THE EFFECTS OF CHANGING PRECIPITATION PATTERNS ON SOIL

MICROBIAL COMMUNITIES AND NITROGEN CYCLING IN THE NEW JERSEY

PINELANDS

by

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ABSTRACT OF THE DISSERTATION

THE EFFECTS OF CHANGING PRECIPITATION PATTERNS ON SOIL MICROBIAL COMMUNITIES AND NITROGEN CYCLING IN THE NEW JERSEY PINELANDS

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I studied the potential effects of a change in the amount, frequency and timing of precipitation on soil microbes and nitrogen cycling in the New Jersey Pinelands. I performed a two year field manipulation of precipitation amount and measured the response of the microbial community, potential net nitrogen mineralization and amino acid production. I found that soil microbes were not affected by rain exclusion or a doubling of rainfall. Nematode densities, but not community composition, were sensitive to precipitation amount. A large accumulation of ammonium in drought plots suggested sustained microbial activity under extreme drought conditions. I observed small changes in potential net nitrogen mineralization due to the effects of soil moisture on diffusion and immobilization. I measured the short-term response of the microbial community to a rewetting of dry soil and found a very rapid (three hour) change in the microbial community. The accumulation of ammonium within drought plots appears to have suppressed fungal biomass following the rewetting event.

In a two year winter study, I found no long-term effect of supplemental winter rainfall on the soil microbial community. Elevated winter precipitation prevented ammonium accumulation, presumably by protecting plant roots from freeze damage. I found that supplemental watering insulates soil microbes from cold stress over the short-term (days), but that mid-winter declines in biomass due to cold soil recovered by the start of spring.

These experiments demonstrate that soil microbial communities in Pinelands soils are highly tolerant of abiotic stressors such as drought, upshock stress and soil freezing. Recovery from these disturbances is extremely rapid, occurring on the scale of hours to days. I conclude that changing precipitation patterns will not have a direct, long-term effect on soil microbial communities. Changes in precipitation patterns are more likely to alter nitrogen cycling rates via the influence on nitrogen diffusion and plant and microbial uptake. Furthermore, precipitation-induced changes in nematode densities may have important implications for nitrogen cycling in the New Jersey Pinelands.

DEDICATION

This dissertation is dedicated to

Bertha Meyrowitz

Joseph Meyrowitz

Miriam Landesman

William Landesman

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CHAPTER 1

Introduction

BACKGROUND

Soil microbes (bacteria and fungi) and their microbivorous grazers are responsible for a large portion of all terrestrial nutrient cycling. Due to the immense diversity of soil microbial communities (Hawksworth 2001, Torsvik et al. 2002), the tremendous amount of biochemical processes performed in soil and technological limitations, the factors controlling soil microbial community composition and nutrient cycling are poorly understood. This is now a topic of intense investigation because a changing global climate may impact soil microbial communities in ways that either ameliorate or further degrade environmental quality.

Some of the most basic questions regarding soil microbial communities remain elusive. For example, we do not have a complete understanding of the abiotic factors controlling soil microbial community composition and nitrogen cycling, primarily because the relative importance of temperature, precipitation, soil characteristics and other factors change over multiple spatial and temporal scales. Much less is known about the influence of microbivorous grazers on microbial communities. These abiotic and biological controls are likely to have far reaching effects on aboveground plant community composition (Moore et al. 2003, Swaty et al. 2004) and atmospheric (Borken et al. 2006a and 2006b) and water quality (Fitzhugh et al. 2001).

There is growing interest in understanding the controls on soil microbial communities and nitrogen cycling because a changing global climate is likely to alter

these processes. Soils represent a very large sink of carbon dioxide, holding more than three times the amount of carbon stored in aboveground biomass and two times the amount in the atmosphere (Eswaran et al. 1993). Any change in soil nitrogen cycling rates caused by long-term changes in climate may alter the rate at which soil carbon is sequestered. Due to uncertainties with climate forecasts, as well as the many complex interactions of climate on soil microbial communities, it is unclear if climate change will lead to high rates of CO_2 flux to the atmosphere or facilitate soil carbon sequestration. The results will likely vary between ecosystems, highlighting the need for more site specific research.

Soil moisture and the potential effects of climate change

Human activity has led to an increase in greenhouse gas emissions that have raised global mean surface air temperatures throughout the 20th century. In North America, air temperatures have increased by $0.7^{o}C$ over the past 100 years, and projected increases over the next century range from $1.8^{o}C$ to $4^{o}C$ (Pachauri and Reisinger 2007). Theory suggests that warming alters precipitation patterns by increasing the water holding capacity of the atmosphere, increasing evaporation rates and disrupting air circulation patterns (Trenberth et al. 2003). Climate models predict that rising surface air temperatures caused by elevated atmospheric carbon dioxide (CO_2) concentrations will result in an increase in precipitation amount in the northeastern U.S. (Baede et al. 2001, Kunkel 2003, Wentz et al. 2007, Willett et al. 2007).

Empirical evidence confirms that precipitation patterns are changing across the United States. Over the past century, atmospheric moisture content has increased as a

consequence of human activity (Willett et al. 2007), leading to increases in average precipitation amount. The increase in precipitation has been particularly dramatic since the early 1970's (Ross and Elliott 2001, Groisman et al. 2004) and strongly associated with El Nino/Southern Oscillation events (Dai and Trenberth 1998). The climate record for southern New Jersey shows that mean annual temperature over the past 30 years (1979 to 2008) was 12.3^{o}C, a 0.8^{o}C increase over the historical (1895 to 1978) average (11.5^{o}C). Over this same 30 year period, average annual precipitation was 1159 mm, or 39 mm higher than the mean from 1895 to 1978 (1120 mm, NJ State Climatologist, http://climate.rutgers.edu/stateclim).

The impact of changing precipitation patterns on soil moisture is uncertain. One expected consequence of an increase in average annual precipitation amount is a corresponding increase in soil moisture. Elevated atmospheric CO_2 may also contribute to long-term increases in soil moisture through small reductions in evapotranspiration (Field et al. 1995, Niklaus et al. 1998). On the other hand, an increase in precipitation frequency and intensity could result in more dry days (Diffenbaugh et al. 2005). Combined with greater runoff, which reduces water infiltration (Trenberth et al. 2003), soil moisture could decline in response to changing precipitation patterns.

Soil bacteria and fungi possess fundamentally different physiological properties that influence their response to a change in soil moisture. It has been known for a long time that fungi are more drought tolerant than bacteria under field conditions (Bhaumik and Clark 1947, Kouyeas 1964, Griffin 1969). While both microbial groups are capable of maintaining a constant internal water content under a broad range of conditions (Harris 1981, Luard 1982), they possess different nutrient acquisition strategies that affect their

survival in soil. Bacteria are aquatic organisms that require water for movement, nutrient uptake and release of waste products (Swift et al. 1979, Harris 1981). Soil drying limits their access to resources while elevated soil moisture facilitates growth and activity. Fungi are less affected by soil drying, as they can extend hyphae through air-filled pore spaces to access moisture and nutrients, and can translocate these resources to water and nutrient-limited cells within their mycelial network (Jennings 1976, Jennings 1990). These properties of soil microbes explain why fungi have been observed to be inversely proportional to soil moisture and bacteria positively correlated with soil moisture (Kouyeas 1964, Wilson and Griffin 1974). More recently, these properties of bacteria and fungi have been observed under natural field conditions (Jensen et al. 2003, Bell et al. 2008, Collins et al. 2008).

While bacteria and fungi are responsible for the decomposition and mineralization of organic matter, there are differences in the types of substrates metabolized and the rate at which they cycle nutrients. Coleman et al. (1983) characterized the bacterial and fungal food webs as representing a "fast cycle" and "slow cycle" in reference to the rate at which nutrients are cycled through each energy channel. The strong association of bacteria with the rhizosphere and their higher abundances in surface soils (Fierer et al. 2003b) suggests that many bacteria are well adapted for growth on low molecular weight carbon substrates (Coleman et al. 1983) and, therefore, a faster cycling of nutrients. The slow cycle in soil, with fungi at the base of the energy channel, is characterized by slower growth rates and the metabolism of recalcitrant organic matter (Dighton 1991, Setälä and McLean 2004, Paul 2007).

Because bacteria and fungi are the basis of two fairly distinct soil food webs (Hunt et al. 1987), a shift in the relative abundance of these microbes should alter the relative abundance of bacterivorous and fungivorous grazers. Microbivorous grazers are responsible for a large portion of bacterial and fungal mineralization (Moore et al. 1988), although bacterivores have a greater stimulatory effect (Clarholm 1985, Ingham et al. 1985). Fungivores are larger, possess longer generation times and a slower metabolism (Rooney et al. 2008). An increase in the relative abundance of the bacterial energy channel should be positively correlated with soil nutrient cycling rates while an increase in the densities of fungi and fungivores should decrease nutrient cycling rates.

Soil nematodes are unique grazers because they can influence community structure and soil processes in both the bacterial and fungal energy channels. Because their C:N ratio is higher than that of their prey, bacterivorous nematodes often excrete excess ammonium or amino acids as a result of grazing activity (Woods et al. 1982, Anderson et al. 1983, Ingham et al. 1985). Bacterivorous grazing also stimulates microbial growth and activity (Ingham et al. 1985) which contributes to accelerated nitrogen mineralization rates. This is because the growing microbial community mineralizes N-containing carbon substrates for energy and biomass accumulation (Chapin et al. 2002). If a change in precipitation patterns were to increase the dominance of the fungal energy channel, the higher prey density may increase the relative abundance of fungivorous nematodes. Fungivorous nematodes have less of a stimulatory effect on nitrogen mineralization rates (Ingham et al. 1985, Chen and Ferris 1999). This is because the C:N ratio of their prey is much higher than that of bacteria. During grazing, a higher proportion of the fungal nitrogen is assimilated into nematode biomass.

Precipitation frequency and dry wet cycles

Observations for the northeastern U.S. indicate that precipitation intensity is increasing (Kunkel 2003, Groisman et al. 2004). This is the result of a buildup of atmospheric moisture due to extreme warming events (Diffenbaugh et al. 2005, Allan and Soden 2008) and greater hurricane activity. Between these more extreme rainfall events there may be an increased number of dry days (Diffenbaugh et al. 2005) which, combined with reduced water infiltration resulting from more intense storms (Trenberth et al. 2003), may contribute to soil drying. Therefore, an increase in precipitation frequency or intensity could increase the frequency and severity of dry-wet cycles in soil.

Because dry-wet cycles are a stressor of microbial communities, a higher frequency of more severe drying and rewetting events could have important implications for soil microbial communities and nutrient cycling. As soils dry, microbes prevent water loss by synthesizing osmoregulatory solutes (Harris 1981). However, when soil is rewet, the higher internal water potential makes microbes more susceptible to water influx and cell lysis. This mechanism explains the observation of cell death during rewet events (Kieft et al. 1987, Halverson et al. 2000). Microbes that survive drying-rewetting events adjust their internal water potential by releasing osmoregulatory solutes into soil (Harris 1981).

Empirical evidence, primarily from laboratory investigations, suggests that Gram positive bacteria and fungi should be well adapted for surviving dry-wet cycles (Harris 1981, Luard 1982, Halverson et al. 2000). These microbes are capable of producing a large suite of osmoregulatory solutes that can maintain a stable internal water potential. The strong cell wall of Gram positive bacteria may provide this microbial group with an

additional advantage during more severe and frequent dry-wet cycles. Therefore, an increase in the frequency of dry-wet cycles could cause a shift in the microbial community under field conditions (Schimel et al. 2007) as demonstrated in some studies (Fierer et al. 2003a).

Winter precipitation and freeze-thaw cycles

There are important seasonal dynamics to changing precipitation patterns that could influence soil microbial communities. Groisman et al. (2004) found that recent increases in precipitation in the U.S. were concentrated in the spring, fall and summer. However, Hayhoe et al. (2006) predicted that most of the annual increase in precipitation amount in the northeastern U.S. will occur during winter. This potential increase in winter precipitation is likely to coincide with a reduction in the number of extremely cold days (Diffenbaugh et al. 2005). Under warmer winter air temperatures, winter precipitation will either melt more rapidly or fall as rain, leading to elevated levels of winter soil moisture.

Studies of microbial processes during winter are usually conducted at extreme northern latitudes or in alpine ecosystems, where winter snow fall creates a continuous insulating cover. Little attention has been paid to winter processes at lower latitudes, where winters are cold enough to freeze soil, but insufficiently cold to sustain snow pack for more than a few days. Soils lacking a winter snow cover are exposed to greater climatic variability and are more likely to experience freeze-thaw cycles (Groffman et al. 2001). There are two primary mechanisms by which freeze-thaw cycles can alter soil nutrient cycling rates. First, freezing and thawing of soil causes lysis of bacterial and

fungal cells (Biederbeck and Campbell 1971, Skogland et al. 1988, Schimel and Clein 1996), which can alter nutrient cycling rates by shifting the relative dominance of the fast and slow cycle. Second, soil freezing disrupts soil aggregates (Steinweg et al. 2008) and damages roots (Tierney et al. 2001). These processes release labile carbon and nitrogen substrates into soil, potentially increasing nutrient cycling rates.

If winter precipitation increases as predicted by climate models (Hayhoe et al. 2006), the elevated soil moisture may increase the severity of these freeze-thaw cycles by creating additional ice lenses that subsequently damage more microbial cells and plant roots. This could result in a reduction in microbial biomass, a shift in the relative dominance of the fast and slow cycle and altered nutrient cycling rates. These changes may be particularly important because they would impact concentrations of plant-available nitrogen during early spring.

OBJECTIVES AND PROJECT OUTLINE

The objective of this project was to evaluate the potential for altered precipitation patterns to impact soil microbial composition and nutrient cycling in the New Jersey Pinelands.

Chapter 2 – Two year rainfall manipulation

During a two year rainfall manipulation experiment, I studied the long-term response of soil microbial communities to a complete two year rainfall exclusion as well as a doubling of precipitation amount. I measured the PLFA composition of the microbial community and measured potential net nitrogen mineralization and amino acid

production rates. These measurements were performed on six occasions, during

September 2006 and 2007, May 2007 and 2008 and July 2007 and 2008.

Chapter 3 – Temporal dynamics and the effects of an extreme dry-wet event

At the conclusion of the two year study, I took advantage of the drought

conditions created to measure the potential effects of an increase in precipitation on the

soil microbial community. To assess the short-term (hours to weeks) response of the

microbial community to a rewet event, I performed these measurements at very fine

temporal scales. The response of microbial communities to a dry-wet event was

compared to the response of microbial communities in natural plots to a similar rewetting

event.

Chapter 4 – Response of nematode communities to rainfall manipulations

In Chapter 4, I present additional findings from the two year rainfall manipulation

experiment by focusing on the response of nematode densities and community

composition. This analysis was performed on samples removed in September 2007, one

year after the start of the experiment, and were analyzed in the context of the response of

the soil microbial community and nitrogen cycling. This project will improve

understanding of how microbivorous grazers respond to changing precipitation patterns

and therefore has implications for the role of top-down control in a changing climate.

This study was a collaborative effort with Amy Treonis at the University of

Richmond. I performed almost all field and laboratory work, including the extraction and

counting of nematodes. Dr. Treonis performed nematode identification and calculation of community indices and contributed to the writing of the results section.

Chapter 5 – Winter Processes

Seasonal dynamics of precipitation and the potential role of freeze-thaw cycles in structuring soil microbial communities were evaluated during a two year experiment during which winter precipitation was elevated. Natural rainfall was allowed to reach control and experimental plots, while experimental plots received supplemental weekly rainfall for nine weeks. These manipulations were performed from January to mid-March of 2007 and 2008. The response of microbial community composition, potential net nitrogen mineralization and amino acid production rates were measured at the end of winter. The timing of these samples was selected to provide an indication of the potential impacts of the treatment on nitrogen availability to plant communities in early spring.

In a second winter experiment, I measured the response of microbial community composition to a period of extremely cold air temperatures that corresponded to the coldest soil temperatures of the winter of 2008. I measured the microbial community before and after the cold event, which lasted for two days. I compared the response of microbes under natural conditions to plots that were watered just prior to the deep freeze event.

APPROACH

This study addresses the potential for changes in precipitation patterns and soil moisture to alter the structure and function of soil microbial communities in the New Jersey Pinelands. I measured soil microbes at the taxonomic resolution of bacteria and fungi. These are very broad taxonomic groups that encompass an extremely large amount of biological and functional diversity. However, there are functional implications of a shift in the relative abundance of bacteria and fungi.

Within the bacterial community, I measured relative abundances of Gram positive and Gram negative bacteria. This too is a very coarse measurement of the microbial community. Due to differences in cell wall composition, these two groups are believed to respond differently to changes in precipitation amount, frequency or intensity. Because Gram negative bacteria are associated with rhizosphere and surface soils (Marilley and Aragno 1999, Fierer et al. 2003b) and Gram positive bacteria are more common in bulk soil (Marilley and Aragno 1999) and at depth (Fierer et al. 2003b), a shift in the relative abundance of Gram positive and Gram negative bacteria may impact nutrient cycling rates.

Changes in the relative abundance of bacteria and fungi may cause a shift in the relative dominance of the fast and slow cycle in soil and, therefore, an overall change in nutrient cycling rates. During this project, I used nitrogen mineralization as one measure of soil nutrient cycling rates. Nitrogen mineralization is the process by which microbes convert simple organic monomers of nitrogen into inorganic forms (NO_3^- and NH_4^+) and potential net nitrogen mineralization represents the balance between mineralization and microbial uptake (Chapin et al. 2002). Because plant communities are often limited by

inorganic nitrogen (Vitousek and Howarth 1991), this measurement is believed to be a good estimate of plant-available nitrogen.

It has been demonstrated that plants in many ecosystems can utilize simple organic forms of nitrogen. Initially, this was found in Arctic soils (Schimel and Chapin 1996) but has now been observed in a diverse range of ecosystems (Schimel and Bennett 2004). One of the first demonstrations of amino acid uptake by plants under field conditions was conducted in a boreal forest with pine trees and ericaceous shrubs (Nasholm et al. 1998). The results indicated that amino acid uptake by plants could be equally or more important than inorganic forms of nitrogen. It was recently determined that amino acids, while not the dominant form of nitrogen used, are an important source of plant nutrition in temperate forests of the northeastern U.S. (Berthrong and Finzi 2006). Theory predicts that amino acids become an increasingly more important nitrogen source for plants as soil nitrogen limitation increases (Schimel and Bennett 2004). I measured amino acid production rates as a second measurement of soil nitrogen cycling and to more accurately estimate the potential pool of plant available nitrogen.

Site Description

This study was conducted in an upland forest of the New Jersey Pinelands at the Rutgers University Pinelands Field Station. The New Jersey Pinelands are located on the outer Atlantic Coastal Plain, one of New Jersey's five physiographic regions that covers most of central and all of southern New Jersey. The uplands are dominated by Pitch Pine (*Pinus rigida*), which covers 50-80% of the Pinelands (McCormick 1998). Other species include shortleaf pine (*Pinus echinata*), black oak (*Quercus velutina*), post oak (*Quercus*

stellata), Scarlet oak (*Quercus coccinea*), chestnut oak (*Quercus prinus*) and blackjack oak (*Quercus marilandica*).

