobacteriaceae sp. (381/404 [94%])* gb|EF219138.1		+		
Edaphobacter sp. (236/252 [93%])* gb|DQ528761.1		+		
Edaphobacter sp. (351/362 [96%])* gb|FJ469913.1		+	+	
Acidobacterium sp. (433/453 [95%])* dbj|D26171.1|ACARRNA			+	+
Edaphobacter sp. (361/387 [93%])* gb|DQ528760.1			+	+
Acidobacterium sp. (349/381 [91%])* dbj|D26171.1|ACARRNA			+	+
Acidobacteria sp. (437/450 [97%])* gb|FJ870383.1			+	+
Acidobacteria sp. (437/450 [97%])* gb|FJ870383.1			+	+
Mycobacterium sp. (406/410 [99%])* gb|AY215317.1		+	+	+

Closest Relative in GenBank (Identities) GenBank Accession Number	**SB-2**	**SB-5**	**SB-13**	**SB-17**
Mycobacterium sp. (490/495 [98%])* gb|AY215317.1		+	+	+
Mycobacterium sp. (385/396 [97%])* gb|AY312276.1		+	+	+
Mycobacterium sp. (474/462 [96%])* gb|AY065649.1	+	+	+	+
Mycobacterium sp. (485/490 [98%])* gb|AY215317.1	+	+	+	+
u. Acidobacteriales clone (416/455 [91%])* gb|FJ870383.1	+	+	+	+
Acidobacteriaceae sp. (451/464 [97%])* dbj|AB245339.1	+	+	+	+
Acidobacteria sp. (431/441 [97%])* gb|FJ870383.1	+	+	+	+
Mycobacterium sp. (471/489 [96%])* gb|AY065649.1	+	+	+	+
Mycobacterium sp. (482/486 [99%])* gb|215317.1	+	+	+	+

u. indicated uncultured

* indicates that the forward and reverse primers were in agreement.

+ indicates presence of the sequence in a particular site, while an empty space indicates that the sequence was not detected in that site

Table 10. Sequence analysis of bacterial bands excised from the DGGE gel of the mineral samples.

Closest Relative in GenBank (Identities) GenBank Accession Number	**SB-2**	**SB-5**	**SB-13**	**SB-17**
u. Bacillaceae clone (236/258 [91%]) gb|AY827066.1	+	+		
Mycobacterium sp. (374/399 [93%])* gb|EUFJ418058.1	+	+		
Desulfotomaculum sp. (219/246 [89%])* emb|AJ294428.1		+		
Acidobacteriaceae sp. (239/261 [91%])* gb|EF219138.1		+		
Mycobacterium sp. (370/397 [93%])* gb|AY312276.1		+		
u. Acidobacteria clone (207/213 [97%])* gb|FJ037418.1	+	+	+	
u. Actinobacterium clone (167/179 [93%])* gb|EU038051.1	+	+	+	
u. Actinobacterium clone (251/269 [93%])* gb|EF221456.1		+	+	
Mycobacterium sp. (165/179 [92%])* gb|EU370526.1			+	+
Mycobacterium sp. (198/215 [92%])* gb|EU703147.1				+
Acidobacterium sp. (358/370 [96%])* gb|FJ870383.1		+		+
u. Acidbacteriales clone (230/253 [90%])* gb|AY174195.1	+			+
u. Firmicutes clone (324/336 [96%])* gb|EU044333.1	+		+	+

Closest Relative in GenBank (Identities) **GenBank Accession Number**	**SB-2**	**SB-5**	**SB-13**	**SB-17**
Acidobacteriaceae sp. (425/464 [91%])* gb|EF219138.1	+	+	+	+
Acidobacterium sp. (330/361 [91%])* dbj|D26171.1|ACARRNA	+	+	+	+
Acidobacterium sp. (305/324 [94%])* dnj|D26171.1|ACARRNA	+	+	+	+
Acidobacterium sp. (292/310 [94%])* dnj|D26171.1|ACARRNA	+	+	+	+
Mycobacterium sp. (447/465 [96%])* gb|AY065649.1	+	+	+	+
Mycobacterium sp. (415/422 [98%])* gb|AY215317.1	+	+	+	+

u. indicates uncultured
* indicates that the forward and reverse primers were in agreement
+ indicates presence of the sequence in a particular site, while an empty space indicates that the sequence was not detected in that site

phylum Cyanobacteria and the genus *Clostridium* were detected in the samples collected two months post-fire (Table 9). The fire disturbance resulted in the potential of proliferation of nitrogen fixing bacterial groups such as Cyanobacteria and *Clostridia*. Interesting OTUs detected in the samples collected 5 months after the fire include bacteria related to members of the genus *Edaphobacter*, from the phylum Acidobacteria. Acidobacteria have been found to thrive in acidic environments (Madigan *et al.*, 1997). In addition, members related to the Firmicutes phylum were detected in samples collected 5 months post-fire. These bacteria, which also include the genus *Clostridium*, are generally gram-positive endospore-formers that have a low G+C content (Madigan *et al.*, 1997). An OTU related to the genus *Desulfotomaculum* was detected in the 5 month severely burned mineral layer samples (Table 10). Members of this genus are sulfate-reducers that are also endospore-formers (Madigan *et al.*, 1997).

3.4 Annual bacterial community shifts as a result of wildfire

To clarify the long term changes in bacterial community structure, soil samples collected approximately on an annual basis were specifically compared. DGGE analysis of the organic layer samples revealed complex banding patterns of the bacterial communities in the disturbed and undisturbed sites (Figure 10a). Cluster analysis demonstrated five groups that formed two main clades (Figure 10b). In the evaluation of the bacterial communities in the organic layer samples from the UB and SB_1 sites, PCA showed that the communities in the SB_1 samples collected 2 months after the fire were spatially distant to those in the SB_1 samples collected over a year after the fire in two-dimensional space (Figure 11). MANOVA revealed that the bacterial communities in the

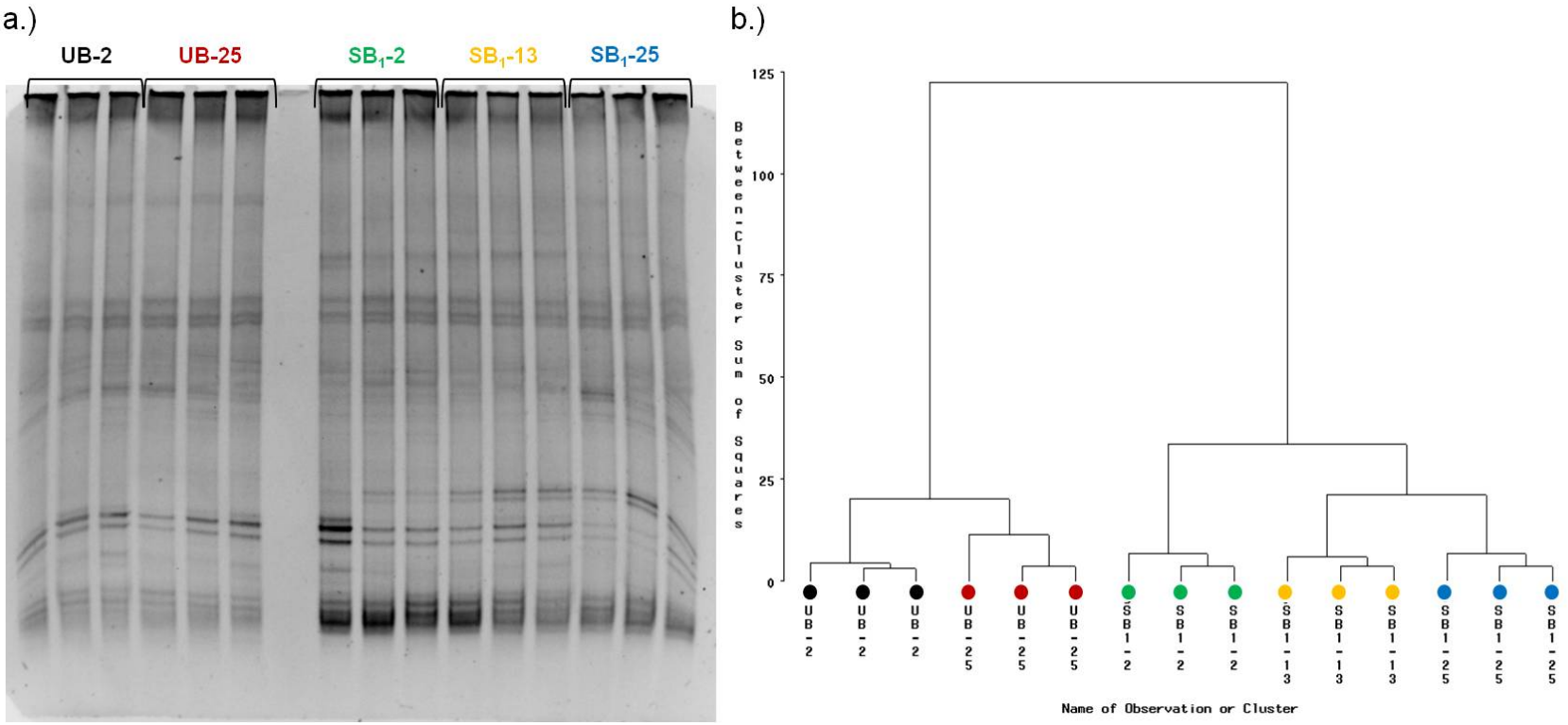

Figure 10. a. DGGE gel of the bacterial community profiles in the organic layer from the UB and SB_1 sites. 425 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months post-fire and from the SB_1 site 2, 13, and 25 months post-fire. For each branch, *n*=3.

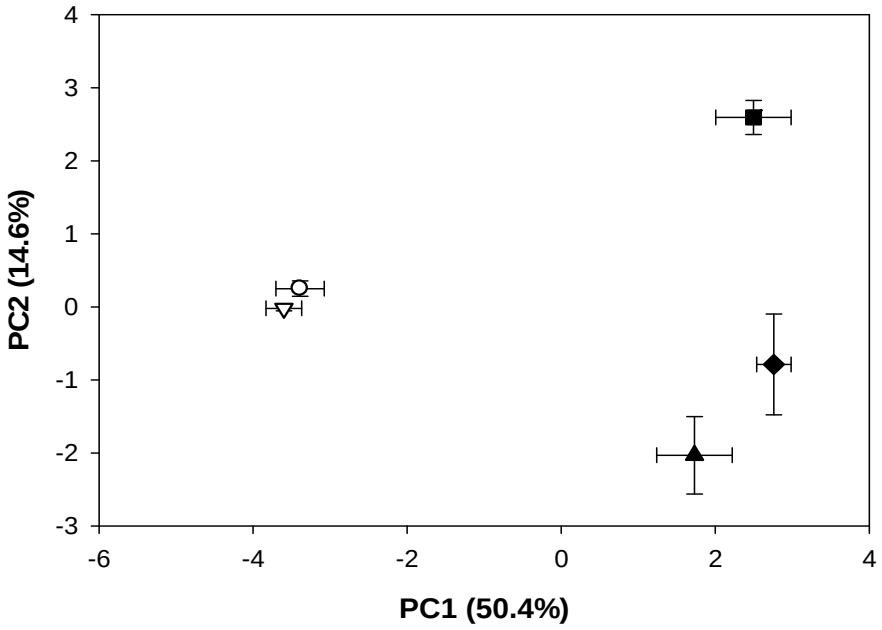

Figure 11. PCA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Organic layer samples from the SB_1 site were collected 2 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

UB samples collected 2 and 25 months after the fire were not significantly different (Table 11). In PC1, which accounted for the 50.4% of the variation in the system, the communities in the UB samples were statistically ($F_{4,10}$=227.16, P<0.0001) different from all of the communities in the SB_1 samples tested in this experiment. With regard to PC2, the bacterial community structure in the UB samples were similar to that of the SB_1 samples collected approximately a year after the fire.

Because the chemical variables did not serve as good indicators of the effects of the wildfire, CANOCO was employed to correlate the chemical and biological parameters tested in the soil samples. Values for manganese, copper, gravimetric water content, and organic matter were omitted from the analysis because they did not demonstrate a significant correlation with the bacterial community patterns in the organic layer soils. Additionally, the nitrogen values for some samples used in the biological analysis were not recorded. CANOCO revealed that pH did not correlate with the bacterial communities in the UB or SB_1 organic layer samples (Table 12). The concentrations of potassium, magnesium, calcium, and boron correlated strongly with the dominant bacteria in the unburned samples ($F_{9,20}$=4.15, P=0.0426), while the concentrations of phosphorus, zinc, and iron correlated with the bacteria associated with severely burned samples ($F_{9,20}$=4.15, P=0.0426) (Table 12).

Additionally, in the evaluation of the bacterial banding patterns in the mineral layer UB and SB_1 samples, cluster analysis reaffirmed that the bacterial communities in the UB samples clustered separately from that in the SB samples, and that the communities in the SB samples collected over a year after the fire grouped most closely (Figure 12). PCA showed that the bacterial community in the UB samples was

Table 11. MANOVA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months after the wildfire. Organic layer samples from the SB_1 site were collected 2, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (50.4%)	PC2 (14.6%)	PC3 (10.2%)
UB-2	a	a	a
UB-25	a	a	b
SB_1-2	bc	b	ac
SB_1-13	bc	a	bc
SB_1-25	c	c	a
	$F_{4,10}$=227.16 P<0.0001	$F_{4,10}$=52.43 P<0.0001	$F_{4,10}$=13.52 P=0.0005

Table 12. CANOCO on the biological and physical data from the organic layer samples collected 2 and 25 months after the fire from the UB site, and 2, 13, and 15 months after the fire from the SB_1 site. For the first canonical variable, $F_{9,20}$=4.15, P=0.0426. The first canonical function explained 92.1% of the variation in the analysis. V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC2, whereas the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC1 (see Appendix 1).

	PC1	**PC2**
V1	-0.8701	0.4929

	pH	**P**	**K**	**Mg**	**Ca**	**Zn**	**B**	**Fe**
W1	-0.0000	-0.4634	0.5867	0.9207	0.7036	-0.4104	0.9150	-0.9385

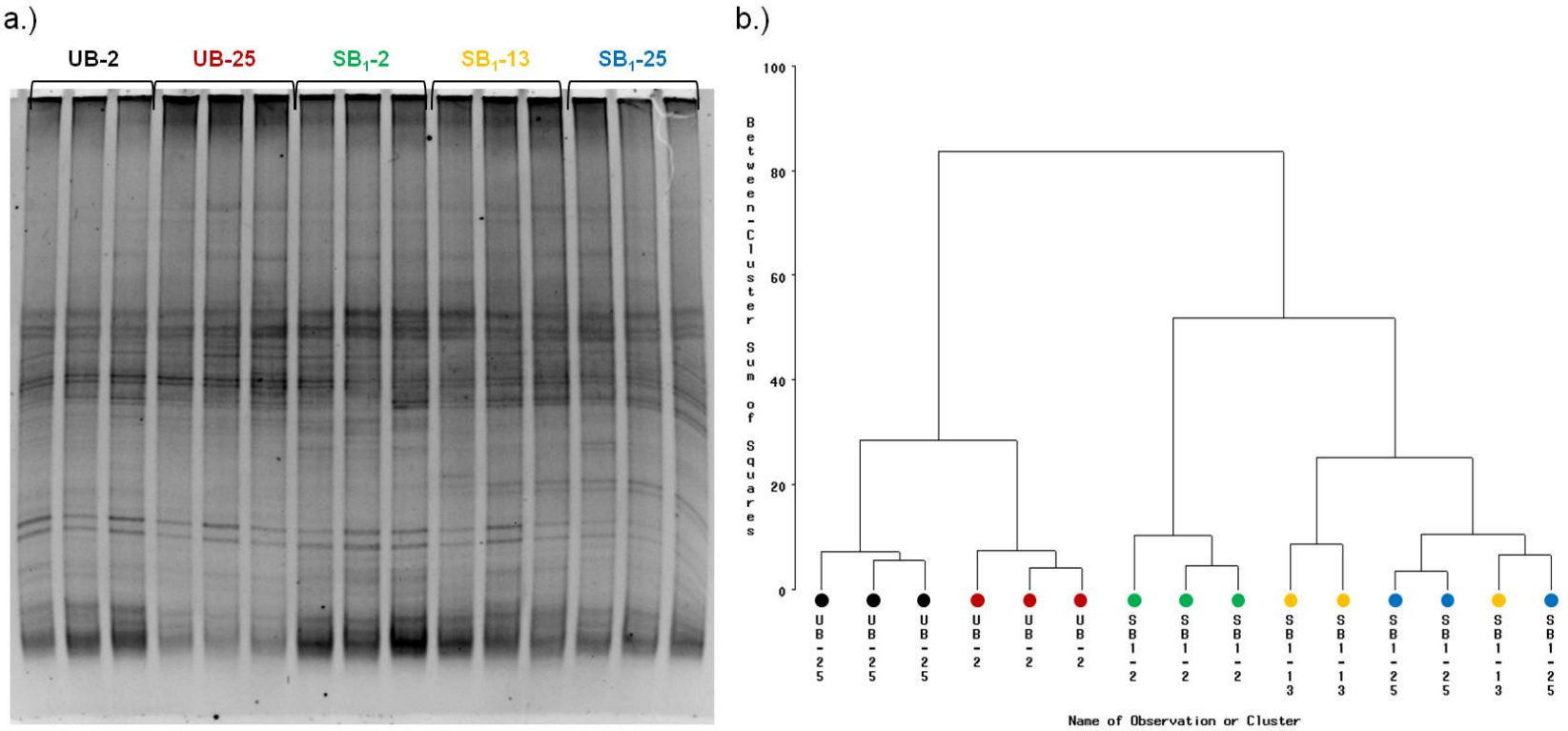

Figure 12. a. DGGE gel of the bacterial community profiles in the mineral layer from the UB and SB_1 sites. 950 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Mineral layer samples from the UB site were collected 2 and 25 months post-fire and from the SB_1 site 2, 13, and 25 months post-fire. For each branch, *n*=3.

segregated from the community within the SB_1 samples from all of the time points (Figure 13). While the DGGE profiles from the UB samples collected 2 and 25 months post-fire were spatially separate on the PCA graph, they were not significantly different in the first two PCs (Table 13). In PC1, the bacterial community in the severely burned samples were statistically ($F_{4,10}$=187.05, P<0.0001) different from that of the unburned samples (Table 13).

Again, CANOCO correlated the chemical and biological parameters tested in the soil samples. Values for organic matter were omitted from the analysis because they did not demonstrate a significant correlation with the bacterial community patterns in the mineral layer soils. Additionally, the nitrogen values for the mineral layer samples were not recorded. CANOCO indicated that the bacterial community in the SB_1 samples associated with PC1, whereas the bacterial community in the UB samples associated with PC2 (Table 14). Interestingly, the bacterial community in the SB_1 samples correlated strongly ($F_{10,22}$=5.89, P=0.0484) with elevated pH values (Table 14).

Sequence analysis of the SB_1 samples from both the soil organic and mineral layers revealed that only members related to the genus *Mycobacterium* and the phylum Acidobacteria were detected in both the unburned and severely burned samples regardless of sampling location or time point (Table 15).