The study site was within an oak-pine stand containing an understory of *Gaylussacia* sp. and *Vaccinium* sp. The site is located at an elevation of 34 meters (http://gisdata.usgs.net/ned/About.htm), on gently sloping (<5%) land (Markley 1971). The soils at this site are classified as Entisols (Evesboro series), from the mesic, coated family of typic quartzipsamments (Markley 1998). These soils have a thicker litter and organic horizon (3- 5cm) than most upland soils in the Pinelands. Below the organic horizon is gray brown sand to a depth of 25cm, followed by a horizon of brown sand (25-40cm). The B horizon (40-76cm depth) is comprised of yellow brown sand and the C horizon (76-152cm) is comprised of light yellow-brown sand (Markley 1998). The differences in color are due to varying concentrations of iron, which are leached from the upper horizons. Although found in low quantities, the most common clay minerals are kaolinite, smectite-aluminum-interlayered dioctahedral vermiculite and mica (Douglas and Trela 1998).

The soils are very sandy and highly leached, with low cation exchange capacity (2.1 to 4.1 milliequivalents per 100 grams) (Tedrow 1998). The dominant mineral in these soils is sand (quartz, SiO_2), along with other materials that are highly resistant to weathering. The strong leaching conditions result in high levels of soluble aluminum, which contributes to the low pH (4.1 to 4.7 in the organic horizon) (Tedrow 1998). Low values for pH, clay content and cation exchange capacity, in conjunction with the sandy, dry nature of the soils has a strong influence on above-ground plant communities.

CONCLUDING REMARKS

This project is unique because all experiments were performed under field conditions. Therefore, the results are an integrative response of many factors, including the highly variable dynamics of climate, rhizodeposition and soil food webs. It is difficult to identify specific mechanisms in field studies, but in conjunction with past laboratory investigations, field studies allow for a deeper understanding of soil microbial community dynamics.

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CHAPTER 2

Response of microbial community structure and production of plant-available

nitrogen to a two year rainfall manipulation in the New Jersey Pinelands

INTRODUCTION

Soil moisture is an essential component of microbial community composition and functioning, and a change in precipitation and soil moisture are likely to alter microbial biomass, community composition and nitrogen cycling. Changes in precipitation amount may affect rates of production of plant available nitrogen by altering the relative abundance of microbes and microbivorous nematodes capable of fast vs. slow cycling of nutrients. It is important to understand the controls on microbial communities and the production of plant-available nitrogen because this may impact critical ecosystem functions such as above and below ground carbon sequestration and soil nutrient retention.

Climate models predict that rising surface air temperatures caused by elevated atmospheric carbon dioxide (CO_2) concentrations will result in an increase in precipitation amount in the northeastern U.S. (Baede et al. 2001, Kunkel 2003, Wentz et al. 2007, Willett et al. 2007). Temperature and precipitation data from southern New Jersey supports these predictions. Mean annual temperature over the past 30 years (1979 to 2008) was 1^oC higher than the historical (1895 to 2008) average. Over this same period, average annual precipitation was 1130 mm, which is 29 mm higher than the century mean (NJ State Climatologist, http://climate.rutgers.edu/stateclim).

An increase in precipitation amount combined with a decrease in stomatal conductance resulting from elevated CO_2 concentrations (Field et al. 1995, Niklaus et al. 1998) should lead to an increase in soil moisture in many parts of the United States. However, climate models also forecast an increase in precipitation intensity (Diffenbaugh et al. 2005, Allan and Soden 2008) which, combined with atmospheric warming, may result in an increase in the frequency of dry days (Diffenbaugh et al. 2005). Furthermore, more intense storms can increase runoff and reduce the total amount of soil water infiltration (Trenberth et al. 2003). Therefore, it is uncertain if altered precipitation patterns will increase or decrease soil moisture levels, and the effect will most likely depend on local conditions (Diffenbaugh et al. 2005).

The idea that changes in precipitation amount should alter soil microbial community composition is based on the assumption that optimal microbial growth and activity among genera occurs at different values of soil moisture. The extremely high diversity of soil microbial communities (Torsvik et al. 2002) makes it impossible to measure the relative abundance of individual species in response to soil moisture, but measurements of the bacteria:fungi biomass ratio and the Gram positive to Gram negative bacterial biomass ratio are relatively inexpensive and easy to perform. Measurements at even this very coarse taxonomic resolution may provide useful insight into how microbial communities and ecosystem function will respond to altered precipitation patterns.

Empirical evidence indicates that the relative abundance of soil bacteria increases under elevated soil moisture conditions (Bell et al. 2008) while fungi dominate carbon and nitrogen cycling in dry soil (Collins et al. 2008). These patterns follow logically from the metabolic and physiological requirements of these two major microbial groups. While

soil bacteria depend on water for movement and nutrient acquisition (Harris 1981), fungi are aerobic organisms that are more tolerant of dry conditions (Griffin 1969). Soil fungi can extend hyphae through air-filled pore spaces to access moisture and nutrients, and can translocate these resources to water and nutrient-limited cells within their mycelial network (Jennings 1976, Jennings 1990). For bacteria, dry pore spaces are barriers to movement, diffusion of resources and nutrient uptake.

Measurements of the bacteria to fungi and the Gram positive to Gram negative bacteria ratio provide functional information about soil microbial communities. Coleman et al. (1983) described bacteria and fungi as comprising the basis of a "fast" and "slow" cycle in soil in reference to the rate at which nutrients are cycled through each energy channel. The "fast" cycle is performed by bacteria, which are adapted for growth on labile substrates, and bacterivores, which possess high metabolic rates. The "slow" cycle in soil, with fungi at the base of the energy channel, is characterized by the metabolism of recalcitrant organic matter (Paul 2007) and by larger organisms with longer life spans and slower metabolism (Rooney et al. 2008). While there many be exceptions to this conceptual model, it provides a useful framework for analyzing the soil microbial community in relation to ecosystem functioning. It has been hypothesized that any shift in the relative dominance of the fast and slow cycles in soil should lead to an overall increase or decrease in nutrient cycling rates (Hunt et al. 1987).

At a slightly higher taxonomic resolution, there may be functional implications for a shift in the relative abundances of Gram positive and Gram negative bacteria in soils. Gram positive bacteria posses thicker and stronger cell walls (Madigan et al. 2003) and can produce a larger number of osmoregulatory solutes than Gram negative bacteria

(Harris 1981). Based on these physiological differences, Gram positive bacteria are believed to be better adapted to drought stress and large fluctuations in soil moisture (Harris 1981, Schimel et al. 2007). Gram negative bacteria may benefit more from elevated precipitation and soil moisture because they are more abundant in surface soils (Fierer et al. 2003), which are more likely to rewet during rainfall (Borken et al. 2003).

Due to differences in substrate affinity, a shift in the Gram positive to Gram negative bacteria ratio in soils may alter nutrient cycling rates. Most Gram negative bacteria appear to grow faster on labile substrates (Fierer et al. 2003, Fierer et al. 2007), suggesting that a higher relative abundance of Gram negative bacteria should be associated with faster nutrient cycling rates. Gram positive bacteria should be better adapted for growth on recalcitrant materials, as they are more common at depth (Fierer et al. 2003) and in bulk soils (Rovira 1965, Marilley and Aragno 1999). This suggests that higher abundances of Gram positive bacteria should be associated with lower nutrient cycling rates.

Nitrogen cycling is often stimulated by top-down interactions (Clarholm 1985, Bonkowski and Brandt 2002) and soil nematodes are one of the most important groups of microbivores responsible for stimulating nitrogen mineralization (Woods et al. 1982, Ingham et al. 1985, Fu et al. 2005) and amino acid production (Anderson et al. 1983). Consistent with the Coleman et al. (1983) model of a fast and slow cycle in soil, bacterivorous nematodes are responsible for much higher rates of nitrogen cycling than fungivorous nematodes (Ingham et al. 1985, Chen and Ferris 1999) and a shift in the relative dominance of bacterivorous and fungivorous nematodes should therefore impact nutrient cycling rates. A change in precipitation patterns could increase the relative

abundance of bacterivores or fungivores if it also shifts the relative abundance of bacteria or fungi. However, all soil nematodes require water for movement and activity (Neher et al. 1999) and a decrease in soil moisture may affect all nematodes equally. It is unclear how nematodes will respond to changing soil moisture in the pinelands because opposing effects of soil drying on nematode densities have been observed (Savin et al. 2001).

Due to the potential effects on microbial community composition, activity and top down interactions, changing precipitation patterns will likely exert a dominant control over nitrogen cycling rates and the release of inorganic nitrogen (NO_3^- and NH_4^+). Previous investigations have consistently found a positive correlation between soil moisture and nitrogen mineralization rates for many soil types (Stanford and Epstein 1974), including an acidic coniferous forest soil (Tietema et al. 1992). Nitrogen is usually mineralized, and not immobilized, under elevated soil moisture because microbes are usually carbon limited (Demoling et al. 2007) and must mineralize nitrogen rich substrates for energy and biomass accumulation (Chapin et al. 2002).

It has often been assumed that plant productivity is limited by the availability of inorganic nitrogen (Vitousek and Howarth 1991), however plants in many ecosystems are now known to utilize simple organic forms of nitrogen such as amino acids (Schimel and Chapin 1996, Nasholm et al. 2000). This includes systems that share similarities with the pine forests of southern New Jersey. Amino acid uptake among pine trees and ericaceous shrubs has been documented (Nasholm et al. 1998), and it was recently determined that amino acids are an important source of plant nutrition in temperate forests of the northeastern U.S. (Berthrong and Finzi 2006). Furthermore, theory predicts that amino acids become an increasingly more important nitrogen source for plants as nitrogen

limitation increases (Schimel and Bennett 2004). Therefore, studies in the New Jersey Pinelands that only consider nitrogen mineralization might fail to detect other processes that produce plant-available nitrogen.

It is important to study the effects of changing precipitation patterns on soil microbial community structure and nitrogen cycling because microbial communities directly and indirectly impact atmospheric carbon concentrations. Rates of production of plant-available nitrogen impact above-ground primary production which ultimately influences long-term carbon sequestration in soil (Jastrow et al. 2005). Processes that increase plant-available nitrogen are of particular importance in low nitrogen soils because plants in such systems may lack the ability to counteract rising atmospheric CO_2 concentrations (Hungate et al. 2006). Soil microbial community composition may directly influences soil carbon sequestration via the chemical composition of microbial cells (Allison et al. 2005). Understanding how soil microbes and nitrogen cycling respond to altered precipitation patterns will improve our understanding of the controls on soil microbial community structure and function and will improve predictive models of the impacts of climate change.

Precipitation manipulation studies have been conducted successfully to measure the potential impacts on soil processes in a variety of temperate ecosystems. For example, in a throughfall displacement experiment in a Tennessee forest, soil respiration and nitrate flux increased with precipitation amount (+/- 33% of actual precipitation), but little change was observed in root biomass (Hanson et al. 2003, Joslin and Wolfe 2003). Exclusion of snow and summer rainfall increased methane uptake in a deciduous forest in Massachusetts (Borken et al. 2006a). In a deciduous forest in Kentucky, a low

precipitation treatment indirectly increased decomposition rates (Lensing and Wise 2006). These studies demonstrate that precipitation can have complex effects on carbon and nitrogen cycling and highlight the need for more research into the effects of precipitation on soil processes.

The goal of this project was to study the impact of changes in precipitation amount and soil moisture on soil microbial community composition and the production of plant-available nitrogen in an oak-pine forest of the New Jersey Pinelands. I hypothesized that manipulating precipitation amount would alter the composition of soil microbial communities, microbivorous grazers and nitrogen mineralization and amino acid production rates. I predicted that the bacteria:fungi ratio would be positively correlated with precipitation amount and that the Gram positive:Gram negative bacteria ratio would be negatively correlated with precipitation amount. I predicted that nematode densities would be positively correlated with precipitation amount. Because elevated precipitation was expected to increase the relative importance of the bacterial energy channel, I predicted that nitrogen mineralization and amino acid production rates would be positively correlated with precipitation amount.

METHODS

Experimental Design

Twelve rainout shelters (2.47m x 1.91m x 0.7m) were constructed to block rainfall from experimental plots. They were distributed so that shrub density was similar below the shelters. Each shelter was covered with clear PVC panels and pitched such that rainwater was diverted into 208 liter tanks adjacent to the shelters. Shelters were assigned

randomly to one of three treatments: "drought" (no rain fall), "ambient" (100% of actual rain applied to the plots) or "high" (200% of actual rainfall applied to plots). A "natural" treatment was established by delineating four additional plots (1.4m x 0.8m) which received no manipulation. The experiment was conducted for two years, between July 2006 and July 2008. The experiment began in July 2006 with three replicates. One additional replicate of each treatment was added in late August 2006, for a total of four replicates.

The flat topography of the study site ensured that all water applications penetrated the soil below the plot with minimal runoff. Visual inspections of the rainout shelters during rain events confirmed that the ground below the rainout shelters remained dry, and soil moisture data confirmed that the treatments were successful (see results section). It is possible that small amounts of natural rainfall reached the edges of the covered area. Therefore, all sampling occurred within a central area of 1.4 x 0.8m, corresponding to a buffer of 0.5m on each side.

Water was usually applied within two days, but almost never more than one week following a rain event. Using a battery operated pump attached to a garden hose, rain water was simultaneously removed from tanks and applied to the plots at a rate of about 1.6mm/minute. The ambient treatment received the contents of one tank, the high treatment plots received the contents of two tanks and no rain was ever applied to the drought treatments. During winter, snow and ice were removed from drought shelters and applied to high treatments. Snow and ice falling on all other shelters were applied to their respective plots.

A maximum of 44 mm of rain could be collected before the tanks overflowed. On four occasions (9/7/06, 11/15/06, 4/20/07, 7/24/07), unusually high rainfall resulted in overflow of the tanks. On two dates (3/1/07, 3/21/07), shelter collapse due to ice accumulation prevented the collection of precipitation. Lost precipitation never reached the sampling area but did contribute to a discrepancy between total amount of water applied to ambient and natural plots. From July 12 to August 30, the time between the start of the experiment and the installation of the fourth replicate, natural rainfall totaled 137mm.

Sampling and soil processing

During the first year of the experiment, samples were removed during September 2006, May 2007 and July 2007. Samples were removed during the same months during the second year of the experiment for a total of six sampling events. Samples were removed from three randomly selected points under each shelter, from the well defined organic horizon (approximately 3-5cm in depth). Three additional cores were removed immediately adjacent to each initial sample. These cores were placed in plastic bags and incubated *in situ* for 30 days. Litter and loose sand were removed from all samples and the organic horizon of the three subsamples were pooled and retained for analyses. Immediately following a sampling event, samples were broken apart by hand and visible roots were removed. Samples were further homogenized first by passing the sample through a 4.75 mm and subsequently through a 2 mm sieve. Soil moisture, percent organic matter and nitrogen analysis were performed on fresh soil. The remaining sample

was stored at $-20^{\circ}C$ in plastic bags and subsamples were removed from the freezer on a later date for microbial community analysis.

Soil moisture was measured on 5 g subsamples from the initial and incubated cores by weighing fresh soil into aluminum containers and drying at $70^{\circ}C$ for 48 hours. Soil moisture ($SM_{PERCENT}$) was calculated as the difference between the fresh weight ($Soil_{FW}$) and dry weight ($Soil_{DW}$) of the subsample, per unit dry weight soil: $SM_{PERCENT} = [(Soil_{FW}) - (Soil_{DW})]/(Soil_{DW})*100$. The percent organic matter content of the initial samples was determined by burning the dried sample at $550^{\circ}C$ for 12 hours. Percent organic matter was estimated as the difference between the initial mass of the dry soil and the mass of the burned sample.

KCl extractions were performed on the initial and incubated cores. These extractions were used to measure inorganic nitrogen pools (NO_3-N and NH_4^+-N). Soil (10g) was added to plastic bottles and mixed with 30mL of 2M KCl. The soil-KCl mixture was placed on a shaker for one hour and filtered through cellulose filter paper (pore size = $8\mu m$). The extracts were stored at $-20^{\circ}C$. Concentrations of NO_3^--N and NH_4^+-N in the KCl extracts were measured on an Astoria Pacific AP-3 auto analyzer (Astoria Pacific International, Clackamas, OR). The sum of nitrates and nitrites were determined by the hydrazine method and ammonium by the phenate method. Nitrite concentrations were negligible and, therefore, the concentration of the mixture was used as the measure of nitrates.

The relationship between soil moisture and inorganic nitrogen, when measured on a *per gram of dry weight soil* basis, resulted in artificially high *r*-squared values (data not shown). This is because sand is associated with infertile and dry soil. As a result,

variability in sand content alone can drive strong relationships between soil moisture and inorganic nitrogen pools. For this reason, ammonium and nitrate pools were calculated on a per gram of *dry weight organic matter* ($\mu g NH_4^+\text{-}N*g\ OM^{-1}$ and $\mu g NO_3\text{-}N*g\ OM^{-1}$) basis, allowing for equal comparison among samples.

Potential net nitrogen mineralization and amino acid production were calculated as the difference in values between the incubated and initial samples (Robertson et al. 1999) using the following equation: $N_{MIN} = [(\mu g*g^{-1}\ NO_3^-\text{-}N_{INCUB} + \mu g*g^{-1}\ NH_4^+\text{-}N_{INCUB})$ to $(\mu g*g^{-1}\ NO_3^-\text{-}N_{INIT} + \mu g*g^{-1}\ NH_4^+\text{-}N_{INIT})]/$ # of days of incubation, where

$NO_3^-\text{-}N_{INIT}$ and $NH_4^+\text{-}N_{INIT}$ = estimated concentration of initial samples

and

N_{INCUB} and $NH_4^+\text{-}N_{INCUB}$ = estimated concentration of incubated cores.

All KCl extracts, with the exception of samples from the fifth and sixth sampling events, were frozen at -20^oC. Because they were not frozen, fungal contamination was observed in the KCl extracts for samples from the fifth and sixth sampling events. Nitrogen mineralization or uptake by fungi in these samples may have caused changes in nitrogen values that were unrelated to the treatments. When analyzing these samples, extracts were filtered so that no fungal material was added to the AP-3 analyzer. To assess the effect of contamination, the analysis of ammonium pools and nitrogen mineralization was performed with and without sampling events 5 and 6. Both analyses revealed consistent results. Therefore, I conclude that the effects of this fungal

contamination were minimal and data from these sampling dates were used in the analysis described below.

The KCl extractions were used to measure amino acid pools (expressed on a *per gram dry weight organic matter* basis) and potential net amino acid production. Amino acids were measured during the first four sampling events only. The concentration of amino acid-N was determined colorimetrically through the reaction of KCl extracts with a 2% ninhydrin solution (ninhydrin and hydrantin in dimethyl sulfoxide and lithium acetate buffer, pH 5.2, Sigma-Aldrich, Inc.). During this reaction, ninhydrin forms a complex with ammonium and the α-amino groups of amino acids, resulting in a deep purple color (Joergensen 1995). KCl extracts were mixed with the ninhydrin reagent (2:1 v/v), heated in boiling water for 30 minutes and diluted in an ethanol-water mixture (1:1 v/v) (Joergensen 1995). Absorbance was read at 570 nm on a Spectronic 20 Genesys spectrophotometer.

The reaction of leucine with ninhydrin is representative of all amino acids, with the exception of proline and hydroxyproline (Rosen 1957). Standard curves were created using a leucine standard diluted in the 0 to 1000 mM range ($y = 0.0151x$ to 0.0175; $r^2 = 0.9998$). To correct for interference by ammonium, standard ammonium curves (0 to 1000 mM) were created ($y = 0.097x + 0.008$; $r^2 = 0.9998$). Interference by ammonium was calculated using the standard curves and the ammonium concentrations measured with the Astoria Pacific AP-3 auto analyzer. The following calculation was performed to estimate potential net amino acid production (AA_{NET}): $AA_{NET} = (AA_{INCUB} - AA_{INIT})/\#$ of days of incubation (Berthrong and Finzi 2006) where

AA_{INCUB} = estimated amino acid concentration of incubated cores

and

AA_{INIT} = estimated amino acid concentration of initial samples.

Phospholipid Fatty Acid Analysis

Phospholipid fatty acids (PLFAs) are found in the membranes of all bacteria and fungi and all species within a soil community possess a unique PLFA composition (Pinkart et al. 2002). Following cell death, PLFAs are believed to be rapidly decomposed by phospholipases. Therefore, the PLFA analysis measures the living soil biomass and is also commonly used to measure the living soil microbial community structure (Pinkart et al. 2002).

The PLFA profile was measured on 3g subsamples stored at $-20^{o}C$. Following the methods of White and Ringelberg (1998), soil lipids were extracted in a one phase solvent of chloroform, methanol and phosphate buffer (1:2:0.8 ratio). The phospholipids were separated on a silicic acid column by sequentially eluting with organic solvents of increasing polarity: chloroform (5mL), acetone (10mL) and methanol (5mL). The phospholipids, dissolved in methanol and amended with a nonadecanoic acid standard (100 μl), were saponified and methylated, forming fatty acid methyl esters (FAMEs). The identity of individuals FAMEs were determined based on their retention time on a gas chromatograph and in conjunction with the MIDI Sherlock Microbial Identification System (Newark, DE). Peak identification was confirmed by a combination of gas chromatography and mass spectroscopy (GC-MS).

Calculation of microbial biomass

The biomass of individual fatty acids was based on the peak area of the nonadecanoic acid (19:0) standard and total microbial biomass was the sum of all phospholipid fatty acids confirmed by GC-MS. Microbial biomass was calculated on a per *gram of organic matter* basis (μg PLFA*g OM^{-1}). This was done because sand has a very low water holding capacity and is associated with low quantities of microbial biomass. When microbial biomass was calculated on a dry weight soil basis, variability in sand resulted in artificially strong correlations between soil moisture and microbial biomass.

Community composition

Three approaches were used to assess soil microbial community composition: the bacteria:fungi ratio, the Gram positive:Gram negative bacteria ratio, and the multivariate analysis of all PLFAs. The biomass of Gram positive bacteria was estimated as the sum of all iso and anteiso branch chain fatty acids (O'Leary and Wilkinson 1988) and the sum of all mono-unsaturated and cyclopropane fatty acids were used to estimate Gram negative bacterial biomass (Wilkinson 1988, Zelles 1999, Table 1). Linoleic acid (18:2ω6) was used as the estimate of saprotrophic and ectomycorrhizal fungal biomass (Frostegård and Bååth 1996). Total bacterial biomass was estimated as the sum of Gram positive and Gram negative bacterial biomarkers and all 10-methyl fatty acids (Zelles 1999).

Physiological indicators

The formation of cyclopropane fatty acids begins at the onset of dormancy and is an indicator of physiological stress (Grogan and Cronan 1997, Pinkart et al. 2002). During cyclopropane ring formation, 16 and 18 carbon mono-unsaturated fatty acids are converted to 17 and 19 carbon cyclopropane fatty acids respectively (Kaur et al. 2005). Three indices were calculated based on this conversion from mono-unsaturated to cyclopropane fatty acids (Table 1). "Stress index 1" was based on the ratio of 16 and 18 carbon monounsaturated fatty acid to 17 and 19 carbon cyclopropane fatty acids. A low index value represents high physiological stress and a high index value represents low physiological stress. The index was calculated separately for each mono-unsaturated fatty acid:

Stress Index 1 = (16:1 ω7c + 18:1 ω7c) ÷ (17:0 CYCLO + 19:0 CYCLO ω8c)

Stress Index 2 = 16:1 ω7c ÷17:0 CYCLO

Stress Index 3 = 18:1 ω7c÷19:0 CYCLO ω8c

Stress indices two and three are subsets of stress index 1. They were calculated to separately analyze the conversion of the 16 and 18 carbon monounsaturated fatty acids.

Nematode density and identification

On September 25, 2007, eight days after the fourth sampling event, additional cores were removed for the analysis of nematode density and community composition. Nematodes were extracted from 50g samples (three cores removed and pooled from

randomly selected points) using a modified Cobb's decanting and sieving method

(s'Jacob and van Bezooijen 1984). Nematodes were separated from soil by submerging

samples in deionized water. The nematode + water mixture was decanted onto a 1 mm

sieve and the nematodes trapped on the sieve were backwashed into a container. The

water passing through the sieve was collected, re-mixed with the soil sample, and the

process was repeated using progressively smaller sieve sizes (422 µm, 125 µm, 75 µm

and 43µm). The collected nematodes in water were poured over a cotton milk filter and

submerged in water just above the filter. This caused the nematodes to travel through the

pore spaces of the filter and into clean, deionized water. The separated nematodes were

concentrated into 100mL of water and all individuals in a 10mL subsample were counted

under a dissecting microscope. Nematode density (ND) was calculated as follows: ND =

$[(\# \text{ individuals})*10]*g \text{ soil}^{-1}$.

Following counting, nematodes were concentrated into 10mL of water and

preserved in 10% formalin. Nematodes were identified to the family level and to trophic

group by Amy Treonis of the University of Richmond. Nematodes within a family were

identified as bacterial feeders (BF), fungal feeders (FF), plant parasites (PP) or omnivores

(OM) based on Yeates et al. (1993). Some general results pertaining to nematode

communities are reported below. See chapter 4 for a more detailed analysis of the

response of soil nematode communities to the treatments.

Meteorological Data

Daily precipitation and soil temperature values were obtained from data loggers

installed by the U.S. Forest Service approximately 100 meters from the study site. Soil

temperature was measured by Campbell Scientific CS107 sensors buried to a depth of 5cm. Precipitation was measured with a Campbell Scientific TE525-LC tipping bucket rain gauge. Meteorological data was recorded on a Campbell Scientific CR23X micro logger. Soil temperature is reported below as cumulative thermal warming days. One warming day was calculated by taking the average half-hourly soil temperature values of the sensors and summing these values over a 24 hour period.

Statistical analyses

The response of soil moisture, microbial biomass, the bacteria:fungi ratio, the Gram positive:Gram negative bacteria ratio, stress indices and nitrogen pools were analyzed with the repeated measures analysis of variance (multivariate approach). Significant differences between treatment means on any given sampling date were based on Tukey's studentized range test. Nematode density was analyzed with a one way analysis of variance followed by the Tukey's studentized range test. Analysis of overall microbial community structure was performed with a principal components analysis of the *relative abundance* of individual PLFAs. For purposes of simplicity, this analysis was performed on drought and high rainfall treatments only. Principal components 1 and 2, which explained 25% and 15% of the variance of PLFAs, were retained for analysis with a repeated measures multivariate analysis of variance.

Water applications may have had unequal effects on soil moisture to and therefore microbial biomass to among plots within the same treatment. This may have resulted in a large error term in the repeated measures analysis of variance that could have masked treatment effects. For this reason, the relationship between soil moisture and microbial

biomass was further analyzed using linear regression. For the regression analysis using all data, soil moisture values were arcsin square root transformed and microbial biomass data was log transformed to meet the assumption of no autocorrelation among variables. The regression analysis was performed on all data and repeated separately for samples collected in September, May and July. The maximum gravimetric soil moisture value recorded in July was never above 22%, while the ranges in September and May were 5% to 42% and 3% to 40% respectively. To allow for an equal comparison between July, September and May, the monthly regression analysis were repeated using only soil moisture values below 22%.

Due to missing data points, nitrogen mineralization and amino acid production rates could not be analyzed with a repeated measures analysis of variance. The relationship between soil moisture and nitrogen mineralization and between soil moisture and amino acid production were analyzed with linear regression analysis. Soil moisture was arcsin square root transformed and nitrogen mineralization data was log transformed to meet the assumption of constant variance. Before log transforming nitrogen mineralization data, a constant of 2 was added to each variable to remove negative values. Statistical analyses were performed using SAS 9.2 and graphed in SigmaPlot.

RESULTS

Soil Moisture

The manipulations resulted in significant differences in treatment means for soil moisture on all sampling dates with the exception of September 2006 and September 2007 (Table 2). Average soil moisture was always lowest in drought plots and highest in

the high rainfall plots. In September of 2006, after two months of water exclusion, soil

moisture in the drought plots was 16%. This value continued to decline throughout the

experiment and after two years the value was 4%. Soil moisture in the ambient, high and

natural plots were similar on most sampling dates, however in May 2008 soil moisture in

ambient plots (24%) was significantly lower than soil moisture in high rainfall plots

(33%). In July 2008 soil moisture in ambient plots (8%) was significantly lower than in

natural plots (10%) and soil moisture values were very low on this sampling date. Soil

moisture in natural plots was always higher than soil moisture of ambient plots and closer

to the value in high rainfall plots (Table 2). This was most likely the result of two factors.

First, there were differences in the frequency of water applications, with natural plots

receiving more evenly distributed rainfall. Second, there were inefficiencies in water

collection that decreased the total amount of precipitation reaching ambient shelters

relative to natural plots.

Microbial biomass in relation to meteorological data

Average microbial biomass within natural plots in relation to precipitation, soil

moisture and soil temperature is summarized in Table 3. During the first year of sampling

(September 2006 to July 2007), average microbial biomass in un-manipulated plots

ranged from 304 to 343 $\mu g * gOM^{-1}$. The range was substantially lower during the second

year (September 2007 to July 2008), with values ranging from 128 to 202 $\mu g * gOM^{-1}$

(Table 3 and Figure 1). When microbial biomass in natural plots was analyzed over time,

separately from other treatments, there was a significant decrease between September

2006 (343 $\mu g * gOM^{-1}$) and May 2008 (139 $\mu g * gOM^{-1}$, p = 0.03) and July 2008 (128

$\mu g*gOM^{-1}$, p = 0.04) (Table 3). Average microbial biomass in natural plots during the September 2006 sampling event was not significantly different from microbial biomass during any other sampling period.

Instantaneous values for soil moisture may not accurately explain changes in microbial biomass because there is a lag in the response of microbial biomass to changes in soil moisture that may last for over three weeks (see Chapter 3). It is possible that soil temperatures follow a similar pattern. To more accurately account for changes in microbial biomass, soil moisture and soil temperature were summed over the two months leading up to each sampling event (Table 3). Soil temperature data was monitored continuously throughout the study period by the U.S. Forest Service. Using this data, total thermal warming for the two months leading up to each sampling event was calculated (Table 3). Soil moisture was not monitored continuously during this study. Therefore, total precipitation, *in lieu* of soil moisture, was calculated for the two months leading up to each sampling event (Table 3).

For September and July, average microbial biomass and instantaneous soil moisture values were much lower during the second year of the study (Table 3). However, while microbial biomass in May was lower during the second year, instantaneous soil moisture values were similar between years (Table 3). In contrast to these instantaneous measurements, total rainfall in the two months leading up to the May 2008 sampling event were 74 mm lower than rainfall in 2007 (Table 3). The timing of the rainfall may explain why soil moisture values were similar despite differences in total rainfall. During the May 2008 sampling event, rainfall was higher in the week prior to sampling relative to rainfall prior to the May 2007 sampling event (Figure 2). Soil

temperatures were considerably higher in the two months prior to the May 2008 sampling

event relative to the May 2007 sampling event (Table 3 and Figure 3). Precipitation and

thermal warming in the two months prior to the September and July sampling events

were similar between years (Figures 4-7).

Microbial biomass (treatment effects)

There were no significant differences among treatment means for microbial

biomass on any sampling date (Table 4). While the difference was never statistically

significant, average microbial biomass in drought plots was lower than microbial biomass

in high rainfall plots during the May 2007, July 2007, September 2007 and May 2008

(Figure 8). In addition to the decline in average microbial biomass within natural plots

(see above), there was also a decline in average microbial biomass when values from all

treatments were averaged. Based on the analysis of variance of contrast variables,

average microbial biomass among all samples in September 2006 (344 µg*gOM^{-1}) was

significantly different from average microbial biomass in May 2007 (258 µg*gOM^{-1}, p =

0.006), September 2007 (240 µg*gOM^{-1}, p = 0.006), May 2008 (148 µg*gOM^{-1}, p <

0.0001) and July 2008 (176 µg*gOM^{-1}, p < 0.0001) (Table 4). There was no significant

difference between average microbial biomass in September 2006 and July 2007 (303

µg*gOM^{-1}).

Only a small fraction of microbial biomass was explained by soil moisture when

all dates and treatments were combined (r^2 = 0.06; p = 0.02; Figure 9). Separately

analyzing the relationship for each month individually, soil moisture explained 22% of

the variation in microbial biomass in July (p = 0.007), 15% in September (p = 0.03) and

9% in May (p = 0.10) (Table 5). Microbial biomass was most sensitive to soil moisture in

July (slope = 1020), while sensitivity in September and May was similar (slopes of 330

and 234 respectively).

Soil moisture explained the greatest proportion of the variation in microbial

biomass in July, and soil moisture during this month was never above 22%. Therefore,

the linear regression analysis for the relationship between soil moisture and microbial

biomass was recalculated using only soil moisture values below 22%. This resulted in an

overall r^2 value of 0.24 for the entire dataset (Figure 10). Using only soil moisture values

below 22%, bacterial biomass was more sensitive to soil moisture than fungal biomass

and soil moisture was a better predictor of bacterial biomass than it was for fungal

biomass (Table 6). Below 22% soil moisture, the amount of variation in microbial

biomass explained by soil moisture was much higher in September (41%) and May

(49%). It should be noted that, due to a low sample size, the power of the May analysis

(0.64) was below the recommended 0.80 threshold. Sensitivity of microbial biomass to

soil moisture was similar between September, May and July (Table 7) when soil moisture

values above 22% were excluded from the analysis.

Microbial Community composition

The treatment effects on the bacteria:fungi and Gram positive:Gram negative

bacteria ratio are summarized in Table 4. The treatments did not result in a change in the

bacteria:fungi ratio or the Gram positive to Gram negative bacteria ratio over time (p =

0.19 and 0.42 respectively for the time*treatment effect) and there were no significant

differences among treatment means on any sampling date. Although not statistically

significant, and with the exception of the May 2008 sampling date, the Gram positive:Gram negative bacteria ratio within the drought treatment was always higher than the ratio within the high rainfall treatment. The average bacteria:fungi ratio within the drought treatment was lower than the ratio in the high rainfall treatment during the first four sampling events but higher during the final two sampling events. These differences were never statistically significant.

There were significant time effects on the average bacteria:fungi ratio and the average Gram positive:Gram negative bacteria ratio ($p = 0.005$ and $p < 0.0001$ respectively). The average bacteria:fungi biomass ratio among all treatments in September 2006 was 8.14 (Table 4). This was significantly higher than the ratio during all sampling events between July 2007 and July 2008, which ranged from 6.15 to 6.80 (Table 4 and Figure 11). The average Gram positive to Gram negative bacteria ratio was highest during the first sampling event (1.38) and was significantly lower during all subsequent sampling events (0.84 to 1.11) (Table 4). While always significantly lower than the September 2006 sampling event, the average Gram positive to Gram negative bacteria ratio increased between the May 2007 and July 2008 sampling events (Table 4 and Figure 12).

The principal components analysis detected a significant effect of time ($p = 0.01$) and a significant between subjects effect ($p = 0.005$) between the drought and high rainfall plots. Mean principal component scores were significantly different during September 2006, July 2007 and May 2008 (Tukey's studentized range test, $p < 0.05$) (Figure 13). The fatty acids most strongly correlated ($R > 0.6$) with principal component 1 were cy17:0, 18:1 w7c, i15:0, i16:0, a15:0, 16:1 ω5c and 16:1 ω7c a (Table 8). Along

the second principal component there was a possible effect of time ($p = 0.07$), but principal component scores were not significantly different among treatment means (Tukey's studentized range test, $p < 0.05$) on any sampling date. The fatty acids most strongly correlated with the second principal component were 14:0, 15:0 and 16:0 (Table 8).

Physiological Indicators

The treatments did not result in a change in stress index 1 or stress index 2 between drought and high rainfall treatments (between subjects effect , $p = 0.12$ and 0.42). There was a significant between subjects effect for stress index 3 (17:1w8c/19:0 Cyclo, $p = 0.03$). Mean values of stress index 3 were significantly different (Tukey's studentized range test, $p < 0.05$) during September 2006, July 2007 and May 2008 (Figure 14). Soil moisture explained a higher percentage of the variation in stress index 3 than stress indices 1 or 2 (Table 9).

Nematodes

Nematode densities were significantly different between drought, ambient and high rainfall treatments (Tukey's studentized range test, $p < 0.05$, Figure 15). Average nematode density in the ambient plots was 533 nematodes per gram of dry soil, which was four times the value in drought plots (132 individuals per gram soil). Nematode densities within high rainfall plots (1002 individuals per gram soil) were almost double the value of ambient treatments. A total of 14 families were identified, of which six

comprised 97% of the community. The majority (85%) of taxa within these families were bacterial or fungal feeders.

Ammonium, nitrate and amino acid pools

Average ammonium concentrations in un-manipulated plots ranged from 33 to 144 $\mu g*gOM^{-1}$ throughout the experiment (Table 10 and Figure 16). The highest average values, when averaged among all treatments, were recorded during the initial (114 $\mu g*gOM^{-1}$) and final (144 $\mu g*gOM^{-1}$) sampling events and the lowest concentrations were measured during the September 2007 and May 2008 sampling events (45 $\mu g*gOM^{-1}$ and 33 $\mu g*gOM^{-1}$ respectively). Within drought plots, average ammonium concentrations increased from 84 $\mu g*gOM^{-1}$ during the first sampling event to a final value of 640 $\mu g*gOM^{-1}$. Ammonium pools measured within drought plots were significantly higher than ambient and high rainfall plots during all but the May 2007 sampling event and were more than four times the value of all other treatments during the last three sampling events (September 2007 to July 2008). During the September 2006 and July 2007 sampling events, ammonium concentrations in natural plots were significantly higher than ammonium concentrations in the ambient and high rainfall plots (Tukey's studentized range test, $p < 0.05$) (Table 10 and Figure 16).

Average nitrate pools, which ranged from 4.0 $\mu g*gOM^{-1}$ to 7.8 $\mu g*gOM^{-1}$ in natural plots, represented 8% of the value of ammonium pools (Table 10). When averaged among all treatments, the highest average nitrate values were recorded during the May 2008 sampling event (7.4 $\mu g*gOM^{-1}$) and the lowest nitrate concentrations were

measured during the final sampling event (4.2 $\mu g*gOM^{-1}$). Treatment means for nitrates were not significantly different on any sampling date.

Amino acid pools in natural plots increased from an average value of 29 $\mu g*gOM^{-1}$ in September 2006 to 110 $\mu g*gOM^{-1}$ in July 2007 and decreased sharply to 57 $\mu g*gOM^{-1}$ in September of 2007 (Figure 17). During July 2007, amino acid pools in natural plots were significantly higher than in ambient and high rainfall plots (64 and 53 $\mu g*gOM^{-1}$ respectively). There were no other significant differences between treatments means throughout the four sampling dates during which amino acids were measured.

Net nitrogen mineralization and amino acid production

The average potential net nitrogen mineralization rates among all samples on each sampling date ranged from -0.14 $\mu g*g^{-1}*d^{-1}$ in July 2006 to 0.94 $\mu g*g^{-1}*d^{-1}$ in July 2007 (Table 10). In drought plots, nitrogen mineralization rates increased between September 2006 and May 2007 (0.46 to 1.06 $\mu g*g^{-1}*d^{-1}$) and then decreased to 0.07 $\mu g*g^{-1}*d^{-1}$ in July 2008 (Table 10). Average amino acid production rates, which were only measured during the first four sampling events, ranged from to 0.04 to 0.16 $\mu g*g^{-1}*d^{-1}$. These values were consistently lower than nitrogen mineralization rates during September 2006, May 2007, July 2007 and September 2007 (39%, 29%, 16% and 10% of the value of nitrogen mineralization respectively) (Table 10).