The bacterial profiles in the organic layer soil collected from the UB and SB_2 sites were evaluated. All of the bacterial communities in the samples clustered according to time point both in the cluster analysis (Figure 14) and PCA (Figure 15). Similar to the UB and SB_1 organic layer results, these data show the bacterial structure in the unburned

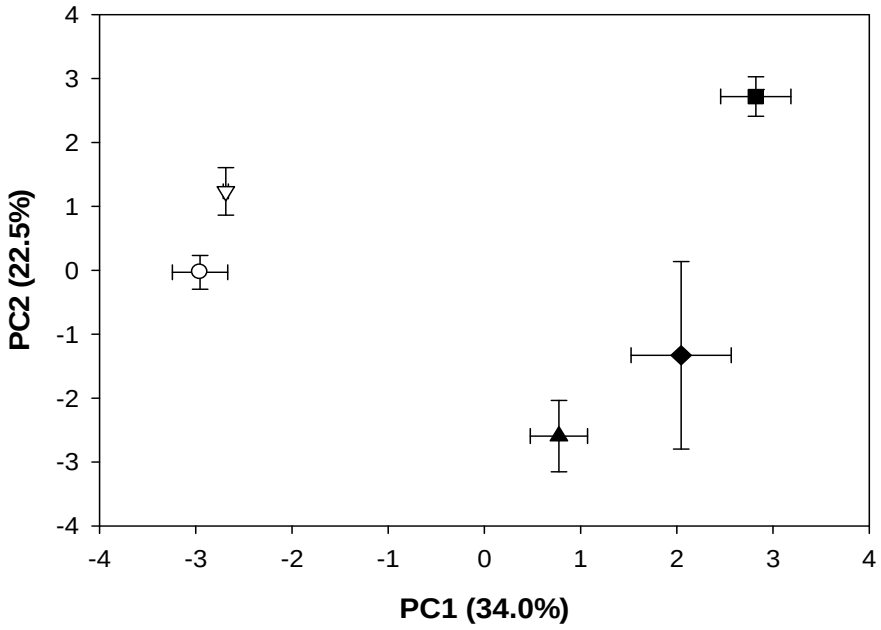

Figure 13. PCA of the DGGE banding patterns. Mineral layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Mineral layer samples from the SB_1 site were collected 2 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 13. MANOVA of the DGGE banding patterns. Mineral layer samples from the UB site were collected 2 and 25 months after the wildfire. Mineral layer samples from the SB_1 site were collected 2, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (34.0%)	PC2 (22.5%)	PC3 (11.2%)
UB-2	a	ab	a
UB-25	a	ac	b
SB_1-2	b	c	c
SB_1-13	b	bd	d
SB_1-25	c	d	c
	$F_{4,10}$=187.05 P<0.0001	$F_{4,10}$=23.58 P<0.0001	$F_{4,10}$=74.77 P<0.0001

Table 14. CANOCO on the biological and physical data from the mineral layer samples collected 2 and 25 months after the fire from the UB site, and 2, 13, and 15 months after the fire from the SB_1 site. For the first canonical variable, $F_{10,22}$=5.89, P=0.0484. The first canonical function explained 78.9% of the variation in the analysis. V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC2, whereas the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC1 (see Appendix 2).

	PC1	**PC2**
V1	0.9893	-0.1460

	pH	**P**	**K**	**Mg**	**Ca**	**Cu**	**Mn**	**Zn**	**Fe**	**B**	**GWC**
W1	0.6306	-0.2857	-0.6683	0.3263	-0.2623	-0.0072	-0.0605	-0.3188	-0.8467	-0.5032	-0.5653

Table 15. Sequence analysis of bacterial bands excised from the DGGE gel of the organic and mineral layer samples.

Closest Relative in GenBank (Identities) GenBank Accession Number	**UB-2**	**UB-25**	**SB_1-2**	**SB_1-13**	**SB_1-25**
Mycobacterium sp. (384/395 [97%])* gb|AY065649.1			+	+	
Mycobacterium sp. (447/453 [99%])* gb|AY215317.1				+	+
Mycobacterium sp. (189/493 [99%])* gb|AY215317.1			+	+	+
u. ***Mycobacterium*** sp. (149/156 [96%]) gb|AY911448.1			+	+	+
Mycobacterium sp. (425/446 [96%])* gb|HM056093.1			+	+	+
Mycobacterium sp. (489/493 [99%])* gb|AY215317.1			+	+	+
Actinomycetales sp. (334/375 [89%])* gb|GQ504237.1	+				
Mycobacterium sp. (480/499 [96%])* gb|AY0650649.1	+	+			
Acidobacteriaceae (455/478 [96%])* gb|EF219138.1	+	+			

u. indicates uncultured
* indicates that the forward and reverse primers were in agreement
+ indicates presence of the sequence in a particular site, while an empty space indicates that the sequence was not detected there

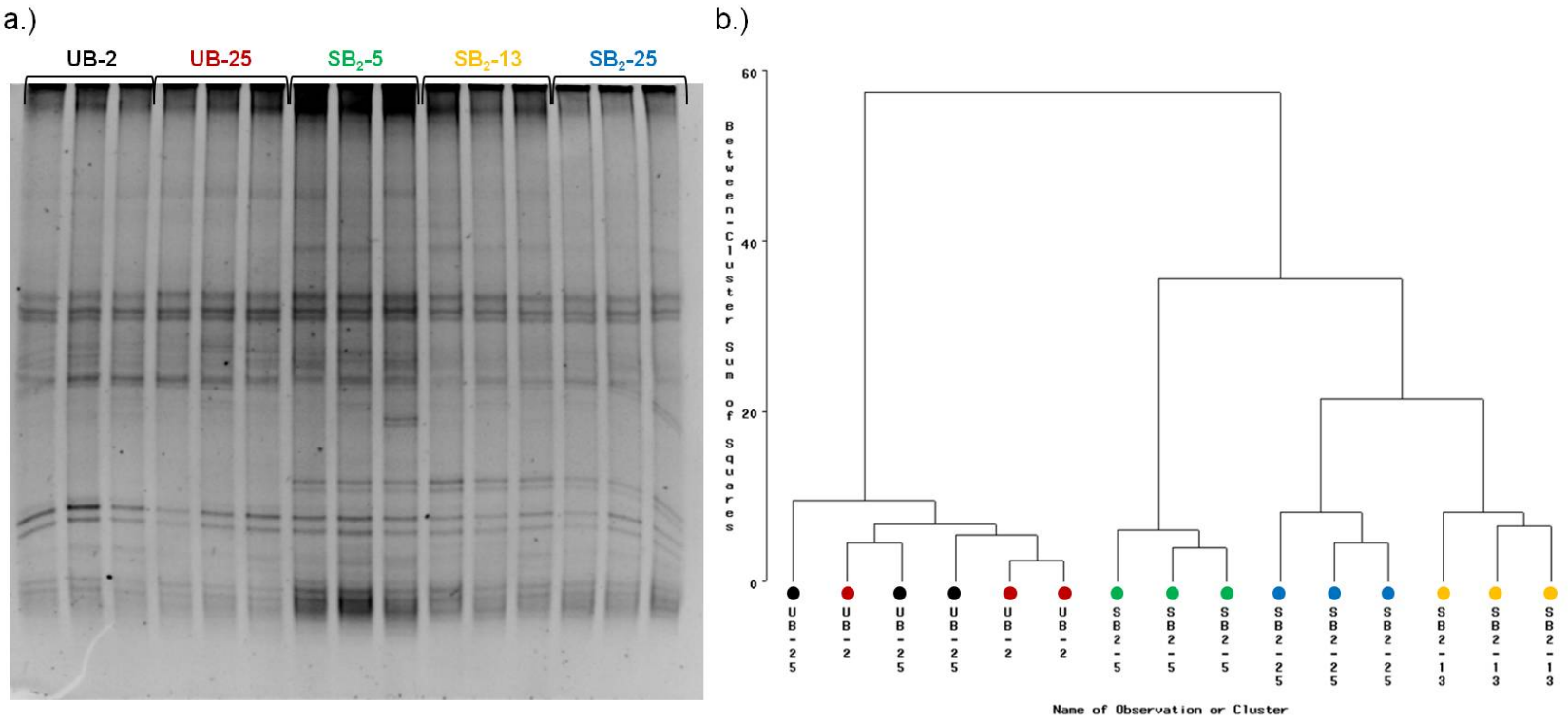

Figure 14. a. DGGE gel of the bacterial community profiles in the organic layer from the UB and SB_2 sites. 500 ng of PCR product were used per well. 55-75% gradient. 55V at 60°C for 17.5 hours. b. Cluster analysis of DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months post-fire and from the SB_2 site 2, 13, and 25 months post-fire. For each branch, *n*=3.

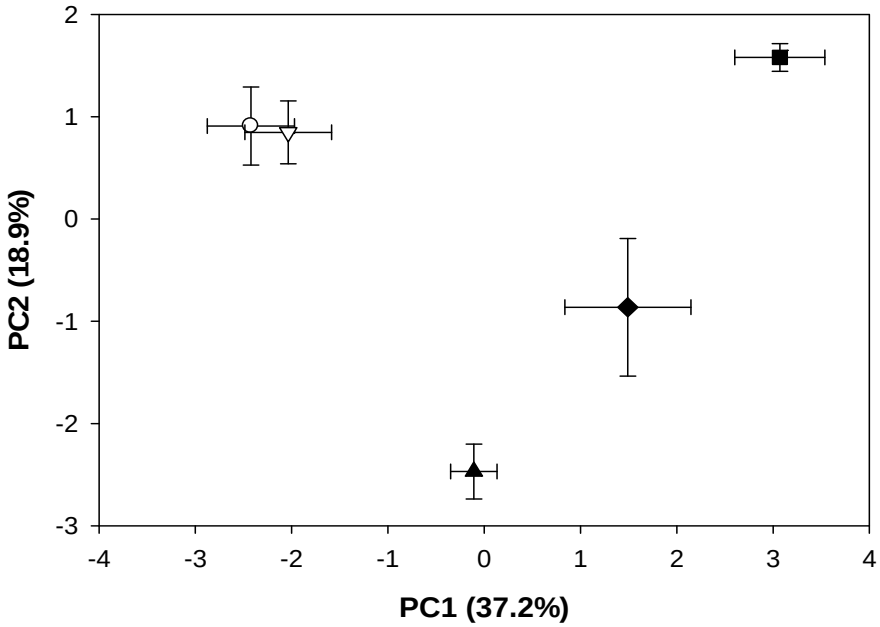

Figure 15. PCA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Organic layer samples from the SB_2 site were collected 5 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

samples collected twice over the span of two years following the fire were not significantly different in PC1 and PC2 (Table 16). As demonstrated by the SB_1 results, the undisturbed bacterial communities were spatially separate from the communities in the SB_2 samples in both PC1 and PC2 (Figure 15). The bacterial community in the SB_2 samples collected 5 months after the fire were also spatially separate from the community in the SB_2 samples collected over a year after the fire (Figure 15). In PC1, the molecular profiles in the samples collected five months after the fire were significantly ($F_{4,10}$=73.08, P<0.0001) different from all other samples collected from both the UB and the SB_2 sites (Table 16). CANOCO did not elucidate the relationship between the organic layer bacterial community structures and the physiochemical variables in response to wildfire perturbation (data not shown).

4. Discussion

4.1 Soil extractable nutrients

The management and conservation of disturbed ecosystems is critical, especially in areas subject to urban sprawl as in southern New Jersey. Historically, wildfires have impacted the New Jersey Pinelands; however, there is limited understanding of how wildfires impact the soil bacterial community, especially in this unique environment also found in various parts of the eastern seaboard (Forman, 1979).

Changes in soil physical and chemical properties were assessed to determine if physiochemical analyses could be used as predictive tools of the impact of fire disturbance on soil biological communities. Due to the low nutrient status of the Pinelands, the cycling of minerals is very important (Collins and Anderson, 1994). Thus,

Table 16. MANOVA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months after the wildfire. Organic layer samples from the SB_2 site were collected 5, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (37.2%)	PC2 (18.9%)	PC3 (10.0%)
UB-2	a	a	ab
UB-25	a	a	a
SB_2-5	b	a	ab
SB_2-13	c	b	c
SB_2-25	d	c	b
	$F_{4,10}$=73.08 P<0.0001	$F_{4,10}$=52.18 P<0.0001	$F_{4,10}$=17.73 P=0.0002

the nutrient cycling patterns post-fire were predicted to have a large impact on the composition of the soil microbial community. Samples sites were selected to control for important factors that have been shown to contribute to the dominant members in a soil system. First, soil type is a soil physical property that has been shown to drive bacterial and fungal community dynamics (Girvan *et al.*, 2003; Sessitsch *et al.*, 2001). To minimize the effect of particle size distribution on the bacterial community profile, all soil samples were collected from the Woodmansie series soil type, which falls in the range of a sandy to loamy sand (USDA, 1971). Therefore, changes associated with the soil properties can be attributed to the impact of fire, rather than to the soil particle size distribution.

Additionally, soil pH has been shown to influence the bacterial community profile, especially on the continental scale. Specifically, pH has been shown to impact the biological community more so than the soil moisture deficit, soil organic carbon, and soil carbon to nitrogen ratio (Lauber *et al.*, 2009; Fierer and Jackson, 2006). pH is considered to be a major influence on the composition of the soil bacterial community because the intracellular pH of most microbes is usually within 1 pH unit of neutral (Madigan *et al.*, 1997). The pH of the forest organic and mineral layers ranged from 3.3 to 4.1 pH units, so the bacteria present were already adapted to a very acidic environment.

In this study, the pH in the soil organic layer was significantly ($F_{7,64}$=15.68, P<0.0001) elevated in the samples collected 2 months post-fire from the SB_1 site and the samples collected 25 months post-fire from the SB_1 and SB_2 sites when compared to the UB site (Table 1). Similarly, the pH values in the SB_1 and SB_2 sites were significantly

(P<0.0001, $F_{7,64}$=11.38) elevated when compared to the UB samples in the mineral layer soil (Table 2). As documented in other burned systems, the differences observed in the samples may be due to the input of ash and therefore the impact of basic cations on the chemistry of the system (Hamman *et al.*, 2007; Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999); however, an immediate increase in the soil pH was not observed, except in the organic layer samples from the SB_1 site collected 2 months after the burn.

Due to the high volatilization temperatures of many soil nutrients, these important elements were predicted to be liberated in the soil as a result of the fire event. Many studies reported a general increase (Murphy *et al.*; 2006b, Knoepp *et al.*, 2004; Neary *et al.*, 1999) or decrease (Castro *et al.*, 2006; Murphy *et al.*, 2006b; Knoepp *et al.*, 2004) of soil extractable nutrients as a result of a burn disturbance. Following the wildfire in the New Jersey Pinelands, the concentrations of soil extractable nutrients such as calcium, magnesium, phosphorus, and iron were variable in the organic and mineral soil layers (Tables 1 and 2). Specifically, the fate of iron is important because it plays a role in cellular respiration, in addition to being a key component of oxygenases and nitrogenases (Madigan *et al.*, 2003). Due to the low cation exchange capacity of the sandy soil, many of the cations that were liberated by the fire event could have been washed away by periods of rain, thereby masking an elevation in cation concentrations two months following the fire. The physical and chemical data from this study and other studies only reflect the variable nature of the soil extractable nutrients and the need for other measures, perhaps of the soluble fractions, to determine the bioavailable nutrients. Additionally, these results show that the soil chemical data alone is not a good indicator

of the effects of the wildfire on the soil community, but rather the biological and chemical data must be resolved together.

4.2 Variations in bacterial community composition on a monthly scale within the first year and a half of the fire

The response of the bacterial community to the wildfire supported our hypothesis that the bacterial community in the severely burned sites shifted drastically within the first year and a half of the fire. Similar to the trends associated with chemical changes from a burn, the reports of the soil bacterial community's response to burn disturbances are conflicting (Hamman *et al.*, 2007; Yeager *et al.*, 2005; Andersson *et al.*, 2004; Choromanska and Deluca, 2001; Neary *et al.*, 1999). In this study, there was a period of time wherein the structure of the detected bacterial community radically changed within the first year and half after the wildfire, as demonstrated in the PCA graphs. Specifically, the bacterial community profiles from each time point clustered separately from the other time points; therefore, the dominant members of the community changed with time after the fire. Furthermore, in both the organic and mineral layers of both sites, PC1 revealed that the samples collected over a year following the fire did not demonstrate significantly different communities. Thus, the dominant bacterial members of the community after the burn maintained their dominance following the first year after the fire. While the data from PC1 suggested that the bacterial community stabilized a year following the fire, the data from PC2 indicated that there were still differences in the bacterial communities. The fire disturbance and temporal changes may have been the driving factors in PC1 and other factors, perhaps seasonal changes, may have been driving the community profile differences associated with PC2. To our knowledge, no other study closely documented

how the bacterial community profile changed within the first year and a half of wildfire disturbance.

Exploration of the bacteria related to the unknown sequences aids in the prediction of bacterial processes occurring in the field at the specific time points following the burn. The presence of bacteria related to Cyanobacteria in the samples collected 2 months following the fire was detected. Perhaps a niche unique to photoautotrophs became available as a result 1) the opening of the tree canopy and/or 2) the loss of key nutrients of the soil system via volatilization or wash-out. The role of autotrophs is extremely important to the overall ecosystem because these microbes conduct key reactions to recycle nutrients, rendering them bioavailable to other members of the ecosystem. For instance, autotrophs fix carbon and serve as food sources for higher organisms. Additionally, members related to the genus *Clostridium* were detected in the organic layer samples collected 2 months after the fire. These bacteria are endospore formers that have the appropriate mechanisms to survive the extreme conditions associated with the disturbance, such as high temperature and low nutrients. Thus, they also have the potential to reinoculate the soil after the perturbation. Due to the acidic forest soil (pH ranged from approximately 3.2 to 4.6), the detection of OTUs related to Acidobacteria was expected and confirmed. The low pH contributed to the proliferation of bacteria related to the genus *Mycobacterium* in all of the severely burned samples. The presence of these organisms is meaningful because they are able to thrive in acidic forest soils and exhibit filamentous-like growth, similar to fungi, in the soil environment. Surprisingly, sequences related to *Bacillus* were not detected even though these are spore forming bacteria that have protection mechanisms to survive extreme

events such as fires. Smith *et al.* (2008) reported detection of DNA sequences related to *Bacillus* in burned samples collected a year after the fire; however, a limitation to this finding is that the only sampling time point in that study was one year following the fire.

Bacteria are impacted by both the direct effects of wildfire such as temperature and immediate loss of nutrients, and the indirect effects such as reduced vegetation and long term nutrient concentration changes. Therefore, these physical and chemical changes select for differences in the microbial communities in the unburned and burned samples. The resilience of these organisms is important because they mediate essential nutrient cycling reactions that support vegetation and water quality.

4.3 Variations in bacterial community composition on an annual scale

The first part of the study raised interesting questions regarding the status of the system as returning to a state similar to that of the unburned system, or rather to an alternate state. To further interpret the trends associated with the DGGE banding patterns, analyses of samples collected 25 months after the fire were necessary to determine when the bacterial community no longer exhibited relevant changes. The experiments examining the bacterial community composition between the unburned and the severely burned samples in both the organic and mineral layers revealed the community changes associated with a severe wildfire. In the long-term bacterial community change experiments, the molecular profiles of the unburned samples were not significantly different over the course of two years, according to the first two PCs. Thus, the dominant bacterial community profiles remained similar in the undisturbed sites over the two year time frame. Conversely, the bacterial communities in the disturbed samples exhibited relevant changes in the two year time period. Shortly following the wildfire,

the bacterial community structure changed in relation to the community in the undisturbed soil. Specifically, the bacterial patterns in the organic layer SB samples were significantly ($F_{4,10}$=227.16, P<0.0001; $F_{4,10}$=73.08, P<0.0001, respectively) different for both SB sites. Interestingly, even though the soil organic and mineral layers contribute to incredibly different soil microsites for bacteria, the patterns associated with the PCA graphs of these samples remained quite similar. This suggests that regardless of depth from the soil surface, the bacterial communities shifted in response to the wildfire.

To address the question of the state of the bacterial community two years following the fire, the communities in the soils collected 25 months after the wildfire were compared. These community profiles in the SB sites reflected a different community from the bacterial communities in the undisturbed site. Thus, rather than returning to a community profile similar to that of the unburned soil samples, the bacterial community is proposed to be approaching an alternate state. In the alternate state, there were a different group of bacteria that became dominant members in the community due to the fire disturbance.