The relationships between soil moisture and nitrogen mineralization and soil moisture and amino acid production rates were analyzed with linear regression analysis. Because microbial biomass was more sensitive to soil moisture below 22% (Figure 10), this analysis was performed separately on samples above and below the 22% soil

moisture threshold. The processes that led to the large accumulation of ammonium in drought plots were most likely different from the mechanisms responsible for positive nitrogen mineralization rates in other treatments (see discussion). For this reason, drought treatments were not included in the analysis of the relationship between soil moisture and nitrogen mineralization. Excluding drought treatments, there was a positive correlation between soil moisture and nitrogen mineralization rates ($r^2 = 0.19$ p = 0.001, Figure 18) below 22% soil moisture, and a negative correlation between soil moisture and nitrogen mineralization ($r^2 = 0.23$; p = 0.006) above 22% soil moisture (Figure 19). There was no correlation between soil moisture and amino acid production (p = 0.22), regardless of whether or not the 22% threshold was used (Figure 20).

DISCUSSION

Decline in microbial biomass

The sharp decline in microbial biomass between years in natural plots is not consistent with precipitation and soil moisture patterns. The largest inter-annual differences in microbial biomass were observed between the May 2007-08 and July 2007-08 sampling events. However, while soil moisture in July 2008 was lower than July 2007, soil moisture values in May 2007 and May 2008 were similar (Table 2). Precipitation in the two months prior to the May and July sampling events were 1.52 and 1.65 times lower in the second year of the study, potentially explaining the sharp decrease in microbial biomass. However, precipitation leading up to September 2007 was 1.45 times lower than the previous year but no significant inter-annual decline in microbial biomass in natural plots was observed. The lack of an association between precipitation

patterns and the decline in microbial biomass through time is supported by the experimental manipulations. Neither rainfall exclusion nor rainfall additions resulted in a significant difference in microbial biomass during the two year experiment (Figure 8). In fact, there was no significant difference between drought and high rainfall treatments in May 2008 when soil moisture in high rainfall plots was more than six times the value of drought plots (Table 2).

No consistent patterns were observed between inter-annual variation in soil temperature and microbial biomass within natural plots. Weekly thermal warming was consistently higher in the two months leading up to the May 2008 sampling event vs. the May 2007 sampling event (Table 3). When soil moisture is not limiting, as was the case in May (Table 2), negative correlations between microbial biomass and soil temperature have been found (Alvarez et al. 1995), suggesting a mechanism by which microbial biomass may have declined in May 2008. However, soil microbial biomass also declined sharply between July 2007 and July 2008 and this decline cannot be explained by differences in soil temperature, which was similar between years (Table 3). Since it is unlikely that two separate mechanisms are responsible for the inter-annual decline in microbial biomass in May and July, the soil temperature data does not provide a complete explanation for the change in microbial biomass. Winter soil temperatures are also inconsistent with the decline in microbial biomass, because soil temperatures were much colder during the winter of 2006-07 than in 2007-08. Furthermore, soil microbial biomass recovers from winter freeze stress by mid-March (see Chapter 3).

A large gypsy moth (*Lymantria dispar*) outbreak during the summers of 2007 and 2008 may have contributed to the large decline in microbial biomass in May and July of

2008. A visual inspection of the canopy during the summer of 2007 revealed approximately 50% defoliation due to gypsy moth herbivory and *Lymantria dispar* were present within the shrub layer of the study site. Defoliation was somewhat less severe in 2008 but gypsy moths were present in large numbers. Gypsy moth consumption may have reduced microbial biomass by causing a reduction in photosynthesis that decreased rhizodeposition (Cardon et al. 2002). Additionally, inefficiencies in feeding resulted in large inputs of fresh litter during the summer of 2007 (observed but not quantified), and therefore a reduction in litter fall during fall of 2007. At the same time that inputs of fresh litter were reduced, the proportion of recalcitrant carbon was increased as a result of gypsy moth frass, which had a C:N ratio (26.3, ±SE 1.36, n=9) that was higher than soil organic matter and was resistant to decomposition (Gray et al. 2009 unpublished data).

The reduction in microbial biomass might be explained by a combination of biological and climatic factors. A reduction in the supply of labile carbon and an increase in recalcitrant substrates in the organic horizon may have had a negative effect on microbial biomass as early as September 2007, when average microbial biomass among all samples was significantly lower relative to September 2006 (Table 4). Unlike the decline in average microbial biomass between September 2006 and May 2007 (Table 4), microbial biomass never recovered but continued to decline. It is possible that the effect carried over until the following spring, when lower precipitation and warmer soil temperatures, combined with a potentially reduced labile carbon pool, may have had a cumulative negative effect on microbial biomass. Finally, the second gypsy moth outbreak during the summer of 2008 may have led to sustained reductions in

rhizodeposition and microbial biomass that were reflected in the low biomass values in July 2008.

Weak correlation between soil moisture and microbial biomass

The rainfall manipulations resulted in significant differences in soil moisture between drought, ambient and high rainfall plots during all but two sampling events (Table 2), but there was never a treatment effect on microbial biomass. Overall, soil moisture was a poor predictor of microbial biomass (Figure 9) and even below the 22% threshold value a large proportion of the variation in biomass could not be explained by soil moisture (Figure 10). Larger declines in biomass in drought plots would have resulted in higher r^2 values and significant differences between treatment means for microbial biomass, but physiological adaptations most likely prevented these large decreases from occurring. The conversion of mono-unsaturated fatty acids to cyclopropane fatty acids (Figure 14) represents one such adaptation. As environmental stress increases, these modifications appear to reduce membrane fluidity, protecting fatty acids from oxidation and ensuring long-term survival of cells (Grogan and Cronan 1997). This is just one of many physiological changes that microbes utilize to tolerate drought conditions. Other modifications in response to drought stress include the alteration of internal water potential (Harris 1981), production of exopolysaccharides (Roberson and Firestone 1992), biofilm formation, sporulation and dormancy. Any of these strategies may have contributed to the less dramatic decline in microbial biomass in drought plots.

At higher soil moisture values, top-down control may have influenced the very weak correlation observed between soil moisture and microbial biomass. There was a

strong and positive response of nematode biomass to precipitation amount (Figure 15)
and most of the nematodes present were microbivores. While grazing limits biomass, it
also maintains microbes in a continuous log phase of growth (Clarholm 1985), and this is
supported by the physiological data, which shows a high value of stress index 3
(representing lower physiological stress) in high rainfall plots (Figure 14). Elevated
grazer biomass and activity in high rainfall plots may have prevented further increases in
microbial biomass, providing further insight into the weak relationship between soil
moisture and microbial biomass (Figure 9).

Sensitivity of microbial biomass below 22% soil moisture

Below the 22% soil moisture threshold, microbial biomass was positively
associated with increased soil moisture, presumably due to the influence of water on the
delivery of limiting nutrients to microbial cells (Stark and Firestone 1995). Similarly,
Almagro et al. (2009) found a threshold value of 10% in a mollisol soil, below which
microbial respiration was sensitive to changes in soil moisture. Above this threshold
value, respiration was more limited by soil temperature. Herron et al. (2009) recently
demonstrated that microbial growth efficiency is different below 5% soil moisture, when
there is a shift towards greater accumulation of biomass C. Because soil texture
influences water potential, these soil moisture values are not directly comparable with
this study. However, they do demonstrate that the microbial response to soil moisture
may be very difference above and below certain threshold values.

Response of microbial community composition to treatments

Microbial community composition could not be distinguished among treatments, suggesting that there was substantial resiliency among the microbial community to changes in precipitation amount. During a seven year rainfall manipulation in a Mediterranean grassland Cruz-Martinez et al. (2009) also found almost no change in microbial community composition. Because these soils were not buffered against climatic variability, they concluded that soil microbes were adapted to drought and moisture stress. Similarly, Almagro et al. (2009) found that microbes in forest soils were less sensitive to changes in soil moisture than microbes from grassland soils and hypothesized that forest soils may be buffered against changes in soil moisture due to such factors as hydraulic lift and root and mycorrhizal foraging. This conclusion is supported by Borken et al. (2006b) who found that the deep penetrating roots of forest trees can maintain activity during an experimental drought treatment.

Taken together, these studies suggests two possible mechanisms that would explain why the microbial community in this study was unaffected by the rainfall manipulations. First, because the organic horizon of these Evesboro soils are relatively thin and sandy, they probably dry faster than other forested soils and experience more variability in soil moisture. The proposed mechanism for community stability in grassland soils (Almagro et al. 2009, Cruz-Martinez et al. 2009) may therefore apply to pinelands soils. Second, deep penetrating tree roots and an extensive mycorrhizal network may have accessed water and nutrients outside of the experimental plots and buffered the microbial community against the experimentally induced changes in

precipitation. This could have led to sustained microbial populations within the rhizosphere.

In response to a drought treatment, Jensen et al. (2003) found an increase in the microbial C:N ratio, indicating a shift towards a more fungal dominated system, however I found no treatment effects on the bacteria:fungi ratio in response to drought stress. Beginning with July 2007, the bacteria:fungi ratio was significantly lower than the ratio during September 2006 (Figure 11). The reduction in the bacteria:fungi ratio is consistent with the hypothesis that fungi are better adapted for survival in dry soil, as natural precipitation values were lowest between July 2007 and July 2008 (Table 2). Because fungi are better adapted for growth on recalcitrant carbon substrates (Paul 2007), it is also consistent with the potential gypsy moth-induced onset of carbon limitation during the fall of 2007.

If the reduction in the bacteria:fungi ratio over time was due to drier conditions favoring fungal survival, the same trend should have been observed in response to the water manipulations. The bacteria:fungi ratio should have been lowest in drought plots and highest in high rainfall plots. While this pattern was observed for four of the six sampling events, the difference was never significant. Using regression analysis, bacterial biomass was found to be more sensitive to soil moisture than fungal biomass (Figure 10), suggesting that the relatively low replication and high variability of soil dynamics may have limited the interpretive value of the repeated measures analysis. The potential gypsy moth-induced carbon limitation may have impacted the bacteria:fungi response by reducing the total amount of dissolved carbon that was mobilized by watering. Without a larger labile carbon pool, the positive effects of watering on bacterial biomass may have

been minimal. Furthermore, the frequency and intensity of water applications, especially

during the drier months, may have resulted in only transient effects on the bacterial

community that were not sustained for more than one week (see Chapter 3).

A reduction in the Gram positive:Gram negative bacteria ratio was observed

between the first and all subsequent sampling events (Figure 12). The largest reduction in

the ratio occurred between September 2006 and May 2007. Interestingly, at the time of

the May 2007 sampling event, gypsy moth herbivory was already substantial, suggesting

that a change in rhizosphere dynamics caused the shift in the Gram positive to Gram

negative bacteria ratio. However, because herbivory was hypothesized to have reduced

the labile carbon pool, this should have resulted in an *increase* in the Gram positive to

Gram negative bacteria ratio. It is therefore unclear what caused the decrease in the Gram

positive to Gram negative bacteria ratio between September 2006 and May 2007.

Between the second and final sampling events, the Gram positive to Gram

negative bacteria ratio increased but at the end of the experiment was still lower than the

ratio in September 2006. The increase in the ratio is consistent with the predicted

response of Gram positive and Gram negative bacteria to moisture stress, as precipitation

amount decreased over this period. The thicker cell wall of Gram positive bacteria,

combined with their ability to produce a larger number of osmoregulatory solutes (Harris

1981) may have led to a selective advantage over Gram negative bacteria (Schimel et al.

2007). Because a treatment effect on the ratio was not observed, this explanation for an

increase in the Gram positive to Gram negative bacteria ratio is questionable. Instead, if

the hypothesized gypsy moth-induced decrease in carbon availability did in fact occur,

this could explain the gradual increase in the relative abundance of Gram positive

bacteria between May 2008 and July 2008. If soil carbon availability decreased, this may have favored the growth of Gram positive bacteria (Fierer et al. 2003).

Principal components analysis revealed that PLFAs within drought and high rainfall treatments were significantly different during September 2006, July 2007 and May 2008 (Figure 13). The drought treatments for these times had lower principal component scores along principal component 1 (x-axis) than the high rainfall treatments from the same sampling date. The same timing was observed for stress index 3, for which there was a significant difference between the drought and high rainfall treatments during September 2006, July 2007 and May 2008 (Figure 14) and suggests that principal component 1 detected a physiological change and not a shift in community structure. This is supported by the fact that, of the seven fatty acids most strongly correlated with principal component 1 ($R > 0.6$), three were used in the calculation of the stress indices (16:1 ω7c, $r^2 = 0.91$; 18:1 ω7c, $r^2 = 0.74$ and 17:0 Cyclo, $r^2 = 0.64$) (Table 8). Along the second principal component, the first sampling event is clearly separated from all other sampling dates (y-axis in Figure 13) and is most strongly correlated with three biomarkers for saturated fatty acids (14:0, 15:0 and 16:0). These fatty acids are universal to all soil organisms and may therefore reflect the general decrease in microbial biomass observed over time.

The assignment of fatty acids to functional groups is questionable, as many of the fatty acids placed in one trophic group are also found in others. For example, Zelles (1999) stated that monounsaturated fatty acids are a good indicator of Gram negative bacteria because they usually represent less than 20% of the PLFA content of Gram positive bacteria. This upper limit could also be interpreted to mean that there is a high

degree of uncertainty in the estimate of Gram negative bacterial biomass. Iso and anteiso fatty acids, used here as biomarkers for Gram positive bacteria, are found in species from the Gram negative *Cytophaga* genus (Zelles 1999), which are widespread in soil (Madigan et al. 2003). While linoleic acid is a unique fungal biomarker, it is also ubiquitous in plant cells (Frostegård and Bååth 1996). The possibility that PLFA analysis may not have been sensitive enough to detect more subtle changes in microbial community composition cannot be ruled out.

Ammonium pools and nitrogen mineralization

In a laboratory study using several different soil types, Stanford and Epstein (1974) observed an accumulation of ammonium at low soil moisture in three sandy loam soils. While these results are consistent with my findings, there does not appear to be a consistent pattern with respect to field studies. For example, on the Patagonia shortgrass steppe nitrate, but not ammonium, accumulated in response to a reduced precipitation treatment (Yahdjian et al. 2006). Intuitively, soil texture should influence the form of nitrogen that accumulates in response to drought. However, Jensen et al. (2003) found a decrease in soil ammonium pools in drought plots, despite the fact that the soils in this study share similar characteristics to pinelands soils. A buildup of nitrogen observed in the organic horizon of a deciduous forest in response to a 30% reduction in rainfall might be consistent with my observations, however the nitrogen form in this study was not specified (Johnson et al. 2002).

The drought treatment had a very strong and positive effect on ammonium concentrations throughout the study (Figure 16). It is unlikely that this nitrogen was

derived from lysed microbial cells because microbial biomass among treatments was always similar. It is more likely that the source of the ammonium was the mineralization of organic matter in conjunction with a reduced population of nitrifying bacteria, which are sensitive to dry conditions (Hastings et al. 2000). A reduced population of nitrifying bacteria would have resulted in low rates of ammonium consumption (Boyle et al. 2008) and the dry conditions should have reduced diffusion of the excess nitrogen, preventing uptake by plants and N-limited microbes (Schimel and Bennett 2004). Plant nitrogen uptake should have been further inhibited by root mortality, which was confirmed by visual inspection of the samples.

I found a negative relationship between soil moisture and nitrogen mineralization above 22% soil moisture (Figure 19) but a positive relationship between soil moisture and nitrogen mineralization below 22% soil moisture (Figure 18). These results are consistent with those of Stark and Firestone (1995), who concluded that microbial uptake of ammonium was more limited by diffusion at higher soil moisture values (note that plant uptake in buried bags is not possible). At lower soil moisture values, they found that ammonium uptake was more impeded by physiological constraints resulting from drought stress (Stark and Firestone 1995). Below the 22% threshold, an increase in grazing pressure, coinciding with the increase in bacterial biomass, may have resulted in higher mineralization rates as well (Clarholm 1985, Ingham et al. 1985).

It should be noted that drought treatments were not used in the regression analysis for the relationship between soil moisture and nitrogen mineralization. They were excluded because the mechanism responsible for the buildup of ammonium in drought plots to reduced plant uptake to was different from the mechanism most likely controlling

the relationship between soil moisture and mineralization in other samples (see above).
Therefore, when drought plots were included in the analysis, no correlation was observed
between soil moisture (<22%) and potential net nitrogen mineralization (data not shown).

Amino acids

Aside from the drought plots, concentrations of amino acid pools were
comparable to ammonium pools (Table 10) although amino acid production rates were
much lower than nitrogen mineralization rates. This may be due to the fact that the buried
bag method for measuring amino acids and nitrogen mineralization severs plant root
connections. This may have reduced protein decomposition by inhibiting the production
of exoenzymes by ectomycorrhizal fungi. Soil pH, which ranges from 4.1 to 4.7 in the
organic horizon of Evesboro soils (Tedrow 1998), may have contributed to the low amino
acid production rates. The activity of leucine aminopeptidase, an enzyme important in the
metabolisms of amino acids (Sinsabaugh et al. 2008), appears to be lowest below a pH of
5.0 (Sinsabaugh et al. 2008). On the other hand, ß-N-acetylglucosaminidase activity,
which is important in the degradation of chitin, is inversely correlated with soil pH
(Sinsabaugh et al. 2008). Chitin is found in fungi and arthropods, both of which are
abundant in Pinelands soils. This is an indication that the production of ammonium was
due to the metabolism of chitin rather than amino acids.

Conclusions

This study demonstrates that soil microbial communities can display exceptional
resiliency in response to long-term drought conditions. Given that microbes can survive

for millions of years in a dormant state (Cano and Borucki 1995), it should not be surprising that only small reductions in microbial biomass were detected during this two year study. The results may have been confounded by gypsy-moth herbivory, which most likely altered the quality and/or quantity of soil carbon. Soil water is important for the distribution of nutrients to microbes, but without an adequate supply of carbon, changes in soil moisture may have had little effect on microbial communities, especially under the high rainfall treatments. Higher grazing intensity in natural and high rainfall plots may have also limited the response of microbial biomass to elevated soil moisture.

It is interesting to note that the reductions in biomass, which ranged from 0 to 30% relative to natural plots, were comparable to reductions observed in much shorter drying experiments. For example, using a similar pine forest soil, Scheu and Parkinson (1994) found less than a 10% reduction in microbial biomass after a 14 day drought treatment. Following a 10 day drought, van Gestel et al. (1991) observed reductions of 26 to 30%. This suggests that soil microbes may quickly adapt to drought conditions with a physiological response that prevents further reductions in microbial biomass or changes in community composition.

As predicted, nitrogen mineralization rates were positively correlated with soil moisture, however this was only found below 22% soil moisture. Because there was no change in the bacteria:fungi or Gram positive to Gram negative bacteria ratio, these nitrogen cycling dynamics could not be related to changes in microbial community composition. One of the most striking results of this study was the large increase in ammonium pools over time within drought plots. This was a clear indication that there was sustained microbial activity within drought plots, even after two years. There were

no dramatic changes in nitrogen mineralization due to the treatments (the range was -14 to 1.06 $\mu g*g^{-1}*d^{-1}$ across all treatments and sampling dates), indicating that the increase in ammonium pools in drought plots was due to modest but sustained nitrogen mineralization rates. However, it is interesting that some of the highest nitrogen mineralization rates were observed within drought plot (see September 2007 and May 2008, Table 10).

The findings from this study suggest that future changes in precipitation patterns will not have a large impact on soil microbial biomass or community composition. However, there may be important implications for the nitrogen cycle, primarily resulting from changes in nutrient diffusion and plant and microbial uptake of nitrogen. If the timing or rates of nitrogen uptake are altered, this could impact plant community composition, carbon sequestration or nitrogen losses via leaching or denitrification. These results must be accepted with caution because an ecosystem-scale change in precipitation may be very different from these plot-scale manipulations.

One important limitation of this study was that all sampling took place more than two days following the most recent water application or natural rainfall event. The response of the microbial community may have happened on a much shorter temporal scale. Furthermore, it is highly unlikely that a two year drought will occur in the near future. Instead, dry conditions would periodically be followed by rainfall which, in the future, may occur with greater intensity. In the next chapter, the potential implications of temporal dynamics and a rewetting of dry soil are considered.

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Table 1. List of fatty acids identified by the MIDI protocol and confirmed by GC-MS (group assignments based on Frostegård and Bååth 1996, O'Leary and Wilkinson 1988, Wilkinson 1988 and Zelles 1999).

Biomarker for	Fatty Acid
Gram positive bacteria	i15:0, a15:0, i16:0, i17:0, a17:0
Gram negative bacteria	16:1 ω7c, i16:1 G, i16:1 I, 17:1 ω8c, cy17:0, 18:1 ω7c, cy19:0ω8c
Bacteria	All above, plus 16:0 10Me, 17:0 10Me, 18:0 10Me
Fungi	18:2 ω6, 9c
Microbial Biomass	All above, plus 18:1 ω9c, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0
Stress Index 1	(16:1 ω7c + 18:1 ω7c)÷(cy17:0 + cy19:0ω8c)
Stress Index 2	16:1 ω7c ÷ cy17:0
Stress Index 3	18:1 ω7c ÷ cy19:0ω8c

Table 2. Response of average soil moisture (±SE) to precipitation manipulations. Letters ([a], [b] and [ab]) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$).

Date	Drought	Ambient	High	Natural
September '06	0.16 (.03)	0.29 (.01)	0.29 (.04)	0.30 (.05)
May '07	0.17[a] (.02)	0.24[ab] (.02)	0.32[b] (.05)	0.33[b] (.04)
July '07	0.09[a] (.01)	0.14[ab] (.02)	0.18[b] (.01)	0.17[b] (.01)
September '07	0.06 (.01)	0.08 (.01)	0.09 (.01)	0.17 (.06)
May '08	0.05[a] (.01)	0.24[b] (.02)	0.33[c] (.02)	0.28[bc] (.01)
July '08	0.04[a] (.01)	0.08[b] (.01)	0.10[bc] (.004)	0.10[c] (.005)

Table 3. Summary of average microbial biomass (±SE) within natural plots in relation to soil moisture (*SM*), total precipitation in the previous two months (*Precip*), soil temperature on the day of sampling (*Temp^1*) and total thermal warming over the previous two months (*Temp^2*). Stars (*) denote significant differences in microbial biomass between September 2006 an subsequent sampling events (analysis of variance of contrast variables). * p < 0.05

Date	*Biomass (µg*gOM^1)*	*SM*	*Precip (mm)*	*Temp^1 (^{o}C)*	*Temp^2 (^{o}C)*
September '06	343 (40)	0.30 (.05)	239	19.2	1407
May '07	304 (31)	0.33 (.04)	217	14.9	568
July '07	329 (29)	0.17 (.01)	142	17.7	1229
September '07	202 (62)	0.17 (.01)	166	17.4	1390
May '08	139 (22)*	0.28 (.01)	143	6.6	729
July '08	128 (28)*	0.10 (.004)	86	18.6	1233

Table 4. Response of average microbial biomass, the bacteria:fungi ratio and the gram positive to gram negative bacteria ratio (± SE) among treatments. Stars (*) denote significant mean differences between September '06 and subsequent sampling events (analysis of variance of contrast variables). * (P<.05), ** (p < 0.01) and *** (p < 0.001).

Date	Treatment	Biomass ($\mu*gOM^{-1}$)	Bacteria:Fungi	$Gram^+:Gram^-$
September '06	Drought	338 (39)	7.2 (0.5)	1.5 (0.1)
	Ambient	378 (25)	7.6 (0.3)	1.3 (0.1)
	High	316 (35)	8.8 (0.9)	1.4 (0.1)
	Natural	343 (41)	9.0 (1.9)	1.3 (0.1)
	Mean	344 (17)	8.1 (0.5)	1.4 (0.04)
May '07	Drought	213 (31)	6.2 (1.2)	0.9 (0.1)
	Ambient	232 (38)	8.4 (0.8)	0.8 (0.1)
	High	281 (18)	8.3 (0.8)	0.8 (0.03)
	Natural	304 (31)	8.1 (0.7)	0.8 (0.01)
	Mean	258 (16)**	7.8 (0.5)	0.8 (0.03)***
July '07	Drought	233 (42)	6.3 (1.3)	1.0 (0.1)
	Ambient	352 (56)	6.4 (0.6)	1.0 (0.1)
	High	300 (38)	6.6 (0.5)	1.0 (0.04)
	Natural	329 (29)	7.9 (1.1)	1.0 (0.04)
	Mean	303 (22)	6.8 (0.5)*	1.0 (0.03)***
September '07	Drought	214 (41)	5.2 (1.0)	1.1 (0.1)
	Ambient	263 (30)	6.7 (0.6)	1.0 (0.04)
	High	281 (46)	6.1 (0.1)	1.0 (0.1)
	Natural	202 (62)	7.2 (0.9)	1.2 (0.1)
	Mean	240 (22)**	6.3 (0.4)**	1.1 (0.03)***
May '08	Drought	119 (198)	7.1 (1.4)	1.0 (0.1)
	Ambient	178 (39)	6.1 (0.3)	1.1 (0.1)
	High	155 (18)	6.1 (0.4)	0.9 (0.1)
	Natural	139 (22)	5.4 (0.7)	0.9 (0.1)
	Mean	148 (13)***	6.2 (0.4)**	1.0 (0.1)***
July '08	Drought	223 (51)	7.0 (1.0)	1.2 (0.1)
	Ambient	154 (41)	6.1 (0.6)	1.1 (0.2)
	High	200 (16)	5.9 (0.3)	1.1 (0.1)
	Natural	128 (28)	5.8 (1.1)	0.9 (0.1)
	Mean	176 (19)***	6.2 (0.4)**	1.1 (0.1)**

Table 5. Results of the linear regression analysis: soil moisture x microbial biomass (all treatments, grouped by month).

Month	r^2	slope	p value
May	0.09	234	0.1
September	0.15	330	0.03
July	0.22	1020	0.007

Table 6. Results of the linear regression analysis: soil moisture x microbial, bacterial and fungal biomass, SM<22%.

Group	r^2	slope	p value
Microbial Biomass	0.24	1030	< 0.0001
Bacteria	0.25	614	< 0.0001
Fungi	0.11	64	< 0.01

Table 7. Results of the linear regression analysis: soil moisture x microbial biomass, grouped by month (SM<22%).

Month	r^2	slope	p value
May	0.46	1024	0.03
September	0.41	1521	0.003
July	0.22	1020	0.007

Table 8. Correlations (Pearson's R) between individual fatty acids and principal component 1 (PC1) and principal component 2 (PC2). Proportions indicate the average relative abundance of each fatty acid among all samples.

			Pearson's R	
Biomarker For	*Fatty Acid*	*Proportion*	*PC1*	*PC2*
Gram positive bacteria	i15:0	0.10	0.76	-0.52
	a15:0	0.02	0.78	-0.38
	i16:0	0.08	-0.77	-0.41
	a17:0	0.01	-0.48	0.08
	i17:0	0.02	0.38	-0.20
Gram negative bacteria	i16:1 I	0.01	-0.40	-0.12
	i16:1 G	0.01	0.15	0.16
	17:1 ω8c	0.01	0.05	0.32
Gram negative bacteria	16:1 ω7c Form A	0.03	0.91	0.03
(and stress markers)	16:1 ω7c Form B	0.02	0.27	-0.17
	cy 17:0	0.02	0.64	0.20
	18:1 ω7c	0.03	0.74	0.37
	cy 19:0 ω8c	0.09	-0.25	0.23
Fungi	18:2 ω6, 9c	0.09	-0.36	0.52
Microbial Biomass	16:1 ω5c	0.01	0.87	0.03
	18:1 ω9c	0.09	-0.46	0.56
	16:0 10Me	0.08	0.21	0.05
	17:0 10Me	0.02	-0.13	0.39
	18:0 10Me	0.03	-0.47	0.01
	14:0	0.02	-0.05	-0.81
	15:0	0.02	-0.54	-0.66
	16:0	0.17	-0.37	-0.62
	17:0	0.01	-0.32	0.43
	18:0	0.03	-0.05	0.52
	20:0	0.01	0.17	0.40

Table 9. Results of the linear regression analysis: soil moisture x stress indices 1 – 3 (all data).

Index	r^2	slope	p value
Stress Index 1	0.31	1.2	<0.0001
Stress Index 2	0.15	2.8	0.0001
Stress Index 3	0.43	0.69	<0.0001

Table 10. Average (± SE) response of nitrates (NO_3^--N), ammonium (NH_4^+-N) and amino acid (AA-N) pools and potential net nitrogen mineralization (*Nmin*) and amino acid production (*AA Prod*) rates among treatments. For nitrogen pools letters ([a], [b] and [ab]) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$) and stars (*) denote significant differences between mean values between September 2006 and subsequent sampling dates (analysis of variance of contrast variables). * ($p < 0.05$), ** ($p < 0.01$) and ***($p < 0.001$)

Date	Treatment	NO_3^-N (µg/gOM)	NH_4^+-N (µg/gOM)	AA-N (µg/gOM)	Nmin (µg/g/d)	AA Prod (µg/g/d)
September '06	Drought	4.7 (1.5)	84 (11)[a]	21 (11)	0.5 (0.4)	0.3 (0.1)
	Ambient	4.0 (0.4)	34 (7)[b]	29 (3)	0.9 (0.3)	0.1 (0.1)
	High	4.4 (0.3)	37 (6)[b]	31 (4)	0.6 (0.4)	0.2 (0.1)
	Natural	4.0 (0.5)	114 (9)[a]	29 (9)	-0.2 (0.3)	0.1 (0.1)
	Mean	4.3 (0.4)	67 (9)	27 (4)	0.4 (0.2)	0.2 (0.03)
May '07	Drought	4.4 (0.7)	114 (13)	18 (5)	0.5 (0.3)	0.3 (0.1)
	Ambient	5.9 (0.4)	51 (21)	34 (11)	0.3 (0.1)	0.1 (0.04)
	High	5.3 (0.8)	86 (15)	55 (15)	0.3 (0.1)	0.1 (0.1)
	Natural	5.1 (0.4)	78 (12)	72 (17)	0.6 (0.6)	-0.02 (0.1)
	Mean	5.2 (0.3)	82 (9)*	45 (8)*	0.4 (0.2)	0.1 (0.1)
July '07	Drought	4.7 (0.4)	139 (11)[a]	76 (2)	0.8 (0.2)	-0.1 (0.1)
	Ambient	4.5 (0.6)	56 (13)[a]	64 (16)	1.0 (0.2)	-0.2 (0.02)
	High	4.7 (0.3)	45 (4)[b]	53 (7)	no data	no data
	Natural	6.9 (1.2)	104 (8)[b]	110 (10)	1.0 (0.3)	-0.2 (0.1)
	Mean	5.1 (0.4)	86 (11)*	76 (7)***	0.9 (0.1)	-0.2 (0.04)
September '07	Drought	5.9 (0.5)	243 (70)[a]	87 (20)[ab]	1 (0.8)	-0.01 (0.2)
	Ambient	6.4 (1.2)	44 (3)[b]	44 (5)[a]	0.2 (0.03)	0.004 (0.04)
	High	5.9 (0.4)	48 (8)[b]	54 (5)[a]	0.2 (0.1)	-0.01 (0.04)
	Natural	4.9 (0.8)	45 (4)[b]	57 (6)[b]	0.1 (0.2)	-0.1 (0.1)
	Mean	5.8 (0.5)	95 (27)	60 (6)***	0.4 (0.2)	-0.04 (0.1)
May '08	Drought	8.0 (1.2)	353 (71)[a]	no data	1.1 (0.2)	no data
	Ambient	7.1 (0.4)	29 (3)[b]	no data	0.5 (0.2)	no data
	High	6.9 (0.7)	83 (7)[b]	no data	0.3 (0.2)	no data
	Natural	7.8 (0.7)	33 (4)[b]	no data	1 (0.5)	no data
	Mean	7.4 (0.5)*	124 (38)*	no data	0.7 (0.2)	no data
July '08	Drought	4.6 (0.7)	640 (182)[a]	no data	0.1 (0.5)	no data
	Ambient	3.8 (0.4)	122 (7)[b]	no data	0.04 (0.1)	no data
	High	4.3 (0.6)	151 (35)[b]	no data	-0.5 (0.2)	no data
	Natural	4.1 (0.6)	144 (10)[b]	no data	-0.2 (0.2)	no data
	Mean	4.2 (0.3)***	264 (70)**	no data	-0.1 (0.1)	no data
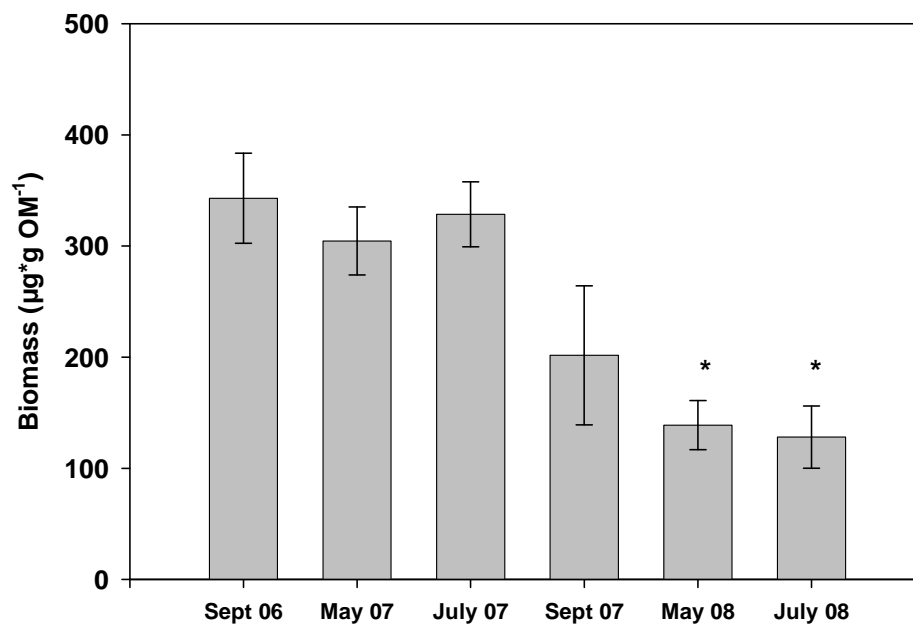
Figure 1. Change in mean microbial biomass (±SE) over time (natural plots only).
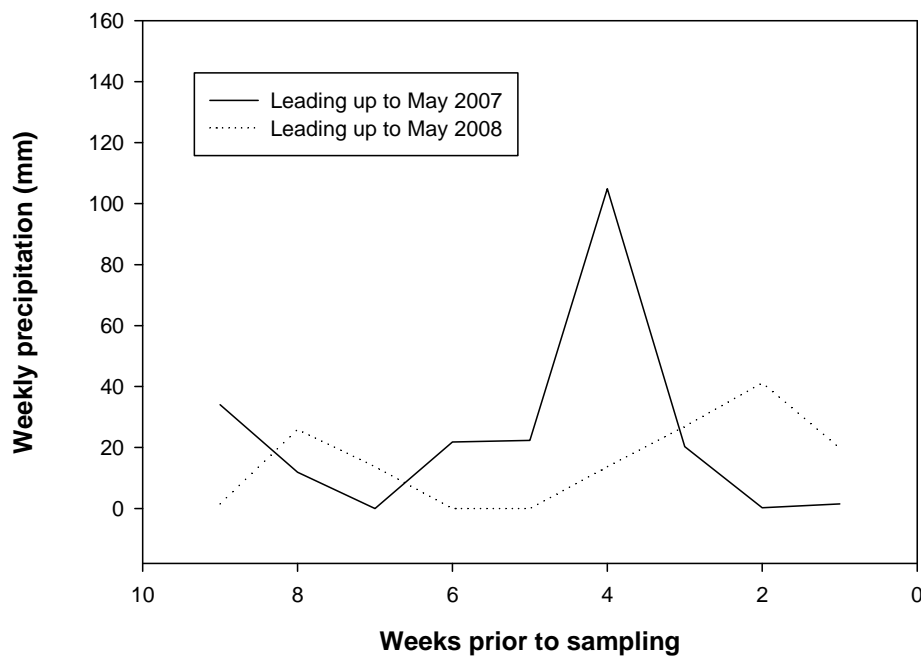
Figure 2. Sum of total weekly precipitation for the nine weeks prior to the May 2007 and May 2008 sampling events. Week 0 corresponds to the week during which samples were removed.
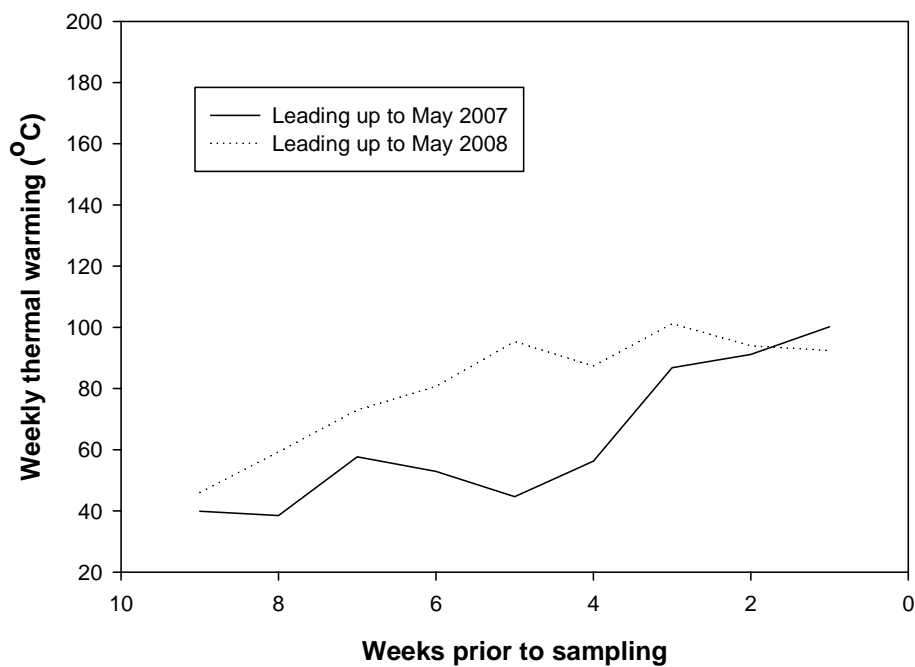
Figure 3. Sum of weekly soil thermal warming for the nine weeks prior to the May 2007 and May 2008

sampling events. Week 0 corresponds to the week during which samples were removed.
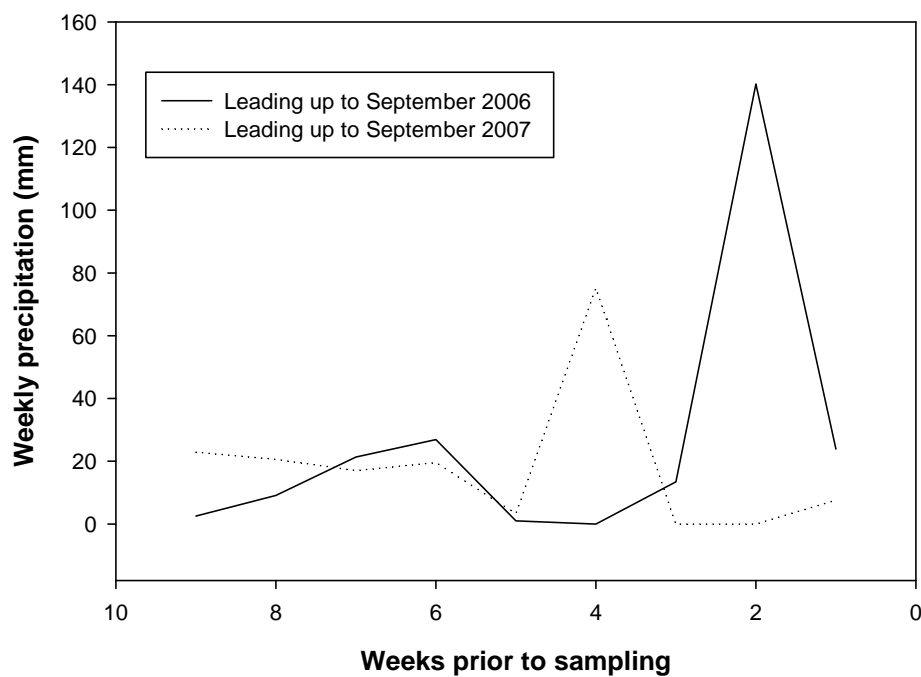
Figure 4. Sum of total weekly precipitation for the nine weeks prior to the September 2006 and September