While unique bands were detected in the bacterial community gels, sequences related to *Mycobacteria* or Acidobacteria were revealed, regardless of treatment. The sequences of the discrete bands varied; however, they did not result in discrete matches because of the detection limit at the genus level with the molecular techniques used. There were differences in the sequence content, but it is unknown if different species emerged as a result of the disturbance. Members related to the phylum Acidobacteria have been shown to be slow-growing bacteria whose growth is regulated by pH (Jones *et al.*, 3009). More specifically, when the pH range was between 3.5 and 4.5, the

abundance of Acidobacteria in the mineral soil was at its highest when compared to soils in the 7.5 to 8.5 pH range (Jones *et al.*, 2009).

From this study, the structure of the microbial community has the potential to serve as an indicator of the effects of fire because together, the chemical and biological data revealed interesting relationships that would otherwise have been missed two ways to analyze the soil system were not used. Bacteria commonly exhibit functional redundancy in that many different species conduct the same chemical reactions. Even though the bacterial community structure has changed as a result of the wildfire, it is unknown whether the plant community is likely to be greatly impacted by the change in the soil bacterial community.

5. Conclusions

Alone, the physicochemical characteristics of the soil post-fire did not provide sufficient information to predict the status of the soil bacterial community following this disturbance. The bacterial community structure was found to respond to the wildfire, and in the two years following the fire, the community structure did not reflect that of the undisturbed soil. These results have major implications for understanding soil bacterial communities in fire-disturbed systems. Our results show the importance of linking biological and environmental parameters. Overall, the fire disturbance greatly impacted the soil microbiota, and it is important to recognize the influence of the belowground soil community on the aboveground forest community.

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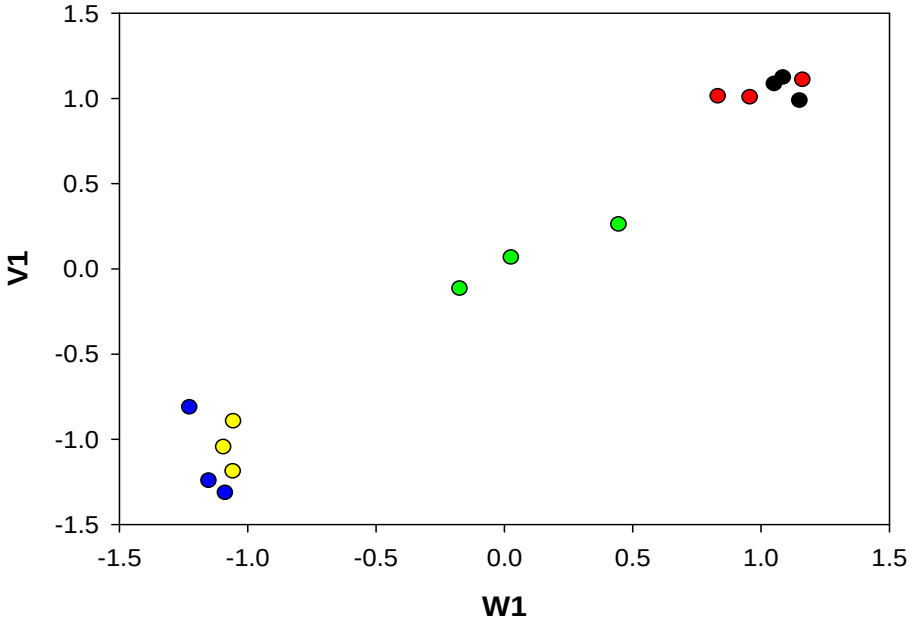
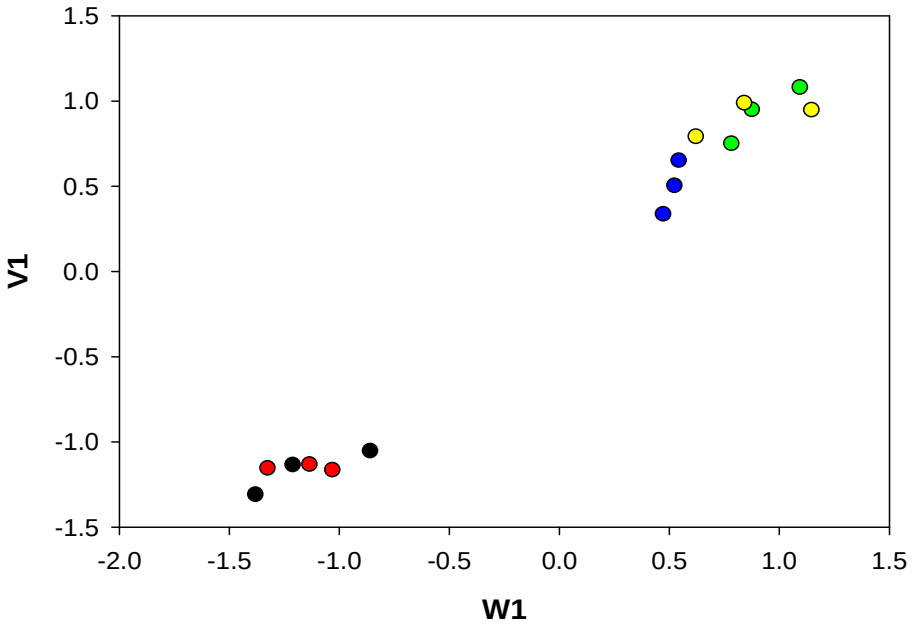
Appendix 1. CANOCO Chart of the Correlation of the Bacterial Community Patterns to Physiochemical Variables in the Organic Layer Soils



Supplemental CANOCO data of the correlation of the bacterial community patterns to physiochemical variables in the organic layer soils. The organic layer soil samples were characterized as: UB-2 (black), UB-25 (red), SB_1-2 (green), SB_1-13 (yellow), SB_1-25 (blue). V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC2, where as the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC1.

Appendix 2. CANOCO Chart of the Correlation of the Bacterial Community Patterns to Physiochemical Variables in the Mineral Layer Soils



Supplemental CANOCO data of the correlation of the bacterial community patterns to physiochemical variables in the organic layer soils. The mineral layer soil samples were characterized as: UB-2 (black), UB-25 (red), SB_1-2 (green), SB_1-13 (yellow), SB_1-25 (blue). V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC2, whereas the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC1.

CHAPTER 3

EVALUATION OF SOIL ARCHAEAL COMMUNITY RESILIENCE FOLLOWING A WILDFIRE IN THE NEW JERSEY PINELANDS

ABSTRACT

Wildfires are common disturbances with profound economic, environmental, and ecological repercussions. However, we have a limited understanding on the effect of severe wildfires on the composition, diversity, and function of belowground microorganisms. The objective of this research was to examine the forest soil archaeal community recovery from a severe wildfire disturbance in the New Jersey Pinelands to reveal which types of archaea were impacted by the fire. Soil samples were collected three times over the span of two years following the fire from the organic and mineral layers of a severely burned site. Samples were also collected from an unburned control site. The wildfire was hypothesized to cause a shift in the dominant archaea based on their ability to respond to changes in their environment as a result of fire. Archaeal-specific primers and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) were used to evaluate archaeal community dynamics following the fire. Data were analyzed with principal component analysis (PCA) and multivariate analysis of variance (MANOVA). Archaeal community analysis revealed that even though there were common bands between the unburned and burned samples, the archaeal communities from the unburned site clustered separately from those of the burned site collected 2, 13, and 25 months after the fire. Based on these indications of changes in

population diversity approximately two years following the wildfire, operational taxonomic units (OTUs) exhibiting major population changes were evaluated. Due to limitations in culturing archaea, the databases were found to be insufficient in providing knowledge regarding the identities of the organisms associated with our samples. Nonetheless, we demonstrated that fire disturbances impact soil archaeal community structure in meaningful ways.

1. Introduction

Archaea were once thought to only inhabit a limited number of extreme environments; however, they have been documented to exist in many moderately-stressed environments, including, but not limited to fir forests in Orgeon (Boyle-Yarwood *et al.*, 2008), tundra wetlands in Norway (Høj *et al.*, 2005), and grassland soils (Nicol *et al.*, 2003). Within these non-extreme environments, Group I Crenarchaeota were thought to be the most abundant and diverse group compared to the other groups of archaea such as the Euryarchaeota and the Korarchaeota (DeLong, 1998). In line with this trend, other studies indicated that the ammonium concentrations in shallow Arctic waters weakly correlated with the abundance of Crenarchaeota (Kirchman *et al.*, 2007). Additionally, plant nutrients and organic carbon were hypothesized to fuel the growth of Crenarchaeota in the Arctic surface waters (Kirchman *et al.*, 2007). Not only have archaea been found to be widely distributed, their occurrence may depend on different energy sources than bacteria (Kirchman *et al.*, 2007).

Despite the limited studies on archeael physiology, Aller and Kemp (2008) suggested that as a group, archaea are indeed physiologically diverse. For instance, they have been found to utilize bicarbonate or carbon dioxide in the North Sea (Wuchter *et al.*, 2003), aerobically oxidize ammonia to nitrite (as a pure culture) (Könneke *et al.*, 2005), and take up dissolved amino acids in the Pacific Ocean and Mediterranean Sea (Ouverney and Fuhrman, 2000). Currently, the role of archaea following ecosystem disturbance is poorly understood.

Forest fires are intense environmental perturbations resulting in a reshaped landscape (Knoepp *et al.*, 2004), an increased release of carbon dioxide to the atmosphere

(Bowman *et al.*, 2009), and changes in soil chemical properties (Hamman *et al.*, 2007; Castro *et al.*, 2006; Murphy *et al.*, 2006; Neary *et al.*, 1999). In addition, declines (Yeager *et al.*, 2005; Choromanska and DeLuca, 2001) as well as no change (Hamman *et al.*, 2007) in the soil microbial biomass following a fire have been documented. A better indicator of the impact of changes in ecosystem properties is the shift in soil microbial community composition. For example, in a study by Smith *et al.* (2008), changes in the bacterial community composition as a result of a wildfire were documented; however, the first sampling point was taken a year after the fire.

There is limited knowledge on the response of microorganisms to forest fires and most studies have focused on prescribed burning (Tuininga and Dighton, 2004; Jurgens and Saano, 1999) and the physiological groups of microorganisms prior to the advent of molecular tools (Acea and Carballas, 1996). Specifically, Jurgens and Saano (1999) determined that an archaeal community in the boreal forest subjected to clear-cutting and prescribed burning contained mainly Crenarchaeota. Limitations to this study include sample collection one year following the prescribed burn in addition to unclear differences between the control and burned samples (Jurgens and Saano, 1999). The study may have overlooked the dramatic shifts of the soil archaeal community within the first year post-fire. Therefore, our study documents the recovery of the soil archaeal community with time following the wildfire with the general objective of achieving better insight into community changes in the critical time within the first year following the fire. Specifically, the objectives were to investigate the archaeal community change with time following a severe wildfire and to determine the status of the community two years following the intense perturbation.

2. Materials and Methods

2.1 Soil

A crown fire burned about 18,000 acres of New Jersey Pinelands forest in May of 2007. Specifically, the Warren Grove section of southern New Jersey was impacted. Site selection was based mainly on soil type (Woodmansie series), fire intensity, and property ownership of the land. All sites had a similar plant community, climate, topography (<0%slope), and loamy sand to sandy soil texture (>89% sand). Soil was collected from a severely burned site and an unburned site. The unburned site served as a basis for comparison since it did not experience the wildfire. The coordinates of the unburned site and severely burned site are N39° 44' 25.3" W74° 22' 8.6" and N39° 43' 39.3" W74° 22' 14.3", respectively (Figure 1). The severely burned site contained severely burned trees and a visible ash layer. To monitor the biological community changes over the course of about two years, soil samples were collected 2, 13, and 25 months after the fire from the severely burned site (SB_1). Soil samples from the unburned site (UB) were collected 2 and 25 months after the fire. A sampling grid was placed at a slightly different location at each time point such that after each time point, the sites varied so as not to retrieve a previously disturbed sample. Soil samples were collected during early-summer and mid-fall. A total of nine soil samples were collected per site. After collection with a sterile spade to the depth of 15 cm, the organic and mineral layers were separated. Samples were well mixed and taken to the Soil Testing Lab (Rutgers University, New Brunswick, NJ) for specific chemical analyses such as pH, phosphorus, potassium, magnesium, calcium, copper, manganese, zinc, boron, and iron

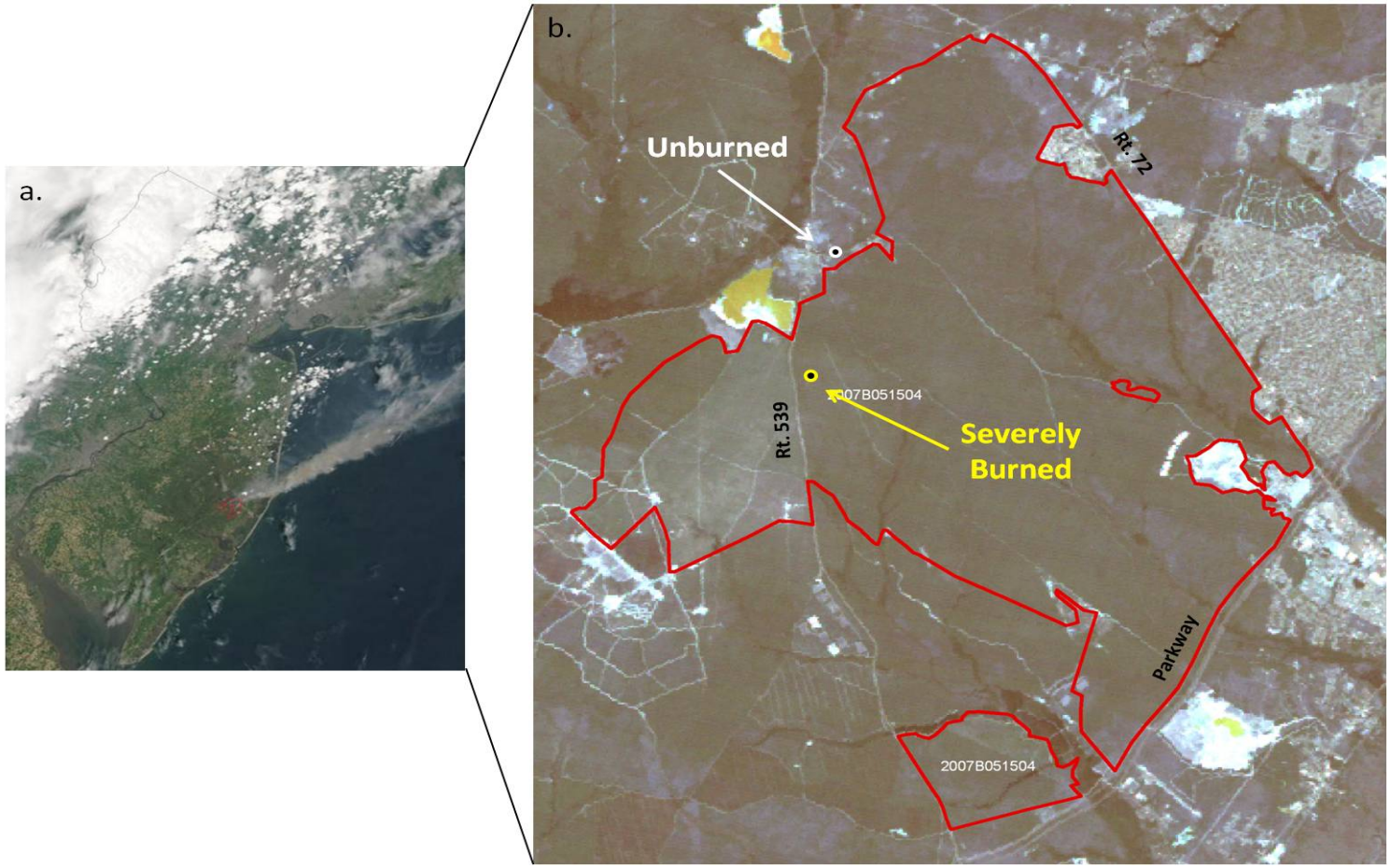


Figure 1. a. Satellite photo of smoke from Warren Grove Fire in the New Jersey Pinelands (May 2007) ©NASA. b. Aerial view of wildfire; Red line indicates outline of fire; Image credit: Inga La Puma.

(measured as soil extractable cations in ammonium-acetate extract solutions) using standard procedures (Sims and Eckert, 2009; Wolf and Beegle, 2009). Additionally, the Soil Testing Lab measured the Total Kjeldahl Nitrogen (TKN), ammonium-N (NH_4^+-N), and nitrate-N (NO_3^--N) only on the organic layer samples (Griffin *et al.*, 2009). For these analyses, only three samples per site were measured. Organic matter analysis was conducted on the soil organic layer with the loss on ignition method and on the mineral layer with the dichromate-oxidation (Walkley-Black) method by the Soil Testing Lab (Rutgers University, New Brunswick, NJ) (Schulte and Hoskins, 2009). Nine samples per site were analyzed. The samples were then stored at -20°C until molecular analyses were conducted.

2.2 DNA extraction and PCR amplification

Total genomic DNA was extracted from 0.3 g of soil with the PowerSoil™ DNA Kit (MoBio, Carlsbad, CA) according to the manufacturer's instructions, amended by eluting the genomic DNA with 10mM Tris buffer (pH of 8). DNA was subsequently analyzed by agar electrophoresis on a 1% agarose gel subsequently stained with ethidium bromide and visualized under UV light. DNA from three of nine samples per site was randomly combined prior to polymerase chain reaction (PCR). In total, three observations per site were obtained. PARCH340 forward (Øvreås *et al.*, 1997) and 934 reverse (Raskin *et al.*, 1994) were used to target the conserved 16S rRNA region of archaea with the PCR cycling parameters from Cytryn *et al.* (2000). To yield 50 µl reaction mixtures, 2 µl of DNA template, 1 µl of each 20 µM primer, 1 µl of 20 µg/µl Bovine Serum Albumen (Roche, Mannhein, Germany), and 45 µl Supermix (Invitrogen, Carlsbad, CA) were used. The amplified products were run on a 1% agarose gel to

determine the concentrations based on lambda HindIII (Invitrogen, Carlsbad, CA). Negative and positive controls, in addition to sequence analysis of the targeted area reduced the occurrence of the primers targeting other genes.

2.3 DGGE

The PCR amplicons were then separated on a denaturing gradient gel electrophoresis (DGGE) gel. Particular bands of interest were excised for further phylogenetic analysis (Muyzer *et al.*, 1993). The same concentration (375 ng to 1,250 ng based on the particular DGGE gel) of PCR products were run on multiple DGGE gels using the DCode universal mutation system (Bio-Rad, Hercules, CA). Denaturing gradients ranged from 35-75%; the 100% denaturant contained 40% formamide and 7 mM urea. Electrophoresis was performed at 60°C at 55 volts for 17.5 hours in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). The DGGE gels were stained with SYBR green I (Sigma Aldrich, St. Louis, MO) and photographed under UV transillumination by using a Kodak Gel Logic 100 gel imaging system. Excised bands of interest were reamplified with the appropriate primer sets and purified with the ExoSAP-IT® Kit (Affymetrix, Santa Clara, CA). The purified products were sequenced with the appropriate primer sets and sent to GeneWiz, Inc. (South Plainfield, NJ) for sequence analysis. Sequences were analyzed with DNASTAR Lasergene™ (Madison, WI) software and compared to sequences found in GenBank by using the BlastN algorithm (Altschul *et al.*, 1990).