2007 sampling events. Week 0 corresponds to the week during which samples were removed.
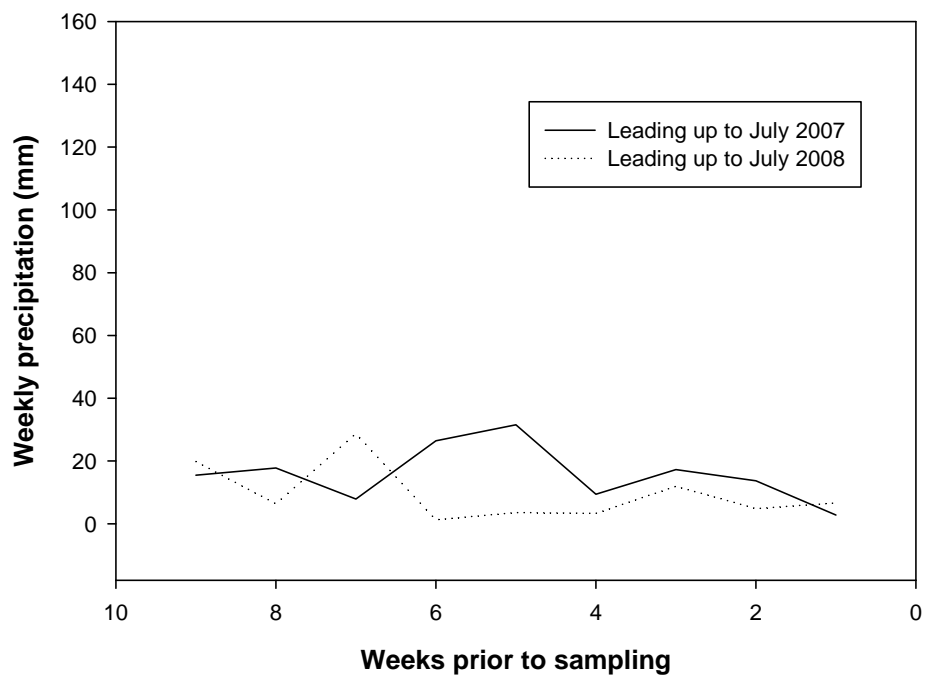
Figure 5. Sum of total weekly precipitation for the nine weeks prior to the July 2007 and July 2008

sampling events. Week 0 corresponds to the week during which samples were removed.
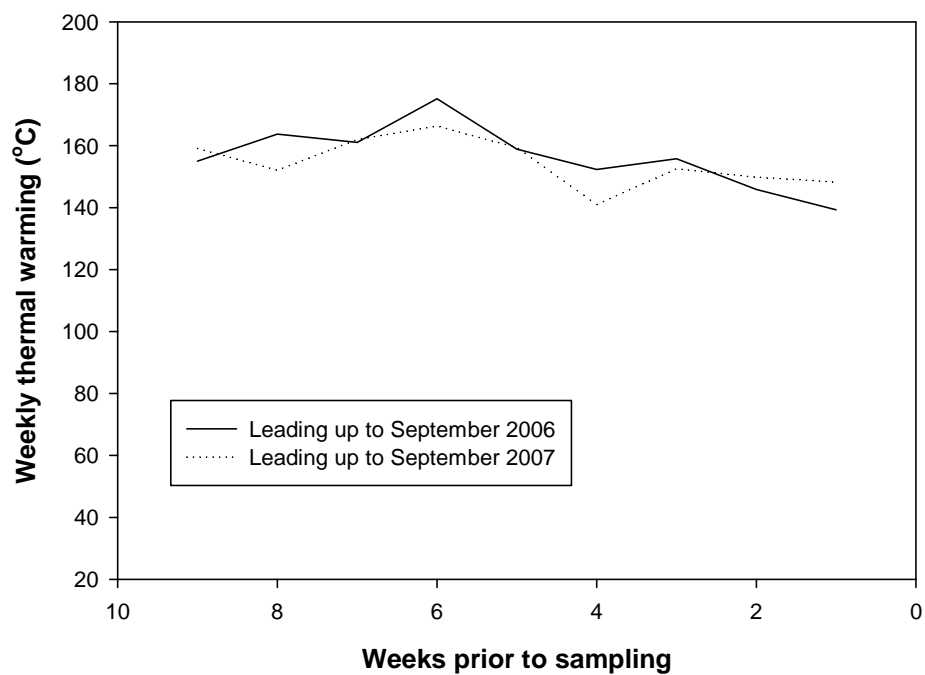
Figure 6. Sum of weekly soil thermal warming for the nine weeks prior to the September 2006 and

September 2007 sampling events. Week 0 corresponds to the week during which samples were removed.
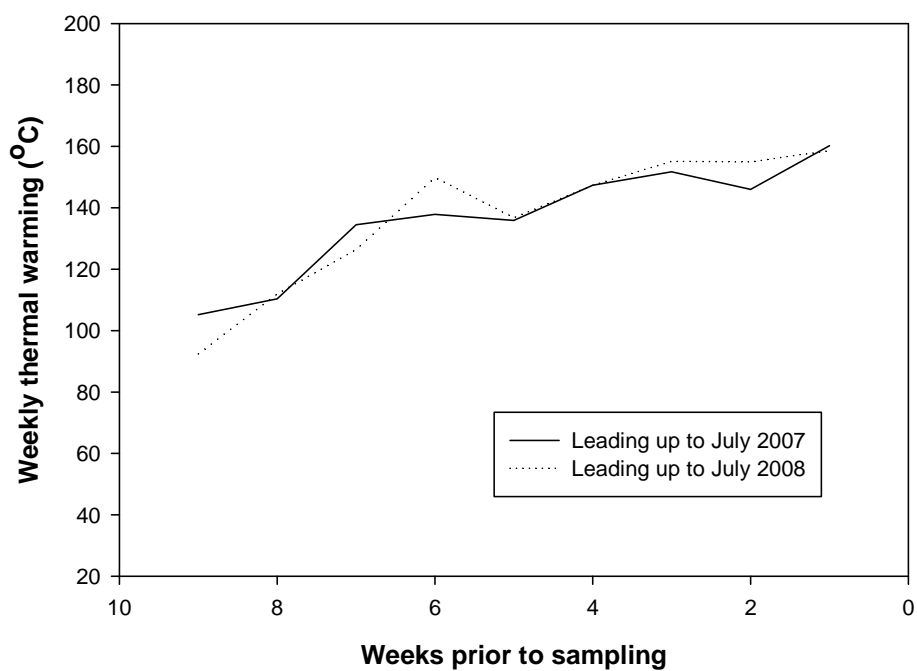
Figure 7. Sum of weekly soil thermal warming for the nine weeks prior to the July 2007 and July 2008

sampling events. Week 0 corresponds to the week during which samples were removed.
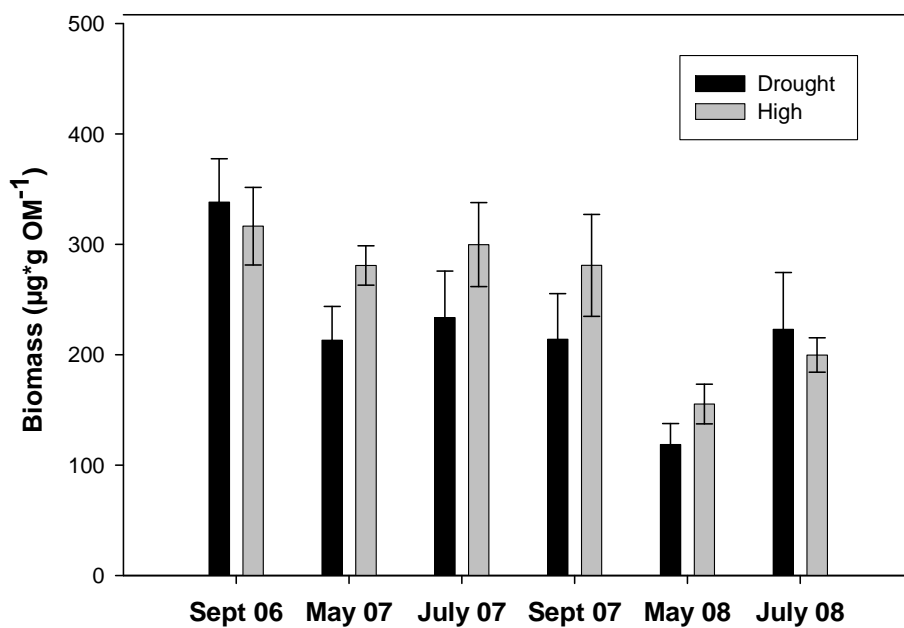
Figure 8. Response of average microbial biomass (± SE) in drought and high rainfall plots. There were no

significant differences between treatment means on any sampling date.
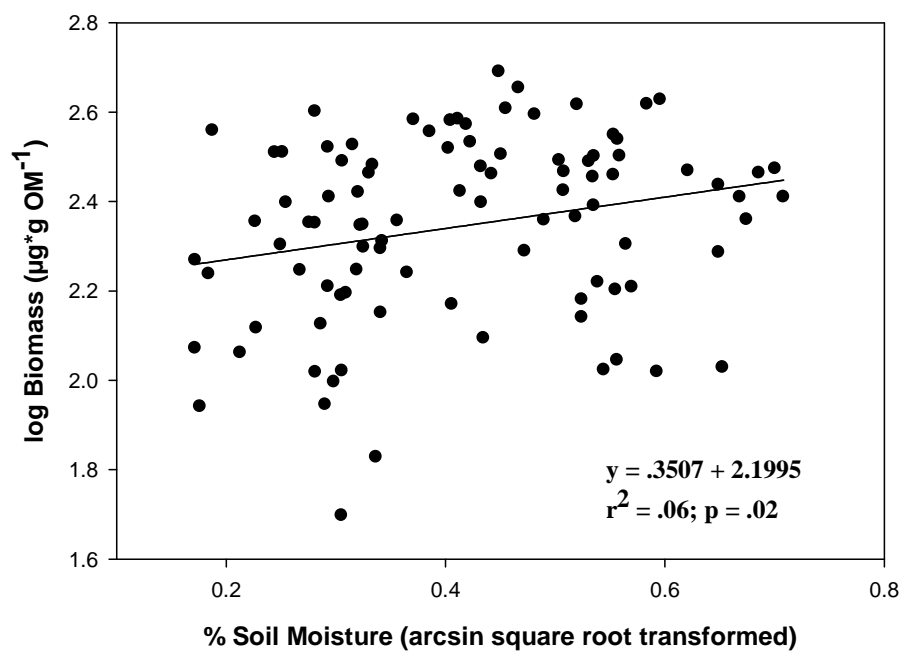
Figure 9. Linear regression analysis of the relationship between soil moisture and microbial biomass (all treatments and all sampling dates).
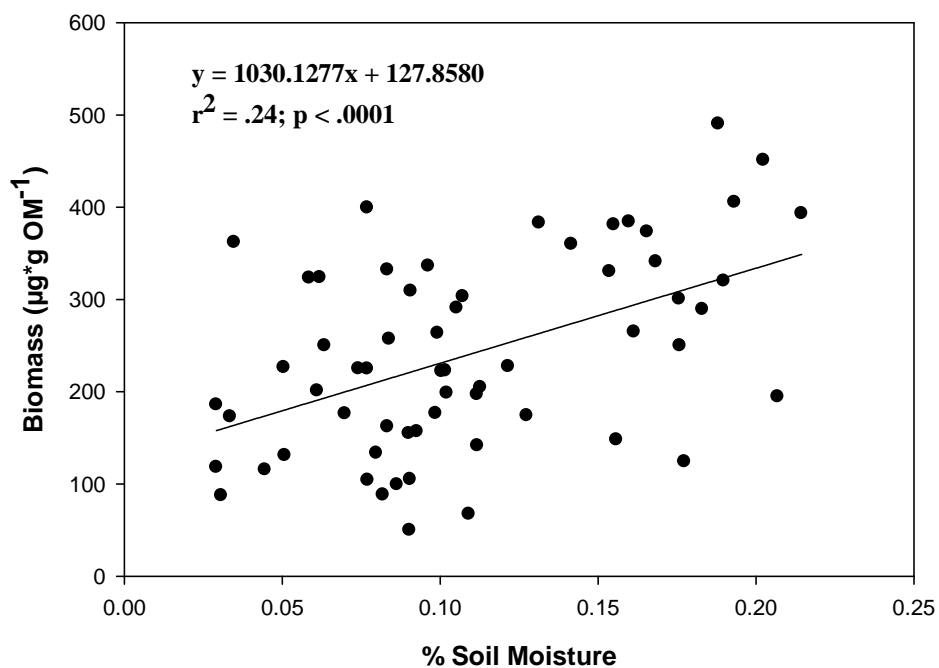
Figure 10. Linear regression analysis of the relationship between soil moisture and microbial biomass (all treatments and all sampling dates, SM<22%).
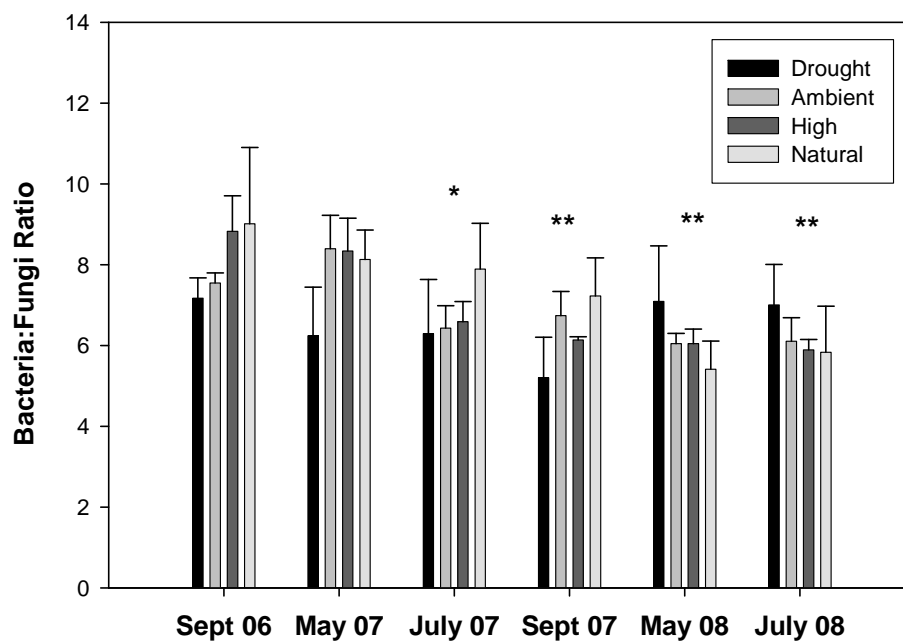
Figure 11. Response of the average (± SE) bacteria:fungi ratio to precipitation treatments. Stars (*) denote

significant differences between mean values in Sept 06 and subsequent sampling dates (analysis of variance

of contrast variables). * ($p < 0.05$) and ** ($p < 0.001$)
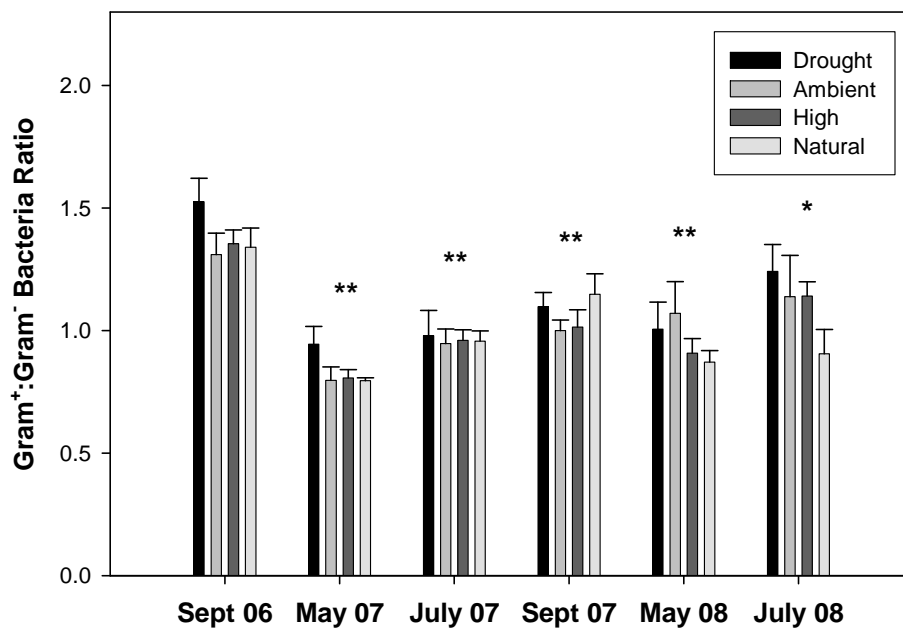
Figure 12. Mean response (± SE) of the Gram positive:Gram negative bacteria ratio to precipitation treatments. Stars (*) denote significant differences between mean values in Sept 06 and subsequent sampling dates (analysis of variance of contrast variables). * ($p < 0.01$) and ** ($p < 0.0001$)
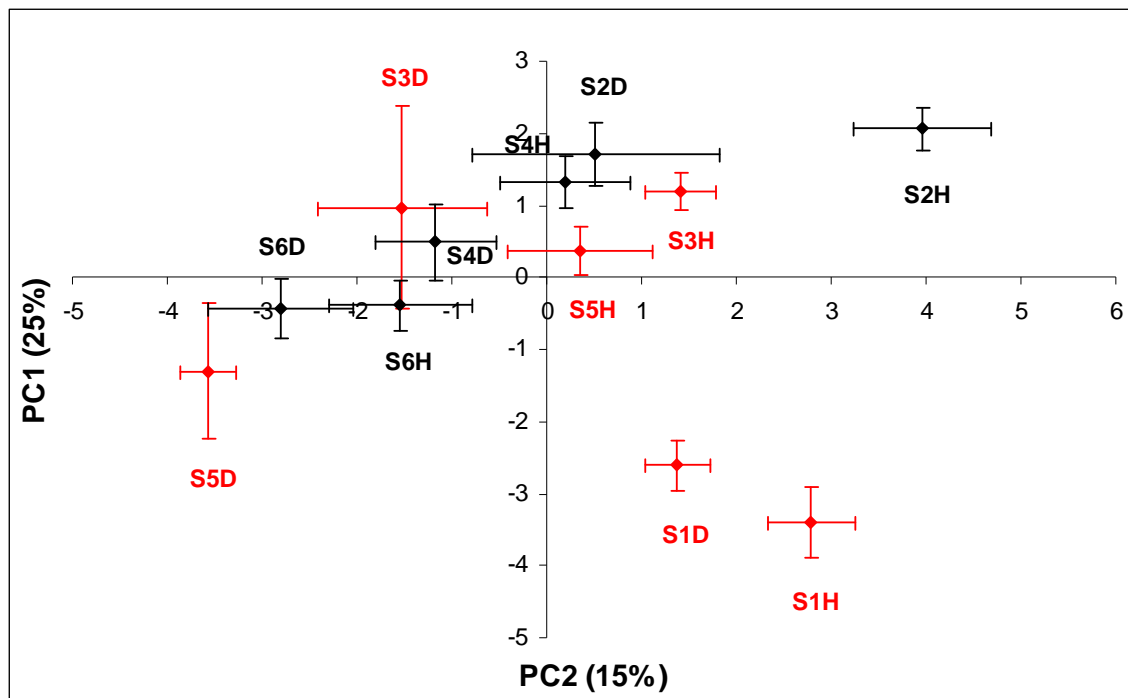
Figure 13. Mean response (± SE) of principal component scores of PLFAs along the first (PC1) and second (PC2) principal components to drought (D) and high (H) rainfall treatments. Numbers represent September 2006 (1), May 2007 (2), July 2007 (3), September 2007 (4), May 2008 (5) and July 2008 (6). Values in red indicate times during which principal component scores were significantly different among treatment means along the first principal component (*x*-axis).
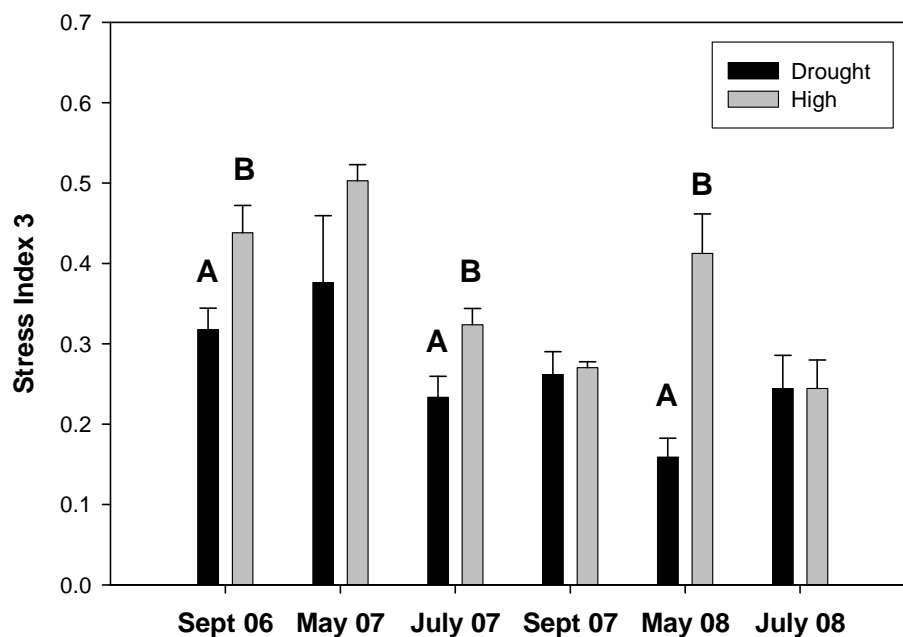
Figure 14. Mean response of stress index 3 (18:1 ω7c ÷ 19:0 CYCLO ω8c , ± SE) to precipitation treatments. Letters (A and B) denote significant differences among treatment means (Tukey's studentized range test, $p < 0.05$).
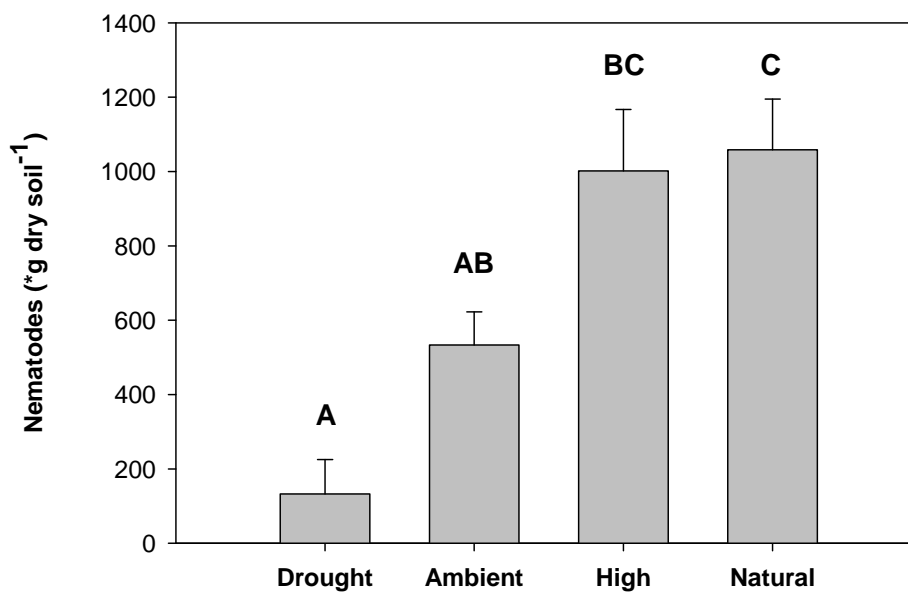
Figure 15. Response of average nematode densities (± SE) to precipitation manipulations during September 2007. Letters (A, B and C) denote significant differences among treatment means (Tukey's studentized range test, $p < 0.05$).
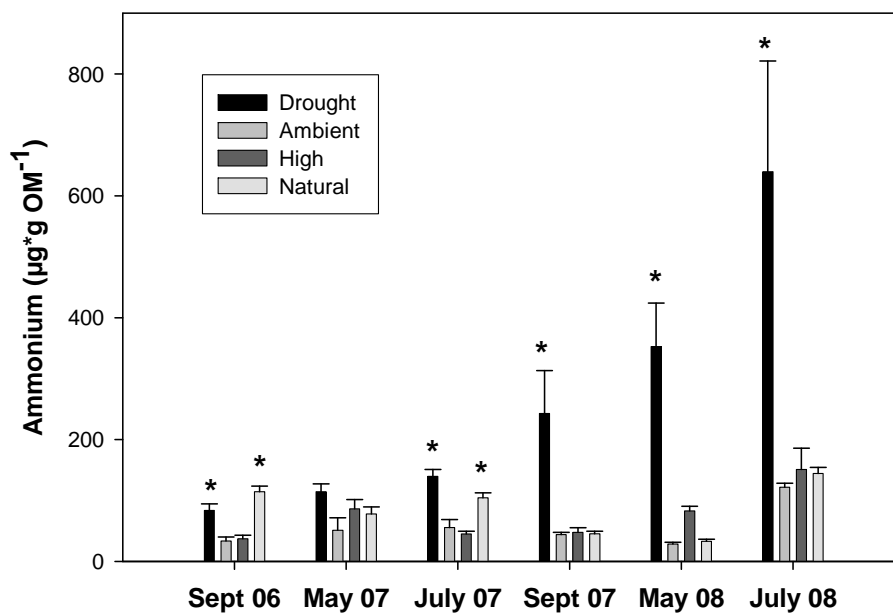
Figure 16. Response of average ammonium (NH_4^+-N) pools ($\pm$ SE) to precipitation manipulations. Stars (*)

denote significant differences between means with other (no stars) treatments (Tukey's studentized range
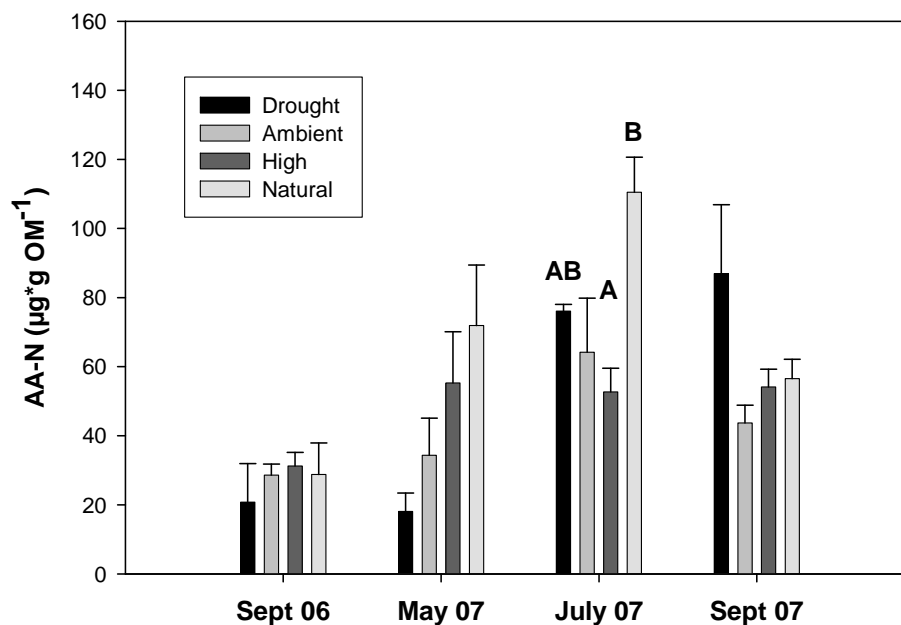
test, $p < 0.05$).

Figure 17. Response of average amino acid (AA-N) pools ($\pm$ SE) to precipitation manipulations. Letters (A

and B) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$).
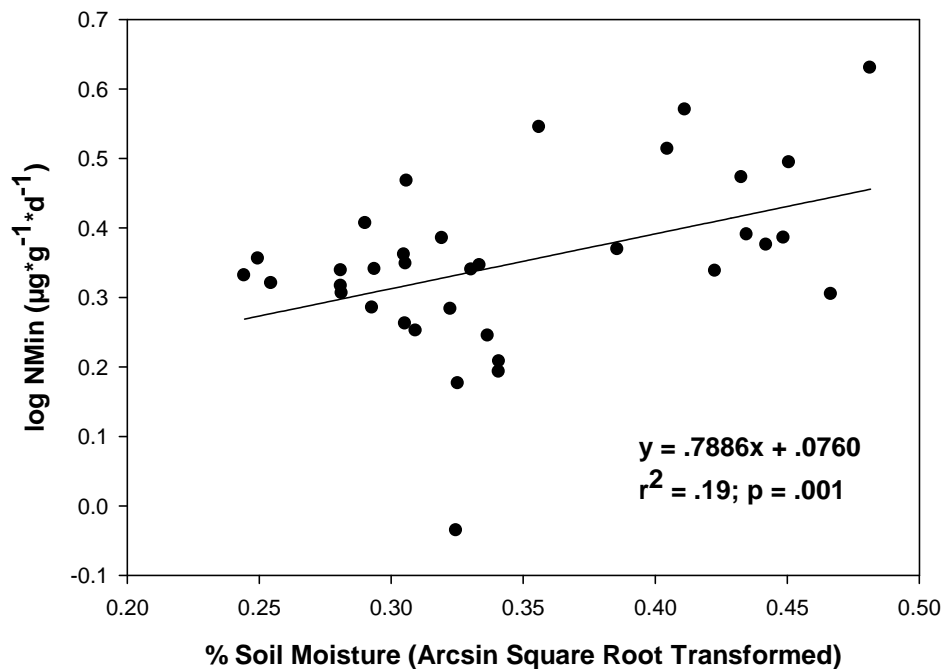
Figure 18. Linear regression analysis of the relationship between soil moisture and potential net nitrogen

mineralization rates, excluding drought treatments (SM<22%).
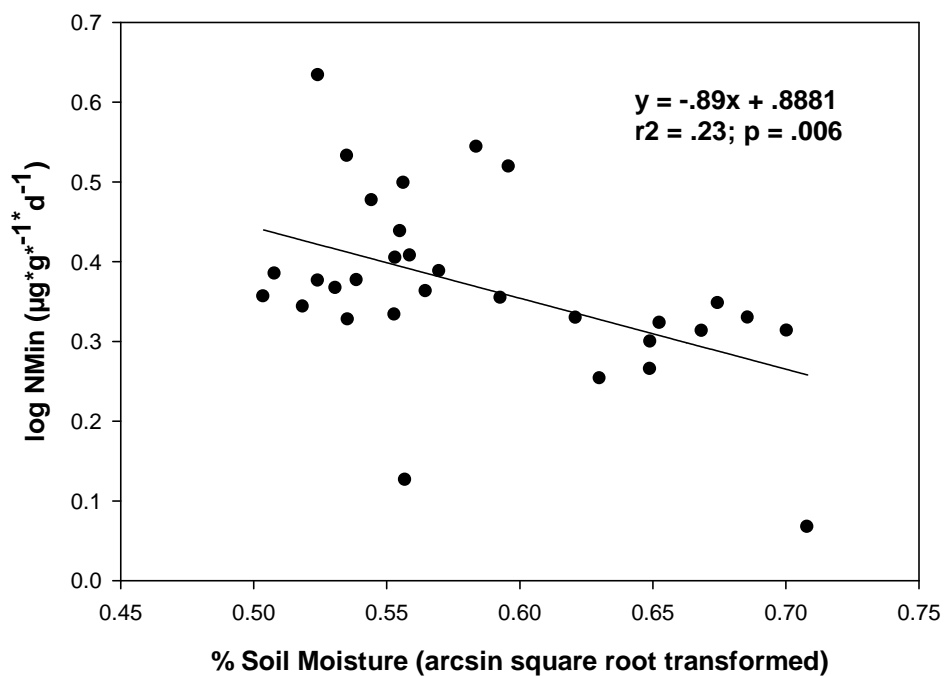
Figure 19. Linear regression analysis of the relationship between soil moisture and potential net nitrogen

mineralization rates, excluding a single drought treatment (SM >22%).
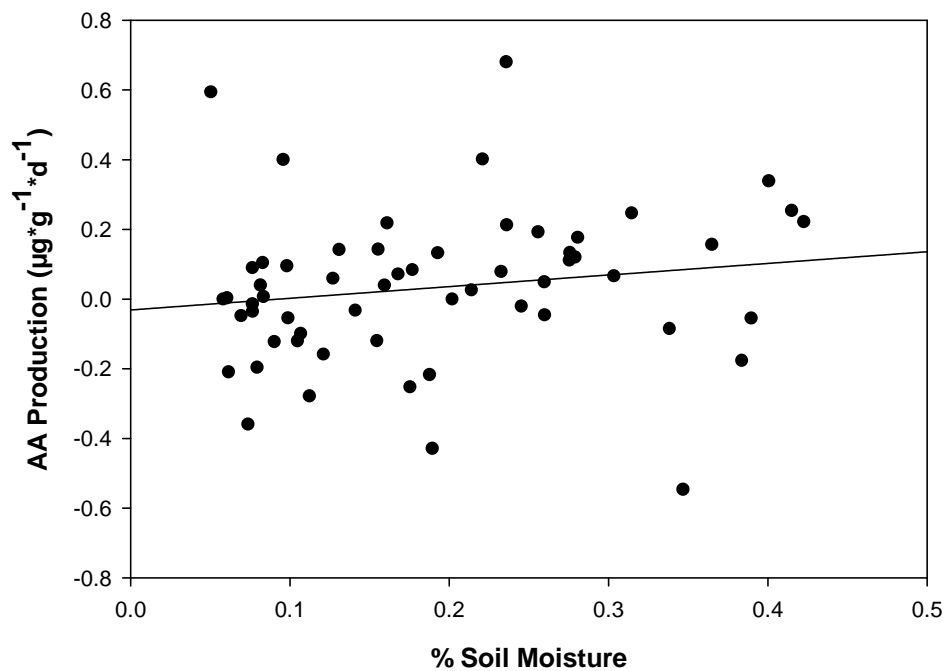
Figure 20. Linear regression analysis of the relationship between soil moisture and potential net amino acid production rates. All treatments and all sampling dates from September 2006 – September 2007 were included in the analysis.

CHAPTER 3

Temporal response of the soil microbial community following a two year rainfall

exclusion in the New Jersey Pinelands

INTRODUCTION

Soil microbial communities are frequently exposed to drying and wetting and have developed strategies for surviving these stressors. Climate models predict that precipitation frequency may decrease and intensity may increase in the northeastern U.S. during the 21st century (Baede et al. 2001, Diffenbaugh et al. 2005, Allan and Soden 2008). Combined with atmospheric warming, this might increase the frequency of dry days (Diffenbaugh et al. 2005) and lead to larger extremes in soil drying and wetting cycles. This could potentially shift the composition of microbial communities towards bacteria and fungi that are more tolerant of large fluctuations in soil moisture. It is important to understand how microbial communities will be affected by extreme drying and rewetting events, because a change in soil microbial community structure could alter rates of carbon (Fierer et al. 2003a, Lee et al. 2004) and nitrogen cycling (Fierer and Schimel 2002) with implications for important ecosystem functions such soil carbon sequestration (Borken et al. 2006) and nitrogen retention (Gordon et al. 2008).

To survive drought stress, microbial cells must maintain equilibrium with the external environment. This can be accomplished by the passive diffusion of water across cell membranes, or by synthesizing osmoregulatory solutes that maintain homeostasis without interfering with enzymatic activity (Harris 1981). Some soil bacteria can survive in dry soil by entering a dormant state, producing exopolysaccharides (Roberson and

Firestone 1992), forming spores or by altering the fatty acid composition of their cell membranes (Chapter 2). These strategies for dealing with drought stress may help to explain why only mild reductions in microbial biomass have been observed in response to drying (Bottner 1985, Scheu and Parkinson 1994, Pulleman and Tietema 1999 and Chapter 2).

Because different microbial taxa possess unique strategies for surviving drought stress, a reduction in soil moisture may alter microbial community composition towards those species that are more drought tolerant. For example, under laboratory conditions, Gram positive bacteria tend survive at much lower soil moisture values than Gram negative bacteria (Harris 1981). This is due to the fact that Gram positive bacteria can synthesize a broader range of osmoregulatory solutes and possess a stronger cell wall that allows for the higher turgor pressure created by osmoregulatory solutes (Harris 1981). Similarly, soil fungi possess a larger suite of osmoregulatory solutes than Gram negative bacteria (Harris 1981). Fungi can also extend hyphae through air-filled pore spaces to access moisture and nutrients, and can translocate these resources to water and nutrient-limited cells within their mycelial network (Jennings 1976, Jennings 1990).

Large precipitation events preceded by dry soil conditions cause a rapid change in water potential that can result in cell lysis (Kieft et al. 1987). To prevent this from occurring, some microbes release the osmoregulatory solutes accumulated during drought periods (Harris 1981). The same adaptations that should improve survival of Gram positive bacteria and fungi under drought stress to stronger cell walls and the ability to produce a larger suite of osmoregulatory solutes to are likely to provide an advantage during moisture upshock stress (Harris 1981, Luard 1982, Schimel et al. 2007). This led

to the hypothesis by Schimel et al. (2007) that, in response to multiple drying and rewetting events, the ratio of Gram positive to Gram negative bacteria should increase (Schimel et al. 2007) and the bacteria:fungi ratio should decrease in soil. Laboratory investigations with soil isolates (Halverson et al. 2000), as well as those summarized in (Harris 1981), support this hypothesis. To the best of my knowledge, this hypothesis has not been tested under natural field conditions.