2.4 Statistical analyses

To analyze the DGGE gels conservatively, each set of statistical analyses were limited to one gel at a time due to the variability between DGGE gels. Therefore,

multiple DGGE gels were not compared, but rather each set of samples were run on individual gels and subsequently analyzed. The DGGE gel banding patterns were converted to a binary matrix as presence and absence of bands (van Hannen *et al.*, 1998). Principal component analysis (PCA) was conducted to condense the information contained in the large number of original variables (banding patterns) into a smaller set of new composite dimensions (principle component [PC] scores) through SAS® (Cary, NC) software (McGarigal *et al.*, 2000). The PC scores were plotted to visualize the data and determine the degree of similarity between samples (Rudi *et al.*, 2007; McGarigal *et al.*, 2000). PCA was followed with multivariate analysis of variance (MANOVA) of the first three PC scores to determine if the effect of the wildfire on the soil archaeal communities was significant (McGarigal *et al.*, 2000). Cluster analysis was conducted to corroborate the results from PCA. Minimum variance clustering was conducted on the DGGE banding pattern data because the data were neat, compact clusters. In addition, proc cluster was used because the data were not continuous measures. One-way analysis of variance (ANOVA) via the Bonferroni (Dunn) t-test was conducted on the banding patterns to determine if fire treatment had an effect on the number of OTUs in the specific denaturing gradient range.

Canonical correlation analysis (CANOCO) related the chemical and the biological variables to determine which chemical features correlated with specific soil archaeal community structure patterns. In this method, two sets of groups (the chemical and biological parameters measures) were used to maximize the correlation between the variables in those groups (Cooper *et al.*, 2006; McGarigal *et al.*, 2000). CANOCO was used to determine which physical variables correlated with the archaeal communities in

the unburned or severely burned soils. The biological variables measured were the archaeal banding patterns, represented as PC1 and PC2 values from PCA. The chemical variables measured include pH, organic matter content, gravimetric water content, in addition to phosphorus, potassium, magnesium, calcium, copper, boron, and iron concentrations (in micrograms per gram). Three concentrations from three samples (the same composite as the DNA samples) were averaged to obtain the same number of observations for the physical data as the biological data. Therefore, three observations per site were obtained.

3. Results

3.1 Investigation of archaeal community change with time in the soil organic layer

3.1.1 Impact of fire on community composition

DGGE analysis of the organic layer samples revealed complex banding patterns representing the archaeal communities in the disturbed and undisturbed sites (Figure 2a). While there were discrete bands associated with either the unburned or severely burned organic layer samples, there were many common bands throughout the DGGE gel (Figure 2a). From the DGGE profile, cluster analysis was conducted to reveal how the samples grouped. The archaeal communities in the organic layer samples clustered into five groups that formed two main clades (Figure 2b). The archaeal communities in the severely burned samples collected over a year following the burn were most closely grouped (Figures 2b and 3). Likewise, the PCA graph indicated a clear division of the archaeal community composition between the archaeal molecular patterns in the unburned and severely burned samples collected from the organic layer (Figure 3). In PC1 and PC3, the archaeal communities in the severely burned samples were

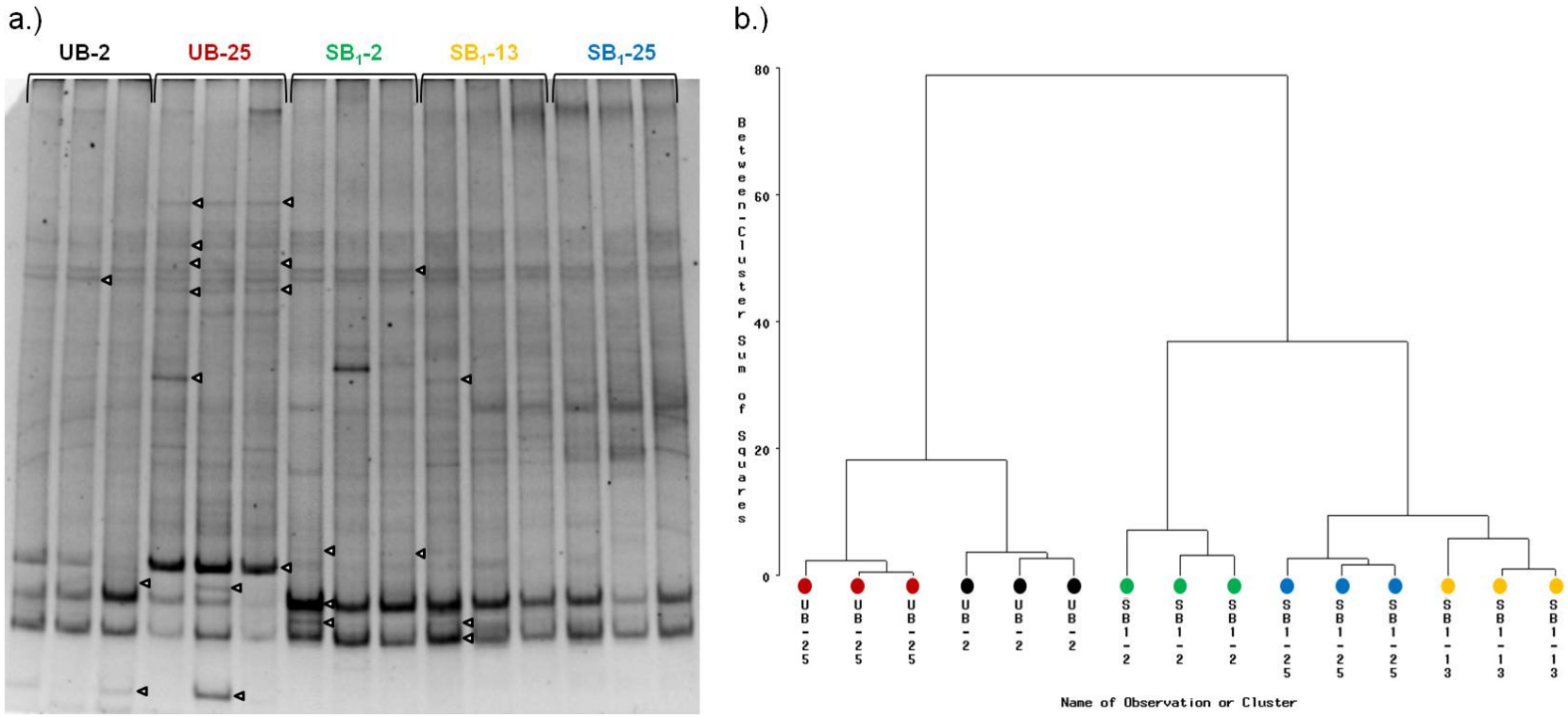


Figure 2. a. DGGE gel of archaeal amplicons from the organic layer of the UB and SB_1 sites. The denaturing gradient was from 35-75%. 375 ng of PCR product was added to each well. Arrows indicate excised bands, 22 in total. b. Cluster analysis of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months after the wildfire. Organic layer samples from the SB_1 site were collected 2, 13, and 25 months after the wildfire. For each group, n=3.

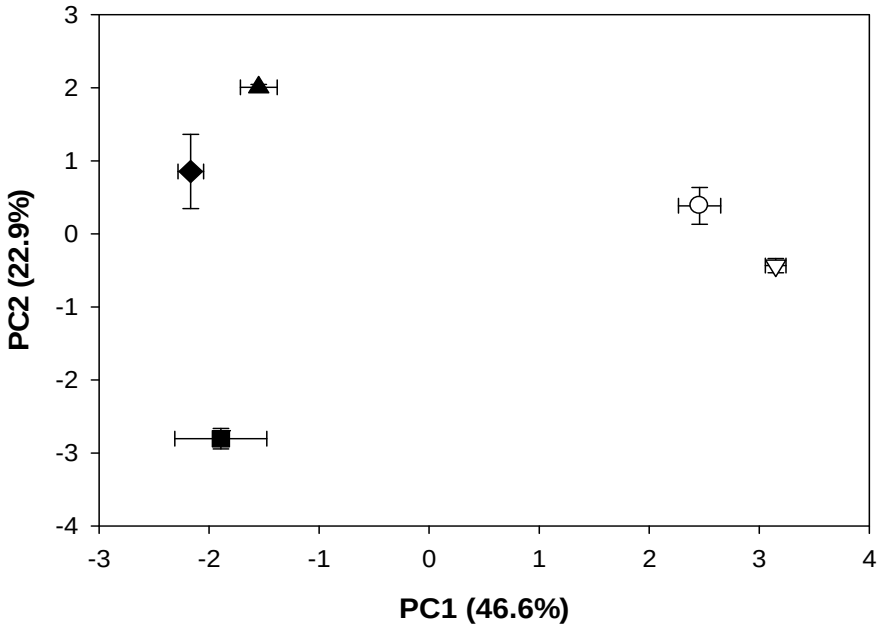


Figure 3. PCA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Organic layer samples from the site SB_1 were collected 2 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

significantly ($F_{4,10}$=381.67, P<0.0001; $F_{4,10}$=84.69, P<0.0001, respectively) different from those in the unburned samples (Table 1). The archaeal patterns in the unburned samples collected two and 25 months post-fire oriented proximal on the PCA graph (Figure 3); however, they differed significantly ($F_{4,10}$=381.67, P<0.0001; $F_{4,10}$=137.78, P<0.0001; $F_{4,10}$=84.69, P<0.0001, respectively) from one another in all three relevant PC axes (Table 1). With regard to the severely burned site, the archaeal communities 2 months following the fire clustered separately from those detected 13 and 25 months after the fire (Figure 3). While these archaeal communities in these samples were distal on the PCA graph, they did not exhibit statistically relevant differences in PC1; however, they were significantly ($F_{4,10}$=137.78, P<0.0001) different from one another according to PC2 (Table 1).

3.1.2 Number of archaeal OTUs in the given denaturing gradient range

One-way ANOVA tested for the wildfire effect on the number of archaeal OTUs in the given denaturing gradient range (35-75% denaturing gradient) in the soil organic layer. No significant difference between the archaeal communities in the unburned and severely burned samples was observed; however, there were significantly ($F_{4,10}$=8.47, P=0.0030) more archaeal OTUs in the samples collected over 13 and 25 months post-fire compared to those collected 2 months post-fire (Figure 4).

3.1.3 Correlation of the chemical and biological variables

CANOCO examined the relationships between the measured physiochemical variables to the archaeal community composition in the disturbed and undisturbed samples to determine which chemical variables most likely selected for the archaeal community composition in either site (Table 2). The physiochemical variables were

Table 1. MANOVA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months after the wildfire. Organic layer samples from the site SB_1 were collected 2, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (46.6%)	PC2 (22.9%)	PC3 (10.4%)
UB-2	a	a	a
UB-25	b	b	b
SB_1-2	cd	c	c
SB_1-13	c	a	d
SB_1-25	d	d	c
	$F_{4,10}$=381.67 P<0.0001	$F_{4,10}$=137.78 P<0.0001	$F_{4,10}$=84.69 P<0.0001

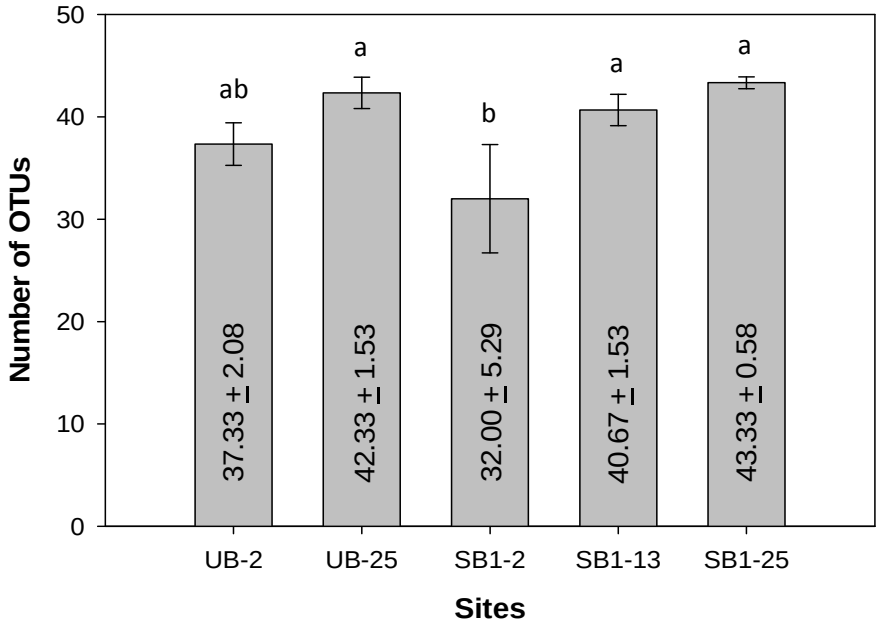


Figure 4. Number of archaeal OTUs within the specific denaturing gradient range detected in the organic layer samples from the UB and the SB_1 sites. For each vertical bar, n=3 and error bars indicate standard deviation. Treatments sharing the same letter are not significantly different from one another in a Bonferroni (Dunn) t Test. In the ANOVA, $F_{4,10}$=8.47, and P=0.0030.

Table 2. CANOCO on the biological and physical data from the organic layer samples collected 2 and 25 months after the fire from the UB site, and 2, 13, and 15 months after the fire from the SB_1 site. For the first canonical variable, $F_{8,18}$=3.40, P=0.0201. The first canonical function explained 90.7% of the variation in the analysis. V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC1, where as the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC2 (see Appendix 1).

	PC 1	**PC 2**
V1	0.9649	-0.2627

	pH	**P**	**K**	**Mg**	**Ca**	**Zn**	**Fe**
W1	-0.2596	0.3930	0.8566	0.0440	-0.1589	0.8621	-0.2318

documented in Chapter 2. Values for manganese, boron, copper, gravimetric water content, and organic matter were omitted from the analysis because they did not demonstrate a significant correlation with the archaeal community patterns in the unburned and burned soils. Additionally, the nitrogen values for some samples used in the biological analysis were not recorded. CANOCO showed that the soil magnesium concentration did not correlate with the archaeal community composition from either treatment. While the concentrations of potassium and zinc strongly correlated with the archaeal banding patterns in the unburned site, the concentration of phosphorus exhibited a weak correlation with the undisturbed archaeal community (Table 2). Alternatively, the archaeal communities in the severely burned site weakly correlated with pH, in addition to the calcium and iron concentrations (Table 2).

3.2 Investigation of archaeal community change with time in the soil mineral layer

3.2.1 Impact of fire on community composition

The archaeal communities in the mineral layer samples exhibited complex banding patterns that were similar to the communities in the organic layer samples (Figure 5a). Discrete bands were observed in both the undisturbed and disturbed samples (Figure 5a). Similar to the organic layer samples, cluster analysis revealed five distinct clusters of the archaeal communities that include the unburned samples collected 2 and 25 months post-fire and the severely burned samples collected 2, 13, and 25 months post-fire (Figure 5b). The archaeal community compositions in the unburned and severely burned clusters were connected by the deepest branch, revealing the disparity between the archaeal community patterns in these samples (Figure 5b).

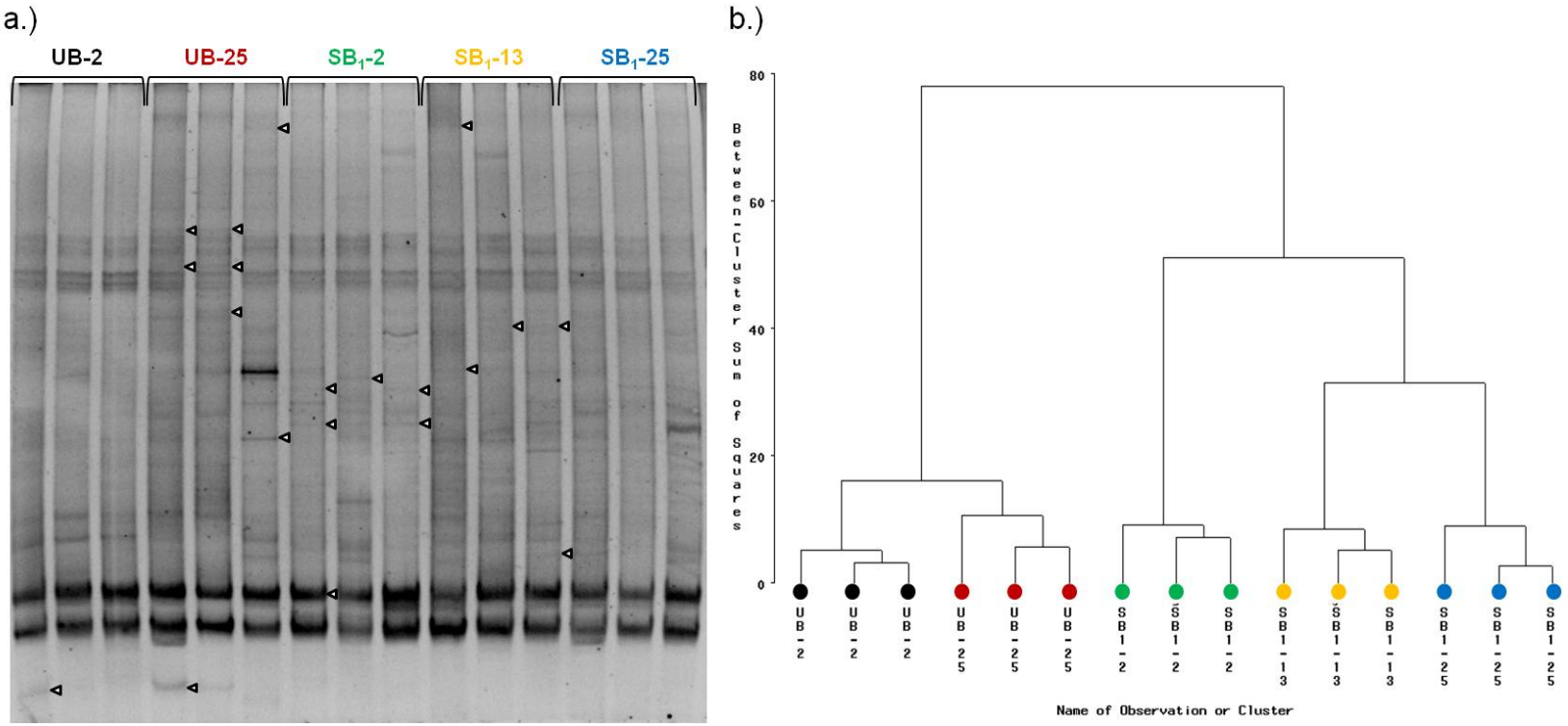


Figure 5. a. DGGE gel of archaeal amplicons from the mineral layer of the UB and SB_1 sites. The denaturing gradient was from 35-75%. 1250 ng of PCR product was added to each well. Arrows indicate excised bands, 20 in total. b. Cluster analysis of the DGGE banding patterns. Mineral layer samples from the UB site were collected 2 and 25 months after the wildfire. Mineral layer samples from the SB_1 site were collected 2, 13, and 25 months after the wildfire. For each group, *n*=3.

PCA corroborated the cluster analysis results in that the archaeal communities in the unburned and severely burned samples clustered separately in two-dimensional space (Figure 6). In PC1, which accounted for 33.0% of the variance in the system, the archaeal profiles of the UB samples were statistically ($F_{4,10}$=173.23, P<0.0001) different from that of the SB samples (Table 3). Unlike the trend observed in the organic layer samples, the archaeal community structure in the UB mineral layer samples did not change significantly over the course of approximately two years in the three statistically relevant PCs (Table 3). Furthermore, the community profiles in the samples from the three severely burned time points were not statistically significant in PC1 (Table 3); however, the SB_1-2 samples were spatially separate from the SB_1-13 and SB_1-25 samples in the PCA graph (Figure 6).

3.2.2 Number of archaeal OTUs in the given denaturing gradient range

One-way ANOVA tested for the wildfire effect on the number of archaeal OTUs detected in the specific denaturing gradient range. No clear trend was observed that associated the number of bands visualized in each particular mineral layer sample to the impact of the fire (Figure 7).

3.2.3 Correlation of the chemical and biological variables

CANOCO did not elucidate the relationship between the mineral layer archaeal community structures and the physiochemical variables in response to wildfire perturbation (data not shown).

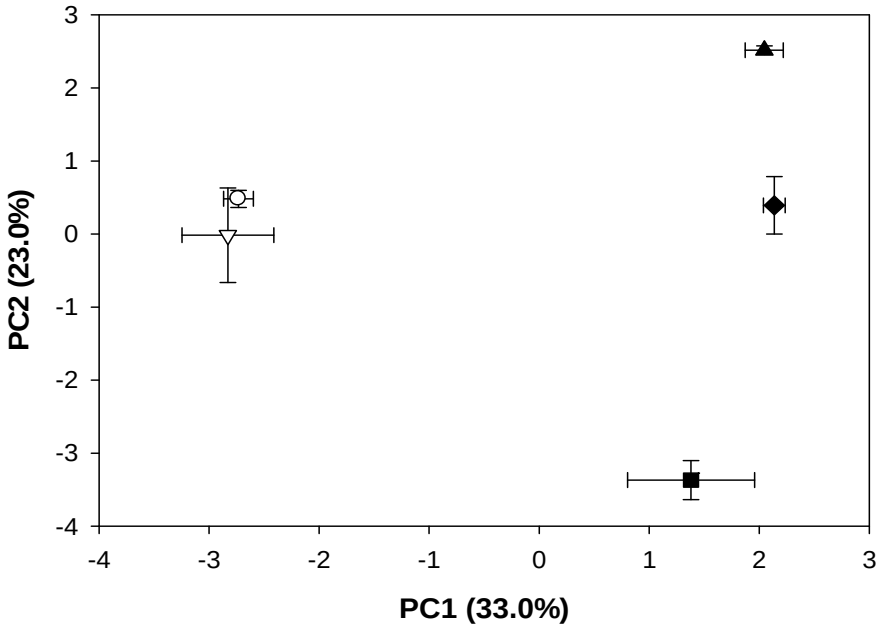


Figure 6. PCA of the DGGE banding patterns. Mineral layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Mineral layer samples from the site SB_1 were collected 2 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 3. MANOVA of the DGGE banding patterns. Mineral layer samples from the UB site were collected 2 and 25 months after the wildfire. Mineral layer samples from the site SB_1 were collected 2, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (33.0%)	PC2 (23.0%)	PC3 (12.5%)
UB-2	a	a	a
UB-25	a	a	a
SB_1-2	b	b	ac
SB_1-13	b	a	b
SB_1-25	b	c	c
	$F_{4,10}$=173.23 P<0.0001	$F_{4,10}$=102.52 P<0.0001	$F_{4,10}$=25.31 P<0.0001

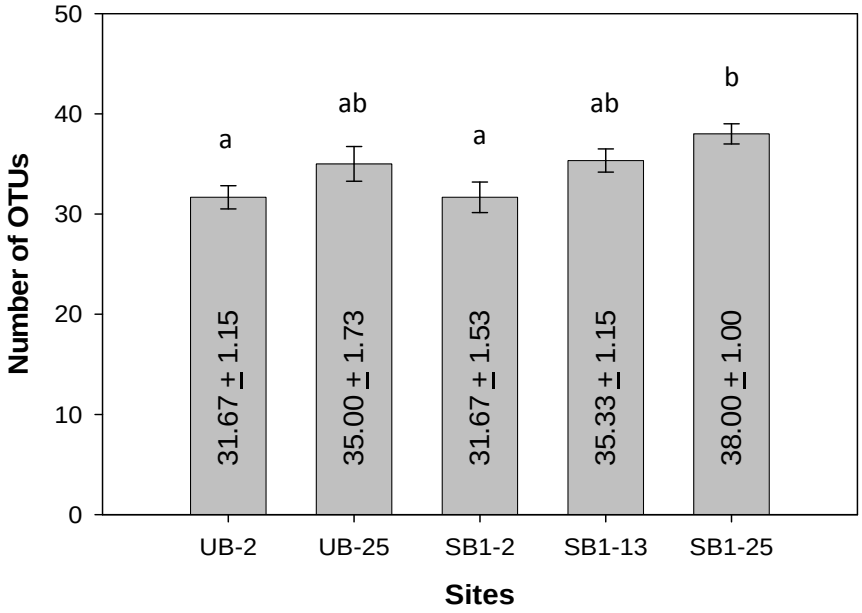


Figure 7. Number of archaeal OTUs within the specific denaturing gradient range detected in the mineral layer samples from the UB and SB_1 sites. For each vertical bar, n=3 and error bars indicate standard deviation. Treatments sharing the same letter are not significantly different from one another in a Bonferroni (Dunn) t Test. In the ANOVA, $F_{4,10}$=12.13 and P=0.0007.

3.3 Archaeal sequence analysis

Discrete bands that were successfully amplified and sequenced demonstrated that eleven sequences closely related to uncultured archaeons, while three sequences closely related to bacteria (data not shown). Negative controls never amplified, while the positive control, *Halobacterium salinarium*, always amplified. In an effort to match the primers to archaeal sequences deposited in the Ribosomal Database Project, we found that both the forward and reverse primers targeted members of the Domain Archaea; however, the reverse primer targeted approximately 0.00718% of the Bacterial sequences deposited in the database. There was nonspecific amplification of the archaeal DGGE primers as noted by Cytryn *et al.* (2000); however, Høj *et al.* (2005) did not detect this discrepancy when studying archaeal communities in arctic wetlands. While sequence analysis of the excised bands was not successful due to limitations in the publically available databases, these archaeal primers targeted members from the phyla Crenarchaeota and Euryarchaeota.

4. Discussion

4.1 Evaluation of the impact of the fire on the archaeal community structure

Previous studies regarding forest burns have generally focused on the microbiological status of the system a year or more following the fire (Smith *et al.*, 2008; Hamman *et al.*, 2007; Jurgens and Saano, 1999) or the impact of less intense prescribed burns (Jurgens and Saano, 1999; Tuininga and Dighton, 2004). Alternatively, the focus of our study was to demonstrate the shift of the archaeal community in the short time period following a crown fire in the New Jersey Pinelands. Archaeal diversity was

evaluated with the PCR-DGGE technique in order to broadly determine the archaeal differences associated with the perturbation.

In these oligotrophic, sandy soils, the archaeal community was expected to behave in a contrary manner to bacterial and fungal communities. Not only do archaea differ from some of its counterparts with regard to cell membrane composition, but also size and microhabitat location (Madigan *et al.*,2003), lending to the idea that the archaea may be well equipped to adapt to perturbations such as forest fires. Furthermore, archaeal diversity was predicted to be lower when compared to bacterial diversity (Aller and Kemp, 2008). Currently, few archaea have been successfully isolated; therefore, database information is limited. Additionally, the community structural changes after a wildfire are not known.

A disturbance of this magnitude was demonstrated to result in archaeal community shifts due to the direct and indirect effects of the wildfire. Specifically, this study revealed that following the environmental perturbation, the archaeal community exhibited meaningful differences in community composition over time, especially two months after the fire. Interestingly, while the organic and mineral soil layer samples were drastically different in terms of physical composition, presence or absence of ash, and depth from the soil surface, the archaeal community exhibited similar trends with regard to how they continued to change with time after the fire. Based on the prediction that the soil archaea in the mineral layer samples may not have been as directly impacted by the fire as the organic layer samples, we did not expect to observe community shifts in these samples. However, the archaeal structure of the mineral layer samples exhibited a similar pattern as the organic layer samples. Thus, the wildfire impacted the archaeal community

dynamics in the surface and mineral soil layers. To reduce the impact of particle size distribution on the microbial community, sites with the same soil texture were selected, since soil texture has been shown to directly impact soil bacterial diversity (Girvan *et al.*, 2003; Sessitsch *et al.*, 2001).

To our knowledge, this is the first study to document how the archaeal community composition was impacted less than a year following a wildfire. Specifically, cluster analysis and PCA were used as complementary analyses to demonstrate that the archaeal communities in the organic layer of the UB samples differed from those of the SB samples. Thus, the archaeal community profiles dramatically shifted in both the soil organic and mineral layers as a result of the direct and indirect effects of the wildfire. Additionally, in the organic layer, there were significantly ($F_{4,10}$=8.47, P=0.0030) fewer OTUs in the SB samples collected two months post-fire compared to the SB samples collected 13 and 25 months post-fire, suggesting that the fire reduced the number of archaeal species in the soil.

4.2 Status of the community two years following the intense perturbation

Surprisingly, after two years, the archaeal communities in the SB samples were still dissimilar to that of the UB samples. They were clustering separately in ordination space, thus suggesting that in the two year time period, the archaeal community structure had not returned to its predisturbance state. Rather, the archaeal community may progress to an alternate state whereby the system is reaching a different stable point due to the stresses associated with the disturbance (Price and Morin, 2009; Schröder *et al.*, 2005; Bertness *et al.*, 2002). We can only suggest that the community is reaching a

stable point because there may be abrupt, but still continuous responses to environmental parameters or long term cycles (Schröder *et al.*, 2005).

It is important to document how the archaeal community shifts as a result of the disturbance because a different community composition may indicate a different functionality of the system (Schröder *et al.*, 2005). For instance, the emerging microbial community may not support the same plant species that the previous community had. Therefore, over time, the ecosystem as a whole would dramatically change. If alternate stable states exist, then the microbial functions of the system will change and perhaps result in varied interactions with the plant community. In disturbed sites such as the one in this study, if there is high diversity with multiple organisms that exhibit functional redundancy, then efficient degradation of organic matter is expected despite population fluctuations (Aller and Kemp, 2008). Therefore, the distribution of microorganisms may not reflect a change in the nutrient cycling processes in the system.

4.3 Correlation of biological and physical variables in the soil organic layer

CANOCO tested for the possible correlations between soil chemistry and biology in the wildfire-impacted forest soil. The fire perturbation was expected to liberate various micronutrients into the soil matrix, where the microorganisms would readily use these nutrients (Hamman *et al.*, 2007; Castro *et al.*, 2006; Murphy *et al.*, 2006b; Neary *et al.*, 1999). Therefore, the various micronutrient concentrations would be expected to have a strong correlation with the archaeal community structure of the SB samples collected two months post-fire. The correlation between the concentrations of phosphorus and the samples may have been more related to time rather than site because the UB-2 and SB_1-2 samples had elevated concentrations of phosphorus, where as the

samples collected over a year after the fire had relatively low concentrations of phosphorus. Soil pH weakly correlated with the soil archaeal community structure in the severely burned samples. To our knowledge, this is the first time that soil pH has been shown to influence archaeal community dynamics. Many studies predict and report that pH is expected to increase as a result of a fire (Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999). The chemical data from this study (see Chapter 2) did not indicate clear trends in pH as a result of the wildfire. Therefore, the correlation between the biological and physiochemical variables provided important information regarding the dynamics of the site as a result of the disturbance. Additionally, the concentrations of calcium and iron were weakly correlated with the soil archaeal community structure in the severely burned samples. Specifically, the pH values were generally lower in the undisturbed samples, while the calcium concentration was not significantly different (as described in Chapter 2). The elevated pH should was expected to liberate iron, making it more bioavailable to the soil microbial community; however, iron concentrations were not significantly different in the organic layer (as described in Chapter 2). Iron was expected to have been liberated as a result of the increase in pH as a result of the fire event (Neary *et al.*, 1999). Overall, CANOCO demonstrated interesting links between the biological community and environmental variables that would have been otherwise missed if they were studied independently.

5. Conclusions

The goals of this study were to investigate the archaeal community change with time following a severe wildfire and determine the status of the community two years following the intense perturbation. This study demonstrated not only how archaeal

community composition changed as a result of a wildfire, but also how the soil archaeal community has not returned to a similar community pattern as in the undisturbed site. Therefore, the archaeal communities in the SB sites may have been approaching an alternate state. The dominant archaea in the severely burned soil samples have shifted, and there may be downstream nutrient cycling changes that may impact other soil microorganisms and the above-ground forest community. Because the soil chemical variables behave differently in various sites, they do not serve as adequate indicators of the impact of perturbation on soil archaeal functions. Therefore, studying the composition of the belowground community is important and ecologically relevant. Future studies should focus on how different genes are expressed as a result of forest fires, and how the soil archaeal community may impact overall ecosystem functioning.

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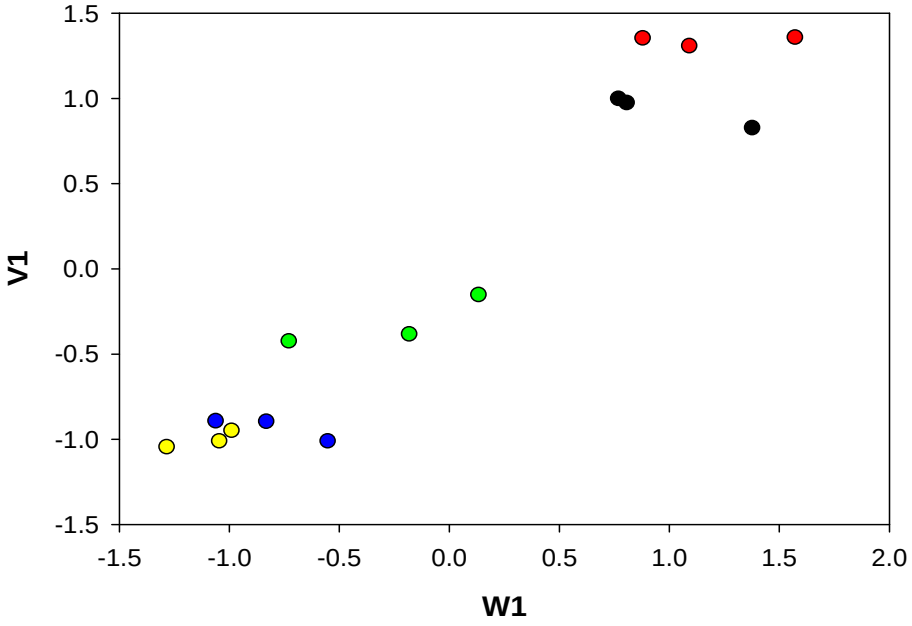
Appendix 1. CANOCO Chart of the Correlation of the Archaeal Community Patterns to Physiochemical Variables in the Organic Layer Soils



Supplemental CANOCO data of the correlation of the archaeal community patterns to physiochemical variables in the organic layer soils. The organic layer soil samples were characterized as: UB-2 (black), UB-25 (red), SB_1-2 (green), SB_1-13 (yellow), SB_1-25 (blue). V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC1, where as the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC2.

CHAPTER 4

FUNGAL COMMUNITIES IN A DISTURBED FOREST SOIL MAY REFLECT THE ALTERNATE STATE HYPOTHESIS

ABSTRACT

Wildfires are common disturbances that will increase in frequency and intensity as a result of conditions associated with the changing climate. These severe environmental perturbations result in profound economic and ecological repercussions. The objectives of this research were to examine the shift of the forest soil fungal community as a result of a severe wildfire in the New Jersey Pinelands and investigate the state of the communities up to more than two years post fire. The soil fungal communities from severely burned samples collected shortly after the fire were expected to be significantly different from both the unburned samples serving as controls and the severely burned samples collected a year or more after the fire. In 2007, a wildfire severely burned approximately 18,000 acres of the New Jersey Pinelands. Soil samples were collected three times over the span of two years following the fire from the organic and mineral layers of a severely burned site and an unburned control site. Microbial community composition was analyzed by principal component analysis (PCA) and multivariate analysis of variance (MANOVA) of molecular fingerprint data from denaturing gradient gel electrophoresis (DGGE) of fungal-specific amplicons. In the organic layer samples, PCA showed that fungal communities from the unburned samples were significantly ($F_{4,10}$=182.85, P<0.0001) different compared to the severely burned samples collected 2, 13, and 25 months following the fire. Conversely, the microbial

communities in the unburned organic layer were not statistically different over the course of approximately two years. Common fungal operational taxonomic units (OTUs) from the unburned and burned organic layer samples related closely to members of the genera *Penicillium* and *Russula*. Interestingly, the fungi in the mineral layer samples exhibited a similar trend to those in the organic layer samples, despite the drastic difference in microconditions in these soil horizons. The fungal communities in the severely burned organic and mineral layer samples were impacted by the wildfire and continued to change in the span of two years as the community recovered from this disturbance. Rather than returning to a pre-disturbance state, the current microbial communities may reflect an alternate state as a result of the intense perturbation.

1. Introduction

Historically, the New Jersey Pinelands have been long influenced by wildfires, dating back to 1632 when Native Americans drove out wildlife through the use of fires (Forman, 1979). These fires have shaped a unique plant community dominated by pitch pine trees and various oak species that can be detected as far south as coastal Maryland and as far north as Maine (Forman, 1979). Not only have wildfires been shown to impact the aboveground plant community, they also impose extreme direct and indirect stresses that select for dominant microorganisms. An example of a direct effect is the elevated temperature of forest fires, ranging from about 200-300°C (Rundel, 1983), while an indirect effect is the liberation of various soil nutrients that may be utilized by the soil biological community or washed away by rain events.

Fungi are significant contributors to the cycling of nutrients in acidic forest systems such as the New Jersey Pinelands and have been found to change the structure of the soil matrix (Bardgett *et al.*, 2005). Due to their ability to degrade complex substrates, they play a key role in the soil system and are key indicators of change. It has been found high temperature of the fire is more lethal to fungi than bacteria, and the increase in pH favors bacteria over fungi (Bárcenas-Moreno and Bååth, 2009; Smith *et al.*, 2008). In the newly alkaline pH, heterotrophic bacteria have been found to dominate over soil fungi (Dunn *et al.*, 1985). While laboratory studies enhance controlled conditions to better understand fungal ecology, fungal community structure should also be studied *in situ*. For instance, Gassibe *et al.* (2011) analyzed fungal community secondary succession following a wildfire dominated by Mediterranean vegetation of *Pinus pinaster*; however,

the first set of samples were collected a year following the fire and there was no unburned control to evaluate the natural variation in the system.

The effects of physiochemical variables are routinely evaluated to explain changes in microbial community structure. For example, the temperature of the fire, in addition to the composition of the plant community can affect nutrient availability post-fire. In an experiment conducted in the Pinelands, the unburned pitch pine litter was nutrient poor when compared to black huckleberry or white oak (Gray and Dighton, 2006). The plant community serves as substrate in the form of decomposed leaves and needles for the soil microbial community. Another potential substrate for the soil microbial community is biochar, the partial oxidation of plant matter. Biochar has been found to be recalcitrant, and therefore, a carbon pool in soils has stimulated efforts geared towards carbon sequestration (Smith *et al.*, 2010; Knicker *et al.*, 1996). Fungi have unique capabilities to utilize nonspecific extracellular enzymes to degrade the biochar.

Short-term and long-term adaptations of the soil microbial community in sites that have experienced a severe wildfire were anticipated to be reflected by changes in the composition of the soil fungal community. With the hypothesis that forest fires considerably modify the soil fungal community, the aim of our study was to demonstrate that the fungal communities in the unburned soil samples were not significantly different from the communities in the severely burned soil samples. We attempt to address the effect of wildfire perturbation on the soil fungal communities by discussing data generated from a severe wildfire in the New Jersey Pinelands. By linking fungal community structure to nutrient concentrations, a better understanding of the impact of the disturbance can be achieved.

2. Materials and Methods

2.1 Study site

In May of 2007, a wildfire burned approximately 18,000 acres of forest in the Warren Grove section of the New Jersey Pinelands (Figure 1). Site selection was based on soil type (Woodmansie series) and the intensity of the fire. All sites had a similar plant community climate, topography (<0% slope), and soil type (>89% sand). Specifically, these forests are typically homogeneous, with vegetation comprising of *Pinus rigida*, *Quercus alba*, *Quercus marilandica*, *Quercus ilicifolia*, *Gaylussacia baccata*, and *Vaccinium angustifolium*, The sandy soil is dominated by quartz (SiO_2), which contributes to the low cation exchange capacity, low pH, and poor nutrient status in the soils of this region (Forman, 1979).

2.2 Soil sampling

Soil samples were collected in a 36 m^2 area from an unburned site and a severely burned site. The approximate coordinates of the unburned site and severely burned site were N39° 44' 25.3" and W74° 22' 8.6" and N39° 43' 39.3" and W74° 22' 14.3", respectively. Soil samples were collected from a different area for each time point. The adjacent, unburned site served as a basis for comparison and it did not have any visible indications of fire. Some characteristics of the severely burned sites included severely burned trees and a visible ash layer. To monitor how the biological community changed over the course of about two years, soil samples were collected 2, 13, and 25 months after the fire from a severely burned site (SB_1). Soil samples from the unburned site (UB) were collected 2 and 25 months after the fire. The samples were collected during early-

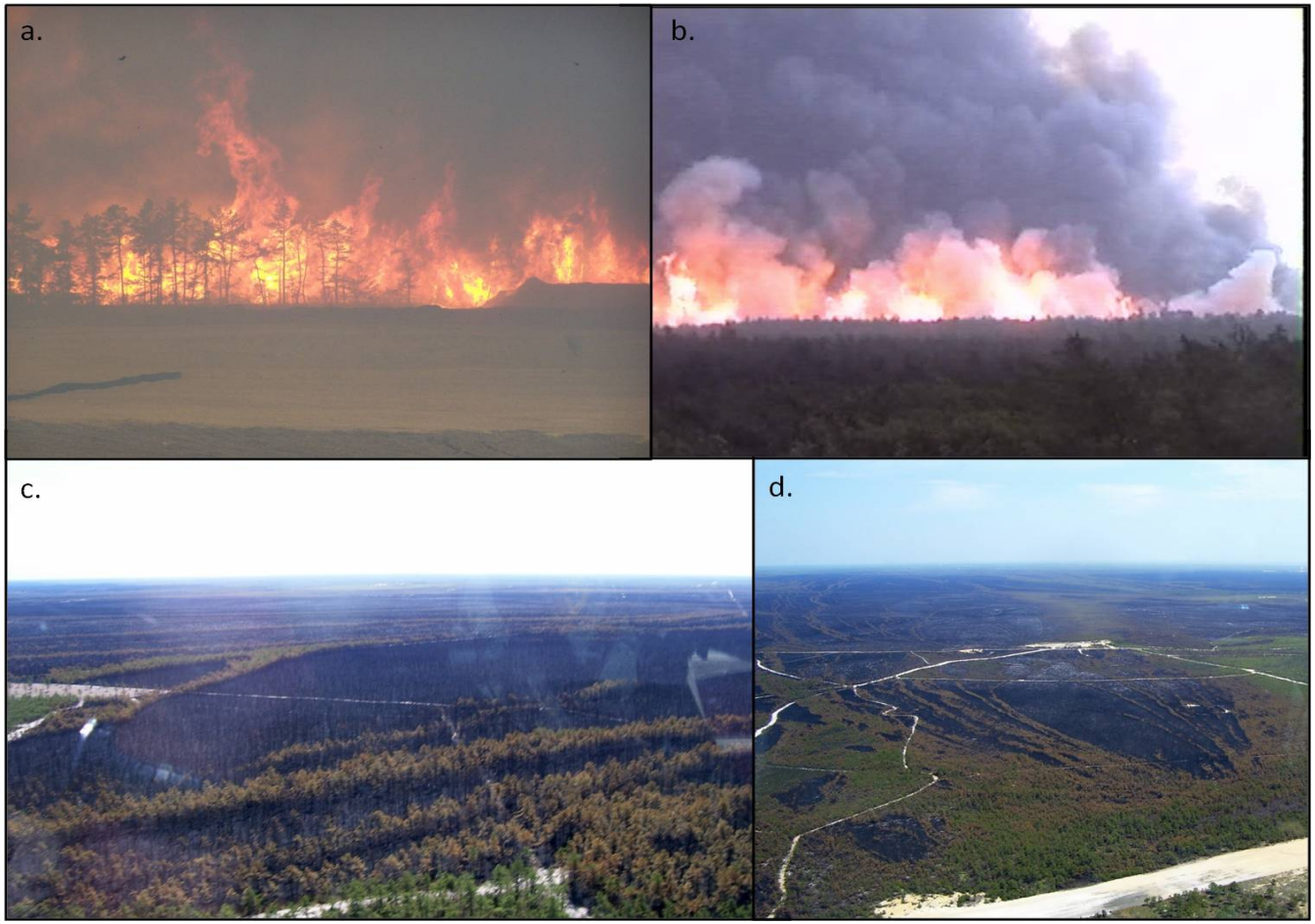


Figure 1. a. Image of the wildfire in May, 2007. b. Smoke plumes as a result of the fire. c. Aerial image of Pinelands forest following the fire. d. Aerial image of the Warren Grove Gunnery Range where the fire originated. Image credit: Nicholas Skowronski.

summer. Nine soil samples were collected per site at a depth of 15 cm, and the organic and mineral layers were separated. All soil samples were well mixed and taken to the Soil Testing Lab at Rutgers University for specific chemical analyses such as pH, phosphorus, potassium, magnesium, calcium, copper, manganese, zinc, boron, and iron (measured as soil extractable cations in ammonium-acetate extract solutions) using standard methods (Sims and Eckert, 2009; Wolf and Beegle, 2009). Additionally, the Soil Testing Lab measured the Total Kjeldahl Nitrogen (TKN), ammonium-N (NH_4^+-N), and nitrate-N (NO_3^--N) only on the organic layer samples (Griffin *et al.*, 2009). For these analyses, only three samples per site were measured. Organic matter analysis was conducted on the soil organic later with the loss on ignition method and on the mineral layer with the dichromate-oxidation (Walkley-Black) method by the Soil Testing Lab (Rutgers University, New Brunswick, NJ) (Schulte and Hoskins, 2009). Nine samples per site were analyzed. The physiochemical results are in Chapter 2. The samples were then stored at -20°C until molecular analyses were conducted.

2.3 DNA extraction and PCR amplification

Total genomic DNA was extracted from 0.3 g of soil with the PowerSoil™ DNA Kit (MoBio) according to the manufacturer's instructions in addition to eluting the genomic DNA with 10 mM Tris buffer (pH of 8). DNA was analyzed by electrophoresis on a 0.9% agarose gel. The gel was stained with ethidium bromide and visualized under UV light. DNA from three of nine samples per site was randomly combined prior to polymerase chain reaction (PCR); in total, three observations per site were obtained. Fungal primers targeting the internal transcribed tracer (ITS) region located between the

18S rRNA and 28S rRNA genes were used because the ITS region yields greater taxonomic resolution than the rRNA regions (Anderson and Cairney, 2004; Viaud *et al.*, 2000). Specifically, ITS1 forward (Gardes and Bruns, 1993) and ITS2 (White *et al.*, 1990) targeted the soil fungal communities. To yield 50 µl reaction mixtures, 2 µl of DNA template, 1 µl of each 20 µM primer, 1 µl of 20 µg/µl Bovine Serum Albumen (Roche, Mannhein, Germany), and 45 µl Supermix (Invitrogen, Carlsbad, CA) were added to each reaction. The PCR cycling parameters for the fungal ITS region included denaturation at 94°C for 5 min., followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by 5 min. extension at 72°C. The amplified products were run on a 0.9% agarose gel to determine the concentrations based on the lambda HindIII standard (Invitrogen Carlsbad, CA). The concentrations of the amplified products were calculated with the Kodak Gel Logic 100 gel imaging system (Eastman Kodak Company, Rochester, NY) software and the amplified products were diluted to yield the same concentration per sample. Positive and negative controls, along with sequence analysis of the targeted area were used to limit the instance of the primers targeting other genes.

2.4 DGGE

The PCR products from the fungal primers were further analyzed through denaturing gradient gel electrophoresis (DGGE). Particular bands of interest were excised for further phylogenetic analysis (Muyzer *et al.*, 1993). The same concentration of PCR products were run on various DGGE gels using the DCode universal mutation system (Bio-Rad, Hercules, CA). The amplicon concentrations on different gels ranged from 350 ng to 1,200 ng of PCR product per DGGE gel. The various denaturing gradients ranged from 25-65%; the 100% denaturant contained 40% formamide and 7

mM urea. Electrophoresis was performed at 60°C at 55 volts for 17.5 hours in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). The DGGE gels were stained with SYBR green I (Sigma Aldrich, St. Louis, MO) and photographed under UV transillumination using the Kodak Gel Logic 100 gel imaging system. Excised bands of interest were eluted in approximately 15 µl of autoclaved QDI water for 24 hours and reamplified with the same fungal primer sets and purified with the ExoSAP-IT® Kit (Affymetrix, Santa Clara, CA). The purified products were sent to GeneWiz, Inc. (South Plainfield, NJ) for sequence analysis. Sequences were analyzed with DNASTAR Lasergene™ (Madison, WI) software and compared to sequences found in GenBank by using the BlastN algorithm.

2.5 Statistical analyses

To analyze the DGGE gels conservatively, each set of statistical analyses were limited to one gel at a time due to the variability between DGGE gels. Therefore, multiple DGGE gels were not compared, but rather each set of samples were run on individual gels and subsequently analyzed. The banding patterns of the DGGE gel were converted to a binary matrix as presence and absence of bands (van Hannen *et al.*, 1998). Principal component analysis (PCA) was conducted to condense the information contained in the large number of original variables (banding patterns) into a smaller set of new composite dimensions (principle component [PC] scores) through SAS® (Cary, NC) software (McGarigal *et al.*, 2000). The PC scores were graphed to visualize the data and determine the degree of similarity between samples (Rudi *et al.*, 2007; McGarigal *et al.*, 2000). A limitation to some ordination techniques such as PCA is that the PC scores may be distributed in the shape of a horse-shoe or arch. The horse-shoe shape may be due to

the fact that the species response to the physical gradient is non-linear (most ordination techniques assume a linear gradient) (Podani and Miklos, 2002). Some groups argue that the horse-shoe is an artifact (Austin, 1985); while others argue that it is a real result of the variation in the data set (Wartenberg *et al.*, 1987). By detrending, the data can be manipulated to flatten the horse-shoe shape; however, this technique may omit real trends in the data. Many groups use PCA to elucidate data from molecular microbial analyses (Krumins *et al.*, 2009; Girvan *et al.*, 2003; Boon *et al.*, 2002). To make sure that the PCA results were not due to artifacts, cluster analysis was performed on the same data set to determine if the trends visualized from PCA could be supported by another statistical technique. Ordination techniques share the same goal of cluster analysis in that they both represent how similar observations lie near each other and dissimilar observations fall far apart in an ordination diagram (Ramette, 2007). Hence, minimum variance clustering was conducted on the DGGE banding pattern data because the data were neat, compact clusters. In addition, proc cluster was used because the data were not continuous measures. Multivariate analysis of variance (MANOVA) was employed on the PC scores to resolve if samples were significantly different (McGarigal *et al.*, 2000).

The chemical and the biological variables were related through canonical correlation analysis (CANOCO) to determine which chemical features correlated to specific soil fungal community structure patterns. In this method, two sets of groups (the chemical and biological parameters measures) are used to maximize the correlation between the variables in those groups (Cooper *et al.*, 2006; McGarigal *et al.*, 2000). CANOCO was used to determine which physical variables correlated with the fungal communities in the unburned or severely burned soils. The biological variables measured

were the fungal banding patterns, represented as PC1 and PC2 values from PCA. The chemical variables measured included pH, gravimetric water content, organic matter content, in addition to the concentrations phosphorus, potassium, magnesium, calcium, copper, boron, and iron (in micrograms per gram). Three concentrations from three samples (the same composite as the DNA samples) were averaged to obtain the same number of observations for the physical data as the biological data. Therefore, three observations per site were obtained.

3. Results

3.1 Impact of the wildfire on the soil fungi in the soil organic layer

The DGGE gel revealed the complex banding patterns of the fungal communities in the organic layer soil samples from both the UB and SB_1 sites (Figure 2a). Due to the complexity of the fungal profiles, cluster analysis was used to visualize any similarities associated with the fungi. The data sorted into five main clades, with the largest separation between the unburned and severely burned samples (Figure 2b). Additionally, the fungal communities in the unburned samples were so similar that one of the unburned samples collected 2 months post-fire clustered most closely with two of the unburned samples collected 25 months post-fire. The fungal communities represented by the severely burned samples collected two months post-fire branched off from the severely burned samples collected 13 and 25 months post-fire, indicating that the fungal communities in the samples collected most recently after the disturbance contained a different community when compared to the other sites (Figure 2b). PCA was used to corroborate the results determined through cluster analysis to gain better insight into how

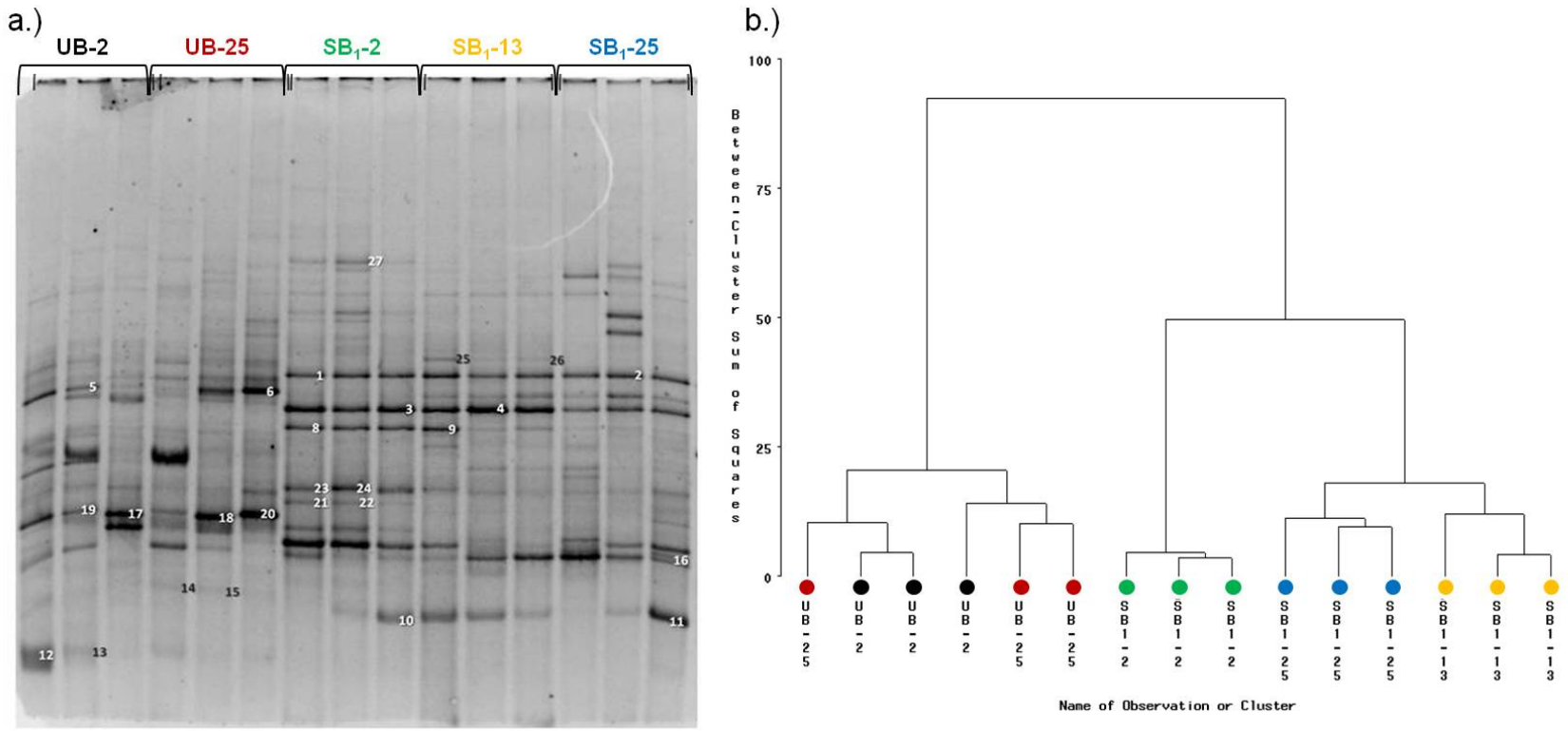


Figure 2. a. DGGE gel of the fungal community profiles in the organic layer from the UB and SB_1 sites. 550 ng of PCR product were used per well. 25-65% gradient. 55V at 60°C for 17.5 hours. Numbers indicate excised bands (note excised band number 7 was from another sample in the gel). b. Cluster analysis of the organic layers samples from the UB and SB_1 sites.

the fungal community structure changed with time following the disturbance, and to determine if the community was returning to the pre-disturbance state. PCA showed that the fungal communities at the UB site composed of a different set of dominant organisms when compared to those members in the SB_1 site (Figure 3). Additionally, the fungal community structure in the SB_1 site from the samples collected 2 months after the fire comprised of the most unique set of community members because they were spatially distal from the other sets of samples on the ordination graph (Figure 3). PC1 accounted for 35.8% of the variation in the dataset. The MANOVA analysis revealed that in PC1, the fungal community profiles of the unburned samples were statistically ($F_{4,10}$=182.85, P<0.0001) different from the profiles of the severely burned samples (Table 1). Also, in PC2, the fungal community pattern was statistically ($F_{4,10}$=55.10, P<0.0001) different from the pattern of the unburned, and severely burned samples collected 13 and 25 months following the fire (Table 1).

Because the soil chemical data alone did not bring insight into how specific soil properties were influenced by the fire, CANOCO was utilized to determine which chemical variables most likely selected for the fungal community composition in either site (Table 2). The physiochemical variables were documented in Chapter 2. Values for manganese, boron, and organic matter were omitted in the analysis because they did not demonstrate a significant correlation with the fungal community patterns from the unburned and burned soils. Additionally, the nitrogen values for some samples used in the biological analysis were not recorded. The CANOCO data suggests that there are some correlations between physical variables and changes in species composition

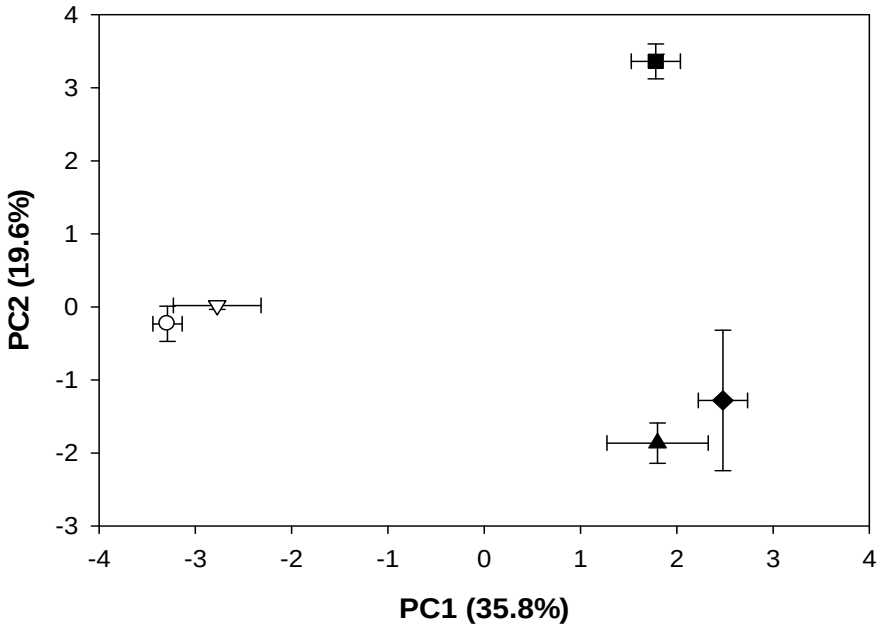


Figure 3. PCA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Organic layer samples from the site SB_1 were collected 2 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 1. MANOVA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months after the wildfire. Organic layer samples from the site SB_1 were collected 2, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (35.8%)	PC2 (19.6%)
UB-2	a	ab
UB-25	a	a
SB_1-2	b	c
SB_1-13	b	bd
SB_1-25	b	d
	$F_{4,10}$=182.85	$F_{4,10}$=55.10
	P<0.0001	P<0.0001

Table 2. CANOCO on the biological and physical data from the organic layer samples collected 2 and 25 months after the fire from the UB site, and 2, 13, and 15 months after the fire from the SB_1 site. For the first canonical variable, $F_{8,18}$=8.83, P=0.0019. The first canonical function explained 95.6% of the variation in the analysis. V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC2, where as the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC1 (see Appendix 1).

	PC1	**PC2**
V1	-0.9902	0.1395

	pH	**P**	**K**	**Mg**	**Ca**	**Cu**	**Zn**	**Fe**	**GWC**
W1	-0.5061	0.6306	0.8749	0.0885	-0.2277	0.4487	0.8420	-0.2848	0.9600

between the UB and SB sites. The fungal species dominant in the UB sites weakly correlated with phosphorus, potassium, copper, zinc, and gravimetric water content. Interestingly, the fungal species in the SB sites strongly correlated with soil pH (Table 2).

Sequence analysis of the excised bands from the organic layer samples revealed organisms related to well-studies saprophytes, mycorrhizal mushrooms, and known antibiotic producers. While fungi related to the mycorrhizal mushroom, *Russula*, were detected in both the UB and SB_1 sites, members related to *Sphaerosporella* were only detected in the SB_1 site (Table 3). Additionally, OTUs related to *Lactarius* and *Leptodontium* were only detected in the burned organic layer samples (Table 3).

3.2 Impact of the wildfire on the soil fungi in the soil mineral layer

Similar to the organic layer samples, the fungal communities in the mineral layer samples exhibited complex banding patterns (Figure 4a). Cluster analysis revealed that each sample set grouped separately, with the largest differences associated with the fungal banding patterns in the UB and SB_1 sites (Figure 4b). In PCA, PC1 accounted for 38.3% of the variation in the data set (Figure 5). While the fungal community pattern in the UB site did not significantly change over the two year sampling period, the pattern in the SB_1 site changed dramatically (Figure 5). More specifically, the fungal community structure in the UB site was significantly ($F_{4,10}$=204.18, P<0.0001; $F_{4,10}$=101.66, P<0.0001) different from the community structure from the SB_1 site according to PC1 and PC2, respectively (Table 4). CANOCO was not significant at the 95% confidence level; therefore, no conclusions regarding the correlation between the biological and chemical variables associated with the mineral layer were made. Sequence analysis of

Table 3. Sequence analysis of fungal bands excised from the DGGE gel of the organic layer samples.

Closest Relative in GenBank (Identities) **GenBank Accession Number**	**UB-2**	**UB-25**	**SB_1-2**	**SB_1-13**	**SB_1-25**
Craterellus sp. (150/160 [94%]) gb|HM468495.1	+	+			
Russula sp. (284/295 [97%])* gb|EU819421.1	+	+			
Trechispora sp. (281/298 [95%])* gb|EU909231.1	+	+			
Penicillium sp. (267/284 [95%])* gb|HQ631040.1			+		
Penicillium sp. (268/281 [96%])* gb|HQ631040.1			+	+	
Gelasinospora sp. (263/264 [99%])* gb|AY681191.1			+	+	+
Lactarius sp. (297/321 [93%])* gb|AY854089.1			+	+	+
Leptodontidium sp. (276/289 [96%])* gb|FJ903294.1			+	+	+
Sphaerosporella sp. (202/213 [95%])* gb|U38587.1			+	+	+
Russula sp. (258/279 [93%])* gb|FJ845435.1					+
Russula sp. (282/291 [97%])* gb|HQ604844.1	+	+			+
Penicillium sp. (232/252 [93%])* gb|HM214448.1	+	+	+	+	+

* indicates that the forward and reverse primers were in agreement.
+ indicates presence of the sequence in a particular site, while an empty space indicates that the sequence was not detected there.

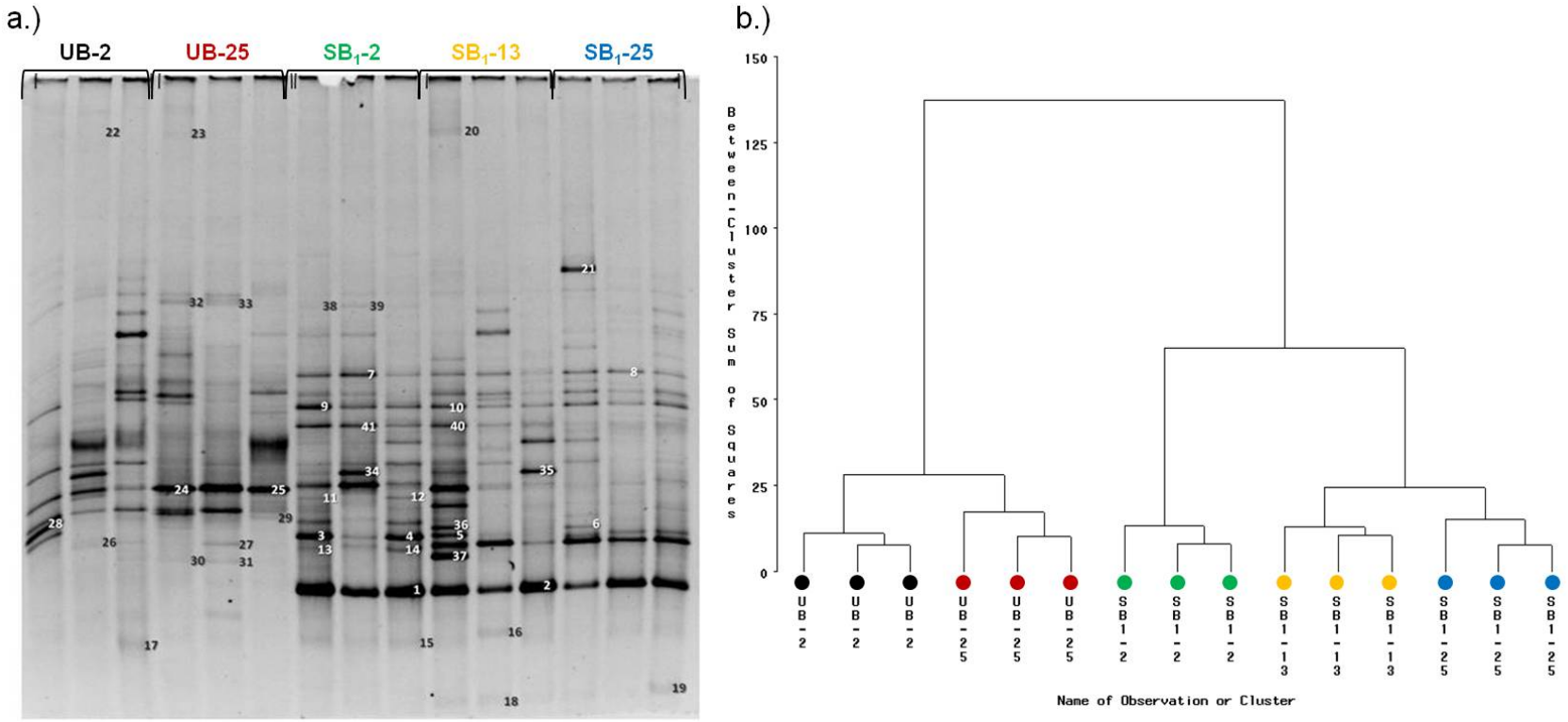


Figure 4. a. DGGE gel of the fungal community profiles in the mineral layer from the UB and SB_1 sites. 550 ng of PCR product were used per well. Numbers indicate excised bands. b. Cluster analysis of the mineral layers samples from the UB and SB_1 sites.

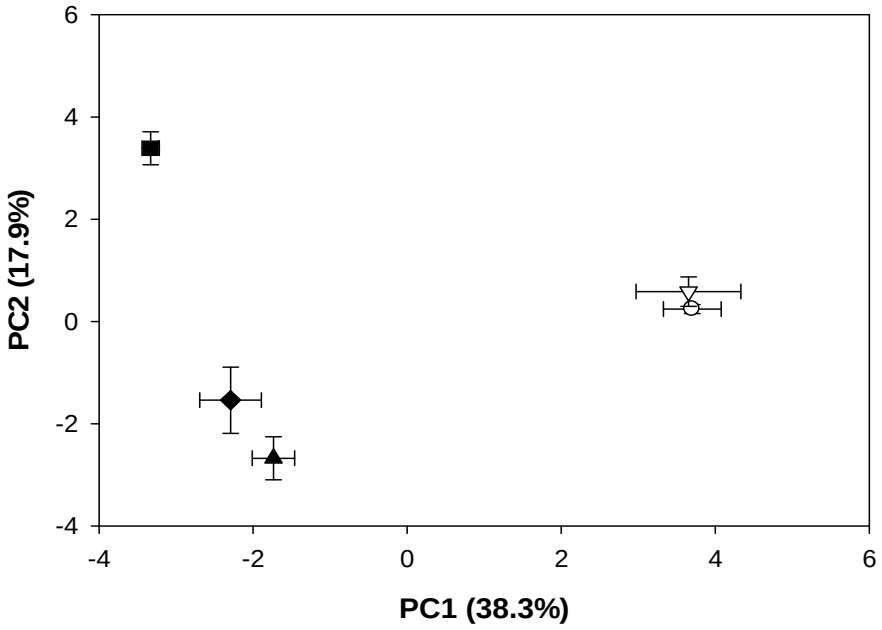


Figure 5. PCA of the DGGE banding patterns. Mineral layer samples from the UB site were collected 2 (open circle) and 25 (open triangle) months after the wildfire. Mineral layer samples from the site SB_1 were collected 2 (closed square), 13 (closed diamond), and 25 (closed triangle) months after the wildfire. For each symbol, *n*=3 and error bars indicate standard deviation.

Table 4. MANOVA of the DGGE banding patterns. Organic layer samples from the UB site were collected 2 and 25 months after the wildfire. Organic layer samples from the site SB_1 were collected 2, 13, and 25 months after the wildfire. Samples with the same letter in the same PC were not significantly different.

Site	PC1 (38.3%)	PC2 (17.9%)	PC3 (8.2%)
UB-2	a	a	a
UB-25	a	a	b
SB_1-2	b	b	c
SB_1-13	bc	c	bc
SB_1-25	c	d	c
	$F_{4,10}$=204.18 P<0.0001	$F_{4,10}$=101.66 P<0.0001	$F_{4,10}$=17.88 P<0.0001

the mineral layer samples revealed that sequences related to *Amanita*, a known mycorrhizal mushroom, were only detected in the undisturbed site, while sequences related to *Lactarius* and *Leptodontium* were detected in the disturbed site (Table 5).

4. Discussion

With the frequency of wildfires increasing across the United States, efforts aimed at understanding the impact of these disturbances on the soil fungal community becomes a central environmental issue. Not only do fungi play a significant role in the degradation of complex plant materials and cycling of nutrients in the belowground soil systems, they also serve as sensitive indicators of environmental changes. More specifically, fungal populations have been found to be more susceptible to disturbance than bacteria by responding more quickly to changes in soil carbon, temperature, and pH (Bárcenas-Moreno and Bååth, 2009). Therefore, it is important to detect the changes associated with fungal community structure following a disturbance to gain insight into the overall health of the system.

In this study, the fungal community structure in the organic and mineral layers shifted as a result of the wildfire disturbance. In a two year time period, the fungal community in the undisturbed site did not change significantly. However, the fungal community in both the organic and mineral layers of the burned site shifted dramatically in that time period. Studies of fungi in soil samples collected from prescribed burns one year post-burn in a Sierra Nevada ponderosa pine forest (Stendell *et al.*, 1999), five months post-burn in a Swedish boreal forest (Dahlberg *et al.*, 2001), in addition to fourteen days post-burn in an Austrailian sclerophyll forest (Chen and Cairney, 2002) showed that ectomycorrhizal fungi were impacted more so in the organic layer when

Table 5. Sequence analysis of fungal bands excised from the DGGE gel of the mineral layer samples.

Closest Relative in GenBank (Identities) **GenBank Accession Number**	**UB-2**	**UB-25**	**SB_1-2**	**SB_1-13**	**SB_1-25**
Amanita sp. (332/342 [98%])* gb| AB015696.1	+	+			
Penicillium sp. (245/274 [90%])* gb|FJ009566.1	+	+			
Penicillium sp. (251/269 [94%])* gb|GU212865.1			+		
Gelasinospora sp. (252/252 [100%])* gb|AY681191.1			+	+	+
Lactarius sp. (232/251 [93%])* gb|AY854089.1			+	+	+
Lecythophora sp. (229/244 [94%])* gb|FJ903377.1			+	+	+
Leptodontidium sp. (252/264 [96%])* gb|FJ903294.1			+	+	+
Penicillium sp. (269/285 [95%])* gb|HQ631040.1			+	+	+
Rusulla sp. (266/267 [99%])* gb|DQ777988.1			+	+	+
Cladosporium sp. (230/241 [96%])* gb|AF393723.2				+	+
Elaphomyces sp. (326/327 [99%])* gb|GU550112.1				+	+
Russula sp. (284/284 [100%])* gb|DQ777996.1	+	+		+	+

* indicates that the forward and reverse primers were in agreement
+ indicates presence of the sequence in a particular site, while an empty space indicates that the sequence was not detected there

compared to the mineral layer. These results are consistent with the observation that soil microbes residing in the soil organic layer are more impacted by wildfire (Neary *et al.*, 1999). However, in this study, there were clear shifts in fungal community patterns in both the organic and mineral layers. Thus, in both soil layers, the wildfire stressed the fungal communities to cause shifts in the dominant members of the communities. These shifts in the fungal community structure can lead to changes in energy pathways, as demonstrated by Bisset and Parkinson (1980).

Overall, indirect, as well as direct effects of the wildfire influenced the fungal community structure. In the upper soil layers of an Australian sclerophyll forest (Bastias *et al.*, 2006) and a Pinelands pine-oak forest (Tuininga and Dighton, 2004), the fungal community composition was found to be impacted by repeated prescribed burning. Specifically, Tuininga and Dighton (2004) studied ectomycorrhizal fungi. Interestingly, Bastias *et al.* (2006) found that the fungal community composition was not impacted by prescribed burning in the 10-20 cm region of the soil. It is therefore necessary to differentiate between prescribed burning and wildfire events. In our study, we demonstrated that the fungal community in both the organic and mineral soil layers shifted as a result of wildfire. Most importantly, the impact of the wildfire was detected up to a year post-fire and if the two and five month sampling periods were absent, the initial dramatic shift would have remained unnoticed.

Because soil geochemistry greatly influences the soil microbial recovery after a fire (Hart *et al.*, 2005), the biological and chemical parameters measured in this system were correlated. In agreement with another study that reported soil temperature, pH, and carbon as the main drivers of the soil microbial community structure in a site (Hamman

et al., 2007), our results indicated that soil pH correlated strongly with the fungal community in the disturbed site. Interestingly, pH correlated with the fungal community shifts as a result to the wildfire; however, it did not correlate with the bacterial community shifts, as documented in Chapter 2. In an early study, pH was found to favor bacterial populations over fungal populations (Bisset and Parkinson, 1980). While Smith *et al.* (2008) reported that the increase in soil pH drives the bioavailability of other nutrients such as phosphorus, calcium, magnesium, and potassium, there was not a strong correlation with the disturbed samples and these soil nutrients in this study. Either the fire did not liberate these nutrients, or they became bioavailable immediately after the fire and subsequently utilized by the soil biological community. The latter is the more likely explanation because at approximately 200°C (the minimum temperature range of most crown fires), soluble nitrogen, sulfur, phosphate, calcium, and magnesium have been shown to become more available to the soil community (Gray and Dighton, 2006). Additionally, fungal diversity has been shown to be influenced by interactions between environmental variables and microorganisms, rather than influenced by reductions in plant diversity (Brodie *et al.*, 2003). The cycling of these elements, driven by the soil biological community, is essential in oligotrophic systems such as the New Jersey Pinelands (Boerner, 1982 in Gray and Digton, 2006).

The second objective was to determine if the fungal community recovered to reflect its pre-disturbance structure, or perhaps change to an alternate state. In the two year period following the fire, the fungal communities in the SB_1-13 and SB_1-25 samples exhibited similarities, yet were disparate from the fungal community structure in the undisturbed site. The alternate state hypothesis, which is commonly used to study the

impact of disturbance on macroorganisms and large microorganisms, was used to reflect the status of the microorganisms as a result of the fire disturbance (Price and Morin, 2009; Bertness *et al.*, 2002). The molecular tools used in this study only provided a snapshot of the community at that particular time point; however, since the fungal communities in the SB_1 samples collected 13 and 25 months after the fire clustered, the communities may be approaching an alternate state. It is possible that as the vegetation matures to mimic the undisturbed site, the soil fungal community structure may also reflect that of the undisturbed site; however, that would not occur until twenty or so years. Exotic plant species can significantly change the soil community structure and function (Kourtev *et al.*, 2003); therefore, the absence of invasive species following the burn negates that factor as a significant influence on the system. As revealed in Chapters 2 and 3, the soil bacteria and archaea did not exhibit such tight clustering; therefore, perhaps the fungal community was the best representative model for the alternate stable state hypothesis.

Sequence analysis of the excised bands from the organic layer samples revealed organisms related to well-studies saprophytes, mycorrhizal mushrooms, and known antibiotic producers. These fungi are important microbes that degrade complex organic matter and perhaps the recalcitrant biochar that resulted from the wildfire. While fungi related to the mycorrhizal mushroom, *Russula*, were detected at both the UB and SB_1 sites, members related to *Sphaerosporella* were only detected in the SB_1 sites. Members of the *Sphaerosporella* genus have been found to be closely associated with jack pine trees in burned soils (Visser, 1995). Additionally, OTUs related to *Lactarius* and *Leptodontium* were only detected in the burned organic layer samples.

In a study evaluating the effect of wildfire on the soil fungal community in a *Pinus pinaster*-dominated forest, Gassibe *et al.* (2011) found that mycorrhizal and saprotrophic fungi both exhibited decreased numbers of species detected following the wildfire. In another study it was found that adding ectomycorrhizal fungi (mainly *Rhizopogon roseolus*) to both burned and unburned ground stimulated the growth of *Pinus pinaster*, which may help in recovery efforts of *Pinus pinaster* plantations in the Mediterranean (Sousa *et al.*, 2011). While our study did not quantitatively reveal the number of mycorrhizal and saprotrophic fungi, it demonstrated that members related to *Penicillium*, *Gelsinospora*, and *Elaphomyces* were able to dominate in the soil surface after the intense perturbation. Additionally, *Cladosporium*, a fungus found to decompose herbaceous litter in its early stages, was not only detected in the burned soil in the Pinelands system, but also in the burned soil of a subalpine coniferous forest (Bisset and Parkinson, 1980).

5. Conclusions

By sampling along a chronosequence of time-since fire, the changes associated with the fungal community patterns were captured. Our study demonstrated that the wildfire dramatically impacted the structure of the soil fungal community. While the fungal community composition in the unburned samples did not change significantly over two years, the severely burned samples exhibited community shifts in this time period following the wildfire. Therefore, these data support our hypothesis that the wildfire had been driving soil community dynamics in the initial two years after the perturbation. Rather than return to the pre-disturbance state, the soil fungal communities reflected an alternate state two years following the wildfire, as proposed by the alternate state

hypothesis. These changes were tracked along with the shifts in the abiotic variables to provide information regarding important factors for biotic recovery in disturbed systems. pH was most strongly correlated to the community structure in the disturbed samples; therefore, represents one of the main abiotic stresses that shaped the fungal community dynamics.

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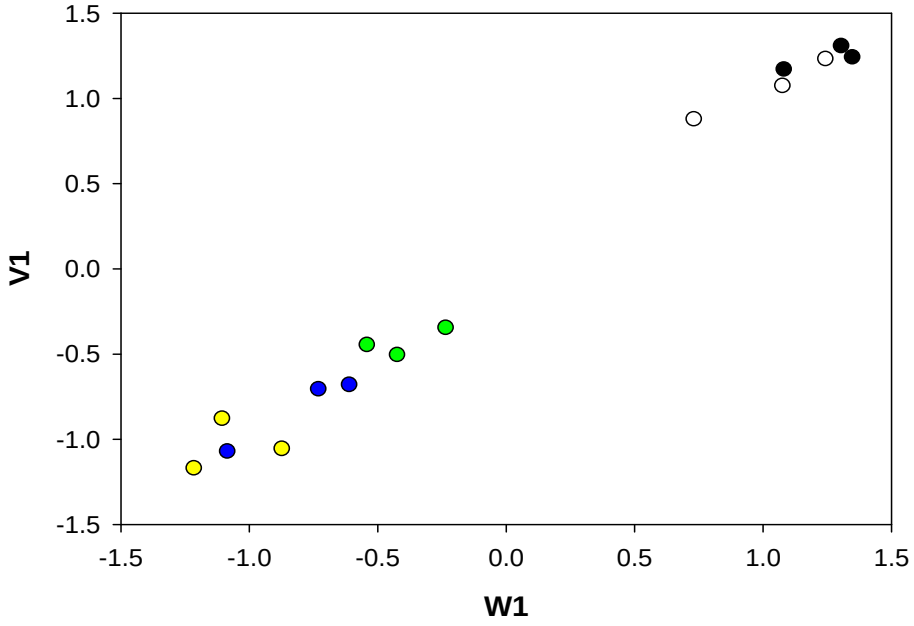
Appendix 1. CANOCO Chart of the Correlation of the Fungal Community Patterns to Physiochemical Variables in the Organic Layer Soils



Supplemental CANOCO data of the correlation of the fungal community patterns to physiochemical variables in the organic layer soils. The organic layer soil samples were characterized as: UB-2 (black), UB-25 (red), SB_1-2 (green), SB_1-13 (yellow), SB_1-25 (blue). V1 was the canonical variable associated with the PC scores. W1 was the canonical variable associated with the physical variables. The UB-2 and UB-25 samples associated with PC2, where as the SB_1-2, SB_1-13, and SB_1-25 samples associated with PC1.

CHAPTER 5

DISCUSSION

Wildfires are common disturbances that dramatically impact the forest nutrient cycling and community composition, both on macro- and micro-scales. While many studies have focused on the impact of fire on vegetation and soil chemistry, studying the impact on the soil microbial community brings great insight into the nutrient cycling and potential predictive capabilities for similar forest systems. The three studies in this dissertation demonstrated the effects of wildfire on soil microbial community composition in the New Jersey Pinelands. Furthermore, the relationship between the physiochemical and biological variables were elucidated to better understand the connection between soil abiotic and biotic factors following a disturbance.

To demonstrate how soil microbial communities respond to disturbance, soil samples were collected from sites within the New Jersey Pinelands ecosystem. A wildfire occurred in May, 2007, subsequently destroying approximately 18,000 acres of forest. The crown fire was hypothesized to cause a shift in the composition of dominant soil microorganisms based on their ability to respond to the fire-induced changes in their environment. Microbes better adapted to the changing conditions were expected to propagate and dominate the community. However, the extent of the change and time for recovery of the community were largely unknown. Some of the soil community might have been maintained because microorganisms were either inactive when the fire occurred or were located in soil sites where the impact of the fire was minimal.

Following this environmental perturbation, possible scenarios included 1) the microbial composition was resistant to the disturbance, 2) the microbial structure changed

as a result of the disturbance, but quickly recovered to the original community composition, 3) the microbial community changed following the disturbance; however, the non-resilient community exhibited similar process rates (functional redundancy) as the original community, and 4) the microbial community not only changed following the disturbance, but the new community was functionally dissimilar to the original one. The goal of this research project was to investigate the status of the soil microbial community following the fire event as per the above-mentioned scenarios. Another aim was to discern the important microorganisms that became dominant players in nutrient mobilization especially because the New Jersey Pinelands contain extremely nutrient poor soils.

The bacterial, archaeal, and fungal communities were hypothesized to respond differently to the wildfire disturbance. Because bacteria and archaea are generally in high abundance, widely dispersed, evolve rapidly, and are metabolically versatile, they were expected to be resilient to the changes associated with wildfires (Allison and Martiny, 2008; Finlay *et al.*, 1997). For instance, bacterial communities commonly exhibit functional redundancy in that many different species utilize similar substrates and conduct similar nutrient cycling processes. The greater the number of taxa that perform a process, the more buffered the process is to environmental perturbations (as cited in Allison and Martiny, 2008). Furthermore, biogeochemical cycling is not limited by genetic diversity (Finlay *et al.*, 1997). Thus, even if many bacteria initially perish as a result of the extreme burn, the functions provided by the biological community may remain the same. Alternatively, the functions could change, which would dramatically influence the concentration of nutrients in the soil that are bioavailable to the flora. There

is redundancy of metabolic functions between species, and therefore, it is reasonable to claim that the capacity of the system does not always decline when species diversity declines (Bardgett *et al.*, 2005; Madigan *et al.*, 2003; Nannipieri *et al.*, 2003). Thus, the functional redundancy of soil bacteria may assist in maintaining the capacity of the system to function despite a decline in species diversity.

Current studies have focused mainly on either the physiochemical parameters or the biological parameters that have been altered as a result of fire. There is a wealth of literature regarding the soil chemical parameters that are impacted by controlled laboratory heating experiments (Sousa *et al.*, 2011; Bárcenas-Moreno and Bååth, 2009), prescribed burns (Jurgens and Saano, 1999; Tuininga and Dighton, 2004; Choromanska and De Luca, 2001), and wildfires (Smith *et al.*, 2008; Hamman *et al.*, 2007; Yeager *et al.*, 2005). Most studies reported a general trend of pH and nutrient concentration fluctuations as a result of a burn event (Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999; Fernández *et al.*, 1997); however, others have detected contrasting patterns (Hamman *et al.*, 2007; Johnson *et al.*, 2007). Two factors contribute to soil chemical variability following fire events. One is the heterogeneous spread of fire based on fuel loads (Neary *et al.*, 1999) because areas of high fuel loads yield aggressive fires. The second is the heterogeneous nature of the soil matrix in that the chemistry of the microhabitats varies spatially.

Additionally, previous studies regarding forest burns have either focused on the microbiological status of the system a year or more following the fire (Smith *et al.*, 2008; Hamman *et al.*, 2007; Jurgens and Saano, 1999) or the impact of less intense prescribed burns (Jurgens and Saano, 1999). While the former studies offered understanding of

complex microbial processes, they neglected the critical time period of the first year following the fire when important microbial community structural changes might be taking place. Additionally, the latter provided insight into fires in general; however, prescribed burns impact the soil community in different ways when compared to wildfires (Choromanska and DeLuca, 2001; Neary *et al.*, 1999). The goal of this research project was to overcome these limitations by sampling within the first year of a wildfire. Furthermore, the biological and physiochemical variables were correlated in an effort to determine the factors that impacted the biological communities.

As presented in Chapter 2, the impact of wildfire on soil chemical and physical variables was examined. The findings demonstrated the unpredictable nature of the soil nutrient concentrations following the fire. Unlike other studies that documented that soil pH and micronutrient concentrations generally become elevated in burned systems (Hamman *et al.*, 2007; Murphy *et al.*, 2006b; Knoepp *et al.*, 2004; Neary *et al.*, 1999), an immediate increase in the soil pH was not observed in this study, except in the organic layer samples from the SB_1 site collected two months after the burn. The mean pH values of these soils ranged from 3.35 to 4.07. Thus, the soil was acidic, selecting for acidophiles based on the environmental conditions, as documented in Chapter 2. Because both chemical and biological analyses were used in conjunction with one another, operational taxonomic units (OTUs) related to members of the phylum Acidobacteria were detected in the undisturbed and disturbed soils.

Additionally, the soil micronutrient concentrations were monitored because members of the soil microbial community mediate chemical reactions to cycle nutrients throughout the forest ecosystem. The nutrient cycling reactions become extremely

important in low nutrient systems such as the New Jersey Pinelands. Due to the high temperatures of wildfires and the high volatilization temperatures of many soil nutrients (Neary *et al.*, 1999), important elements were predicted to be liberated and subsequently available to soil microbes as a result of the disturbance. Therefore, microbes capable of utilizing the liberated nutrients may have dominated shortly after the fire. Many studies have reported a general increase (Murphy *et al.*; 2006b, Knoepp *et al.*, 2004; Neary *et al.*, 1999) or decrease (Castro *et al.*, 2006; Murphy *et al.*, 2006b; Knoepp *et al.*, 2004) of soil extractable nutrients as a result of a burn disturbance. The increase of micronutrients post-fire was not observed, possibly due to the high percentage of sand which contributed to the extremely low cation exchange capacity of the soil. Perhaps many of the cations that were liberated by the fire event were washed away by periods of rain; therefore, masking an elevation in cation concentrations two months following the fire. This scenario would also impact the composition of microbes in the severely burned soils, particularly in the months immediately following the fire event.

Additionally, nitrogen, an important soil nutrient, was predicted to be impacted by the forest fire because it has a relatively low volatilization temperature (Neary *et al.*, 1999). Thus, nitrogen concentrations were hypothesized to be very low in the severely burned sites immediately after the fire, selecting for nitrogen fixers to assimilate nitrogen gas to bioavailable forms. In accordance with this hypothesis, members related to Cyanobacteria and *Clostridia*, known nitrogen fixers, were detected in the samples collected two months after the wildfire. The forest canopy was destroyed in the fire; therefore, there were the presence of lichens able to conduct important photosynthetic reactions that otherwise would not occur in the undisturbed site. Additionally, members

related to Cyanobacteria were detected in the biochar on the bark of the *Pinus rigida* trees (data not shown). The proliferation of these bacteria on the trees may have been the source of inoculum on the surface of the burned soil.

The results of Chapter 2 indicated both the short term and the long term impacts of fire on soil bacterial communities. Specifically, the soil bacterial fingerprints changed with time in the severely burned samples, as described through cluster analysis and PCA of bacterial amplicons subjected to PCR-DGGE techniques. While the short term study highlighted the bacterial community shifts within the first year and a half of the fire, it did not answer important questions regarding the status of the community compared to the undisturbed community. To answer this question, the second part of Chapter 2 tested the impact of the wildfire on the soil bacterial community structure in a two year time period. Interestingly, after two years, the bacterial community pattern in the severely burned samples did not yet reflect that of the unburned samples, but rather maintained a dominant community that was different from the undisturbed site. The rate of change of the bacterial community appeared to be slowing after one year following the fire. These results suggest that the bacterial community structure in the disturbed sites could be approaching an alternate state. While the analyses were helpful in obtaining some knowledge regarding what microbes were present in the samples, the methods used to detect unique OTUs present in either the unburned or burned sites were not sensitive enough to detect differences at the species level, mainly in the genus *Mycobacterium* and phylum Acidobacterium. Therefore, primers that target specific groups of bacteria may provide a more sensitive measure of the particular groups of bacteria that were more

impacted by the fire. This study achieved better insight into what types of bacteria were most impacted by the fire, mainly the Mycobacteria and Acidobacteria.

Because the chemical data alone in the sites were not very informative, the biological and physiochemical parameters were correlated. Surprisingly, a correlation between the bacterial community in the soil organic layer and pH was not detected, contrary to the findings of other studies (Lauber *et al.*, 2009; Fierer and Jackson, 2006). The results from Chapter 2 supported the hypothesis that the wildfire disturbance shifted the soil bacterial community composition with time following the fire. Additionally, Chapter 2 discerned that the soil chemical data alone is not a reasonable indicator of the effects of the wildfire on the soil community, but rather the biological and chemical data must be resolved together. Therefore, examination of the soil biological community in addition to the chemical features associated with the changing soil is paramount to determine the impact of the disturbance on the soil system.

The findings from Chapter 2 sparked curiosity regarding the impact of the fire disturbance on members from the other domains of life. Bacteria are but one type of organism living in the soil; there are so many more that could potentially be impacted by the fire in different ways. Therefore, the influence of wildfire on soil archaeal community patterns was explored. The archaeal communities were hypothesized to exhibit a different response than the bacteria due to differences in cell wall chemistry and/or metabolic capabilities (Madigan *et al.*, 2003). Alternatively, the bacterial and archaea may have exhibited similar trends following a disturbance because they are relatively the same size and may have the same predators (Madigan *et al.*, 2003). The findings from Chapter 3 suggested that the archaeal communities exhibited similar

patterns as the bacterial communities with regard to the fire disturbance. Specifically, the archaeal communities from the unburned site clustered separately from those of the burned site collected 2, 13, and 25 months after the fire. Based on these indications of changes in population diversity approximately two years following the wildfire, archaeal OTUs exhibiting major population changes were evaluated. Due to limitations in culturing archaea, the databases were found to be insufficient in providing knowledge regarding the identities of the organisms associated with the samples. Another limitation to this study is the use of molecular tools in the search of archaeal samples. Because both bacteria and archaea share the 16S rRNA gene, primers thought to be specific to archaea were capable of amplifying members of bacteria (Cytryn *et al.*, 2000). Despite these limitations, the fire disturbance was shown to impact soil archaeal community structure in meaningful ways. In both the organic and mineral soil layers, the archaeal community composition changed with time as a result of the fire and has not returned to the pre-disturbed state. Specifically, the rate of change in archaeal community composition was slowing down one year after the burn. In the time period between the first and second year after the burn, the archaeal community dynamics were not changing significantly; therefore, the nutrient cycling reactions may have remained the same as well. Studies focused on investigating the functions of the system that may have been impacted by the wildfire would answer if the cycling reactions were affected. Furthermore, the archaeal community composition was not resistant to the environmental perturbation, but rather dramatically changed after the disturbance. To our knowledge, this is the first study to document how the archaeal community composition was impacted by a wildfire within a

two year time period, specifically comparing the undisturbed and disturbed community profiles.

The similarities in the community patterns of bacteria and archaea may be attributed to the similarities of size and metabolic capabilities of these microorganisms; therefore, examination of representative eukaryotes was essential. Furthermore, Chapter 4 explored the changes in fungal community structure as a result of the wildfire. The findings indicated that the fungal communities exhibited dramatic community structure shifts as a result of the disturbance in both the organic and mineral soil layers. Unexpectedly, the fungi exhibited similar community shifts as the bacteria and archaea. Therefore, the wildfire impacted members from all three domains of life. While this project examined these domains separately, it is important to consider the soil environment *in situ*. Specifically, these microorganisms were living in conjunction with one another in the soil matrix, experiencing similar nutrient and perhaps predatory stresses. Additionally, in the soil environment, fungi have developed antibiotic proliferation strategies to compete against bacteria for nutrients and space. Therefore, it is important to consider that the patterns associated with fungal communities as a result of the fire may reflect the patterns associated with bacterial communities. For instance, if *Penicillium*, a known β-Lactam producer, proliferated immediately following the fire, then there may have been a selective pressure against gram positive bacteria that lack β-Lactamase. Thus, antibiotic resistant bacteria may have been selected over nonresistant bacteria or the horizontal gene transfer of antibiotic resistance genes may have increased. Exploration of how the number of antibiotic resistance genes changed as a result of the

fire would resolve if antibiotic resistance increased shortly after the fire due to the proliferation of antibiotic-producing fungi.

Interestingly, the response of the fungal community best reflected the alternate state hypothesis when compared to the bacterial and archaeal community dynamics because the fungal communities in the severely burned soils collected one and two years following the fire clustered tightly in the PCA graphs. The bacteria and archaea were expected to respond to the environmental changes associated with the wildfire and quickly adapt by returning to the pre-disturbed state before more evolved microorganisms such as fungi. The results from this dissertation suggested the opposite. One, none of the communities reflected that of the undisturbed site two years following the wildfire. Two, the fungal communities in the severely burned soils appeared to reach the alternate state before the bacterial and archaeal communities. Specifically, while the fungal communities had not reached a similar community composition to the undisturbed site, they stabilized one year following the fire, while the archaeal and bacterial communities did not exhibit such a clear pattern.

It is important to note that this study utilized DNA extraction techniques, rather than RNA, to explore community composition; therefore, both the active and inactive members of the communities were surveyed. Despite this limitation, DNA analyses brought insight into knowledge of the system because community changes were detected in the time period following the wildfire. Additionally, even though soil is heterogeneous when compared to other environmental media, the soil particle size distribution contained approximately 89% sand. Therefore, the relatively homogeneous soil and vegetation (comprised of two predominant tree species), in addition to the lack of invasive plant

species limited the selective pressures other than the wildfire that could have been driving soil community dynamics. The main observational differences between the undisturbed and disturbed sites were the age of the vegetation and visible ash layer, both attributed to the wildfire. Therefore, our findings from this study provided interesting results regarding how forest soil microbial communities responded to wildfire.

Together, the data from these three studies reveal the complex interactions occurring under natural conditions, in addition to the complexity surrounding disturbance events. Though intricate, this field study provided valuable information regarding microbial community structure under natural conditions. Our project provided support for the hypothesis that wildfires impact the soil microbial communities in such drastic ways that they do not return to the pre-disturbance state.

Based on the four possible scenarios illustrated in the beginning of the discussion, the bacteria, archaea, and fungi were best represented by the third and fourth scenarios. For instance, the microbial community changed as a result of the disturbance and either the community was functionally similar or dissimilar from the undisturbed community. The implications of alternate states are far reaching because the functioning of the microbial communities may change. If the functions change, then the rate at which the microbes utilize nutrients, in addition to the rate of release of essential nutrients to other soil community members would be altered. Thus, over a longer time period, the vegetation may be different, or perhaps invasive species would infiltrate the forest ecosystem. Further investigation of functional genes would discern the nutrient cycling reactions that were impacted by the wildfire. Additionally, functional gene analysis would reveal if the communities were functionally redundant following the fire. With

this knowledge, better predictions could be made in other forest systems that experience wildfire, or may experience wildfire in the future due to conditions associated with the changing climate.

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CONCLUSIONS

Studies relating to the continued stability of the forest ecosystem are important for the prediction of how microorganisms and nutrient cycles are impacted by disturbances such as fire. The results of this study revealed that fires greatly influence the community structure of soil microbes.

With an increase in frequency and intensity of forest fires in the future, the need for research concerning the stability of the ecosystems after the fire will increase. Policy targeting these issues of climate change conditions and forest fires will promote more sustainable and mutually beneficial relationships between humans and disturbed environments.

Specific Conclusions

1. Chemical data alone did not serve as adequate indicators of the effects of fire on the soil community composition and status of the soil system.
2. Representative members from all three domains of life were shown to be impacted by the wildfire that occurred in the New Jersey Pinelands in 2007.
3. While the microbial communities in the undisturbed sites did not exhibit community shifts in the two year time period following the fire, the microbial community structure in the severely burned sites demonstrated meaningful fire-induced changes.
4. Rather than return to the pre-disturbance state, the soil microbial community structure appeared to reflect an alternate state two years following the burn.
5. Interestingly, the microorganisms residing in the organic and mineral soil layers exhibited similar structure patterns as a result of the wildfire. Thus, the direct, as well as

indirect effects of the wildfire affected the soil microbial communities deeper in the soil profile than originally expected.

6. Sequence analysis served as a useful tool to gain insight into the operational taxonomic units that dominated after the burn.

7. Correlations between the measured soil physiochemical and biological variables allowed for a better understanding of the specific environmental parameters that selected for the observed changes associated with the soil biological community structure post-fire.

CURRICULUM VITAE

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