When dry soil is rewet, microbial growth and activity is fueled by an increase in the pool of labile carbon substrates. There are several mechanisms by which this can occur. First, cell death resulting from the rapid increase in water potential releases cell contents into the soil (Kieft et al. 1987). Second, microbes that are not killed by upshock stress release osmoregulatory solutes accumulated during drought stress (Harris 1981). Upon rewetting, these become substrates that stimulate bacterial growth and activity (Fierer and Schimel 2003). Third, rewetting dry soil releases labile carbon from organic matter (van Gestel et al. 1991, Appel 1998, Wu and Brookes 2005), providing an additional potential energy source for microbial growth. The quality and quantity of carbon released, in conjunction with mechanisms of microbial adaptation to dry-wet cycles, are likely to influence the microbial community composition resulting from a rewetting event.

During a two year field study in the New Jersey Pinelands, I measured the response of microbial biomass and community structure to both rainfall exclusion and a doubling of precipitation amount. No differences in microbial biomass or community composition were observed between drought plots and other treatments (Chapter 2). These findings were based on six sampling events, during three seasons and over a two

year period. The lack of a response of microbial biomass and community structure to the manipulations may have been related to the temporal scale of sampling. During the two year study, all samples were removed more than 48 hours to and usually more than five days to after the most recent water application. However, other studies have observed changes in microbial biomass within three days of a rewet event (Gordon et al. 2008) and a change in microbial community composition within hours (Scheu and Parkinson 1994, Steenworth et al. 2005). These short-term effects were gone after only a few days (Gordon et al. 2008, Scheu and Parkinson 1994). During the two year rainout shelter study, had sampling events occurred immediately after the water applications, larger treatment effects on microbial biomass and community structure might have been detected.

There were two purposes to this study. The first was to determine the temporal scale over which soil microbes respond to precipitation events under natural field conditions. I hypothesized that, in response to watering, soil microbial biomass and community structure would change within hours but that the effect would not last beyond two days. The second purpose of this study was to analyze, under field conditions, the response of a soil microbial community under severe drought stress to a large watering event. I hypothesized that a rapid increase in soil moisture would negatively impact Gram negative bacteria while providing a selective advantage for the growth and reproduction of Gram positive bacteria. I further predicted that fungi are more tolerant of upshock stress than bacteria and that, within drought plots, the bacteria:fungi ratio would decrease in response to water additions. In natural plots, which received no previous manipulation, I predicted that water applications would have no effect on the Gram positive:Gram

negative bacteria ratio or the bacteria:fungi ratio relative to their values before watering and in comparison to drought treatments.

METHODS

Experimental Design

This study was conducted at the conclusion of a two year rainout shelter study (Chapter 2) during which all rainfall was excluded from four shelters (2.47m x 1.91m x 0.7m and with a 1.4m x 0.8m sampling area). Four additional "natural" plots (1.4m x 0.8m) received no rainout shelter and no manipulation. During this experiment, all drought and natural plots were rewet with rain water, using precipitation that was collected during the 25 days between the end of the two year study and the start of this study. Approximately 200 liters (40mm) of rain water was applied to drought and natural plots at a rate of approximately 1.6mm/minute. Following water applications, drought and natural plots were covered with clear PVC panels to prevent additional rainfall from reaching the plots throughout the three week sampling period.

Less than one hour prior to watering, samples were removed from all plots. A total of six additional sampling events were conducted following the water applications. These sampling events took place at three hours, eight hours, one day, two days, one week and three weeks following the water applications. To ensure that the timing of sampling events in relation to the water applications was consistent between plots, initial sampling and water applications were performed on two plots at a time. This was done by randomly selecting two plots, one from each treatment. Samples were removed to a depth of approximately 3-5cm from three randomly selected points under each shelter. Litter

and loose sand were removed and the organic horizon was retained for analyses. The three sub-samples were pooled and immediately homogenized and processed. Samples were broken apart by hand and visible roots were removed. Samples were further homogenized first by passing the sample through a 4.75 mm and subsequently through a 2 mm sieve. Homogenized samples were divided into subsamples and immediately analyzed for soil moisture (5g) and percent organic matter determination (5g).

Soil moisture was measured by weighing fresh soil into aluminum containers and drying at 70^oC for 48 hours. Soil moisture ($SM_{PERCENT}$) was calculated as the difference between the fresh weight ($Soil_{FW}$) and dry weight ($Soil_{DW}$) of the subsample, per unit dry weight soil: $SM_{PERCENT} = [(Soil_{FW}) - (Soil_{DW})]/(Soil_{DW})$. The percent organic matter content of samples was determined by loss on ignition at 550°C for 12 hours.

Microbial biomass and community composition were assessed using the phospholipid fatty acid analysis on 3 g subsamples stored at -20^oC. Following the methods of White and Ringelberg (1998), soil lipids were extracted in a one phase solvent of chloroform, methanol and phosphate buffer (1:2:0.8 ratio). The phospholipids were separated on a silicic acid column and phospholipid fatty acids were cleaved from the lipids and methylated. The identity of individual fatty acid methyl esters were determined by a combination of gas chromatography and mass spectroscopy. The biomass of individual fatty acids was calculated based on the peak area of the nonadecanoic acid (19:0) standard. Microbial biomass, expressed on a per *grams organic matter* basis (μg PLFA * g OM^{-1}), was calculated as the sum of all phospholipid fatty acids identified by the MIDI protocol and confirmed by mass spectroscopy. Community

composition was assessed based on the Gram positive:Gram negative bacteria ratio and the bacteria:fungi ratio (Table 1, see Chapter 2 for additional details).

At the conclusion of the three week sampling period, litter biomass was estimated by removing all litter within three 0.3m x 0.3m sampling grids. The litter was dried at 105°C for 48 hours, sieved through a 1 cm sieve and weighed. Litter biomass of the plots was estimated from the average of the three subsamples.

Statistical analyses

The response of microbial biomass, the bacteria:fungi ratio and the Gram positive to Gram negative bacteria ratio measured during the two year study (Chapter 2) was re-analyzed with the repeated measures analysis of variance (multivariate approach), using only drought and natural plots as treatment levels. Differences between treatment means were assessed with the Tukey's studentized range test ($p < 0.05$). The analysis of variance of contrast variables was used to compare the first (September 2006) sampling event to all subsequent sampling events.

The effect of the water applications on soil moisture, microbial biomass, the bacteria:fungi ratio and the Gram positive:Gram negative bacteria were assessed with a repeated measures analysis of variance (multivariate approach). Means separation was analyzed with the Tukey's studentized range test ($p < 0.05$). The difference between the initial and all subsequent samples was assessed with the analysis of variance of contrast variables. The relationship between soil moisture and microbial biomass was further analyzed with linear regression analysis, performed separately on samples removed

within the first two days of the experiment and on samples removed between two days and three weeks following the rewet event.

Litter biomass data was analyzed with a one way analysis of variance. Significant differences between treatment means was based on the Tukey's studentized range test ($p < 0.05$).

RESULTS

Microbial communities (two year study)

Throughout the two year study, the treatments did not result in a significant difference in microbial biomass between drought and natural plots (between subjects effect, $p = 0.46$) on any sampling date (Figure 1). Based on the analysis of variance of contrast variables, there was no difference in the treatment response at any sampling time relative to the September 2006 sampling event. However, average microbial biomass among both treatments declined during the second year of the study resulting in a significant mean difference between the September 2006 and September 2007 ($p = 0.06$), May 2008 ($p = 0.0007$) and July 2008 ($p = 0.02$) sampling events (Figure 1).

The two year rainfall manipulation did not change the bacteria:fungi ratio between treatment means (between subjects effect, $p = 0.52$) (Figure 2). There was no overall time by treatment effect ($p = 0.24$) and no overall effect of time ($p = 0.41$). The average ratio between May 2007 and July 2008 was always lower than the September 2006 mean but the difference was never significant at the $p < 0.05$ level (Figure 2). The rainfall manipulations did not alter the Gram positive to Gram negative bacteria ratio between drought and natural plots (between subjects effect, $p = 0.10$) (Figure 3).

However, there was a significant effect of time ($p = 0.02$) and the average ratio among all treatments in September 2006 was significantly higher than the average in May 2007 ($p = 0.0003$), July 2007 ($p = 0.002$), September 2007 ($p = 0.0008$), May 2008 ($p = 0.0003$) and July 2008 ($p = 0.01$) (Figure 3).

Soil Moisture

Immediately prior to watering, average soil moisture in drought (2.4%) and natural (10%) plots were significantly different (Tukey's studentized range test, $p < 0.05$, Table 2). There was a rapid increase in soil moisture in all plots three hours after watering and average soil moisture between drought and natural plots was significantly different at three and eight hours following the rewet event (Figure 4). Between three hours and two days, soil moisture remained fairly stable in natural plots but continued to rise in drought plots. After two days, soil moisture values in drought and natural plots were similar (15.1% and 16.7% respectively). Between two days and one week following the rewet event, soil moisture values decreased by an average of 47% in drought and 36% in natural plots and the difference between treatment means was not significant (Figure 4). After three weeks, average soil moisture was 4% in drought plots and 5% in natural plots.

Effect of the rewet on microbial biomass

At the conclusion of the two year experiment, 25 days prior to the rewet event, microbial biomass in drought (223 µg*gOM^{-1}) and natural (128 µg*gOM^{-1}) plots was not significantly different (July 2008 in Figure 1). There were further declines in microbial

biomass in the three weeks leading up to the rewet event. Less than one hour prior to watering, average microbial biomass in drought plots was 74 $\mu g*gOM^{-1}$ and average microbial biomass in natural plots was 99 $\mu g*gOM^{-1}$ (Figure 5).

As a result of the rewet event, there was a significant between subjects effect on microbial biomass between drought and natural plots (p = 0.005, Figure 5). After only three hours, average microbial biomass in natural plots increased by 74% while there was an increase of only 9% in drought plots. After eight hours, microbial biomass in natural plots was more than double the value in drought plots (Table 2). Between one and two days following the water application, there was no significant difference in average microbial biomass between treatments. The treatment means were again significantly different after one week, but not after three weeks. Relative to the value just prior to watering, average microbial biomass among all plots was always significantly higher after the water applications (Table 2).

Community composition

The initial average bacteria:fungi ratio in drought and natural plots was 6.3 and 5.6 respectively, and this difference was not statistically significant. The treatments resulted in a significantly different response to watering between initial values and the ratio after three and eight hours (analysis of contrast variables, p = 0.003 and 0.01 respectively, Table 3). After three hours, there was an increase in the bacteria:fungi ratio in drought plots to 7.7 and a decrease in the ratio in natural plots to 4.9 (Figure 6). Although treatment means were again significantly different after eight hours (Tukey's studentized range test, p < 0.05), there was a trend towards converging ratios, and after

one day the average bacteria:fungi ratio was almost identical between treatments (6.1 and 5.8). Between one day and three weeks, the bacteria:fungi ratio was stable within natural plots and more dynamic within drought plots. The ratio within drought plots increased between one day and one week and then decreased between one and three weeks (Figure 6). As a result, the contrast analysis revealed a treatment effect between the initial sampling event and the ratio after two days and one week ($p = 0.006$ and 0.009 respectively, Table 3). After three weeks, there was no significant difference between treatment means and the average bacteria:fungi ratio among all plots was not significantly different from the initial value.

The change in the bacteria:fungi ratio was driven primarily by an increase in fungal biomass in natural plots and a suppressed response of fungal biomass within drought plots (Figure 7). A significant difference in the treatment response of fungal biomass over time was apparent after three hours, eight hours and one week (analysis of variance of contrast variables, Figure 7).

The test for between subjects effects on the Gram positive:Gram negative bacteria ratio was suggestive of a significant treatment effect ($p = 0.07$) however the average values were similar on most sampling dates and the Tukey's analysis showed no significant difference between treatment means at any time (Figure 8). The more striking result was a decrease in the ratio between both treatments after eight hours ($p = 0.05$, Table 3). The ratio then increased between eight hours and one week, at which time it was significantly higher than the initial value ($p = 0.03$, Table 3). While the ratio continued to increase in drought plots between one and three weeks there was a decrease in the ratio in natural plots over this same period. After three weeks the average Gram

positive:Gram negative bacteria ratio among all plots was not significantly different from the initial ratio.

Regression Analysis

Linear regression analysis revealed no relationship between soil moisture and microbial biomass when analyzed using only samples removed after two days (Figure 9). Using initial samples and samples removed within the first day of the rewet event, soil moisture explained 47% of the variation in microbial biomass ($p = 0.006$, Figure 10).

Litter Biomass

Average litter biomass of drought plots (932 g/m^2) was significantly higher than average litter biomass of natural plots (510 g/m^2) ($p = 0.03$, Figure 11).

DISCUSSION

Implications for the two year rainfall study

The microbial response to the rewet event was extremely rapid. Relative to the initial sampling event, microbial biomass, the Gram positive:Gram negative bacteria ratio and the bacteria:fungi ratio all changed within eight hours. Treatment effects, which were detected for microbial biomass and the bacteria:fungi ratio, occurred within three hours. Most of these significant differences were undetectable after one day. These results help to explain why no significant treatment effects were detected during the two year study (Figures 1 to 3 and Chapter 2) and highlight the importance of considering temporal dynamics when sampling soil microbial communities. During the two year study, had

samples been removed within one day of a large rainfall event or water application, it is likely that greater differences would have been detected between the drought and other treatments.

While the treatment effects of the rewet were mostly gone after one day, there was a significant difference in treatment means for microbial biomass and the bacteria:fungi ratio during the one week sampling event (Figures 5-6). This suggests that, during the two year study, significant differences in microbial biomass among treatments should have been detected on samples removed within one week of watering. There were four sampling events during the two year study which occurred within one week of the most recent water application, but no treatment effects were ever detected. Three of these sampling events followed much smaller water inputs than the ones applied in the current study, suggesting that precipitation amount influences the duration of the microbial response. This would be consistent with the findings of Steenworth et al. (2005), who found no affect of a mild dry-wet treatment on microbial biomass in sandy agricultural and grassland soils.

Microbial biomass

Previous investigators have observed a rapid response of the microbial community to rewetting of dry soil. For example, Bloem et al. (1992) found that rewetting increased bacterial growth rates for about 5 days. Using an organic podzol soil, (Lund and Gøksoyr 1980) identified a fast growing bacterial population that rapidly increased in response to rewetting and a slow growing population that increased gradually over a ten day period. Similarly, other studies have determined that soil drying

has a greater negative effect on metabolically active microbes, while older, dormant and less metabolically active ("metabolically old") cells are more drought tolerant (Bottner 1985, van Gestel et al. 1993). These studies help to explain the different response of microbial biomass within drought and natural treatments. In natural plots, the rapid (three hour) increase in microbial biomass (Figure 5) is consistent with a fast growing microbial community that responded immediately to the increase in soil moisture. In drought plots, the lack of a similar rapid increase in microbial biomass is most likely due to a large reduction in the population of metabolically active microbes.

The more gradual increase in microbial biomass in drought plots over the three week period is consistent with a slow growing "metabolically old" community, as hypothesized by Bottner (1985) and Lund and Gøksoyr (1980). A similar slow growing community may have been responsible for the gradual increase in natural plots between eight hours and three weeks (Figure 5). The studies performed by Bottner (1985) and van Gestel et al. (1993) used soils with a higher clay content and a well developed aggregate structure. The "metabolically old" microbes in those studies might have been protected from drought by residing within small water-filled pore spaces created by the aggregate structure of soil (Elliott and Coleman 1988). Due to a very low clay content, these microsites should be far less abundant in pinelands soils and, therefore, a much larger proportion of the "metabolically old" microbial community would have been exposed to the drought conditions. As a result, the extreme drought should have negatively affected this slower growing microbial community as well, explaining the consistently higher microbial biomass within natural plots and the significant difference in microbial biomass after one week (Figure 5).

The lack of an immediate response of microbial biomass in drought plots could have been the result of osmotic shock resulting from the rapid increase in soil moisture. In response to the two year drought, microbes would have accumulated osmoregulatory solutes to prevent water loss. Upon rewetting, this would have caused a rapid influx of water, which may have overwhelmed cells before they could adjust by releasing the osmoregulatory solutes (Harris 1981). These lysed cells could have caused an initial delay in the increase in microbial biomass, but the release of labile carbon from cell contents would have eventually fueled microbial growth of surviving microbes. This would be consistent with my predictions but cannot be proven with the existing data.

Factors unrelated to microbial physiology may have influenced the different response between drought and natural plots. There was a very large accumulation of litter in drought plots (Figure 11), which appears to have formed a physical barrier to rainfall and prevented a more rapid increase in soil moisture. While soil moisture rapidly increased within both treatments after three hours, soil moisture remained stable in natural plots until day two while it continued to gradually increase in drought plots over the same time period (Figure 4). This gradual increase in soil moisture in drought plots may have been the result of water trapped in the litter layer being slowly released into the organic horizon. This might have resulted in a slow delivery of dissolved nutrients to the microbial community, explaining the more gradual increase in microbial biomass.

Microbial biomass may have in fact increased rapidly in drought plots but could have been masked by methodological limitations. It is well known that microbial cells lysed during soil drying become an energy source for surviving microbes upon rewetting (e.g., Bottner 1985, Kieft et al. 1987). Because microbial biomass was similar between

treatments prior to the rewet (July '08 in Figure 1), this source of carbon should have been available in relatively equal quantities between treatments. On the other hand, an important assumption of the PLFA analysis, that fatty acids are quickly decomposed following cell death (Pinkart et al. 2002), may have been violated. In the drought plots, decomposition rates were severely reduced, as evidenced by the accumulation of litter biomass (Figure 11). Fatty acids from lysed cells could have remained undecomposed in the soil and would not have been distinguished from living biomass by the PLFA method. Microbial cells activated by the rewet may have decomposed this potentially abundant and phosphorus-rich substrate. If this occurred, then an increase in microbial biomass due to the rewet would have been counteracted by a decrease in soil fatty acids, resulting in the appearance of no change in microbial biomass.

The extent to which the decomposition of fatty acids was reduced by drought, if at all, is unclear. While litter biomass data is indicative of reduced decomposition in the litter layer, the increase in ammonium pools and positive nitrogen mineralization rates over the two year study (Chapter 2) points to sustained microbial activity in the organic horizon. However, extremely low soil moisture values, and the observation of no nitrogen mineralization during the July 2008 sampling event ($0.07 \ \mu g * g^{-1} * d^{-1}$ in drought plots, Chapter 2) suggests that microbial activity may have been much lower towards the end of the experiment.

Community composition

I predicted that the rapid change in water potential within drought plots would negatively impact soil bacteria and favor the growth of fungi. Instead, there was a sharp

increase in the bacteria:fungi ratio in drought plots and a decrease in this ratio within

natural plots (Figure 6). This was primarily the result of a large and rapid increase in

fungal biomass in natural plots and a more suppressed response of fungi in drought plots

(Figure 7). An increase in the bacteria:fungi ratio was also observed within six hours of

rewetting a sandy, organic podzol that had been dried for only 14 days (Scheu and

Parkinson 1994). The increase in the bacteria:fungi ratio in drought plots may have been

driven by differences in the size of the labile carbon pool. Two likely sources of elevated

carbon pools in drought plots include the release of osmoregulatory solutes from living

microbial cells (Fierer and Schimel 2003) and root mortality (confirmed by visual

inspection of samples but not quantified). Additionally, the drought plots contained

almost twice the litter biomass of natural plots (Figure 11), providing an additional source

from which labile carbon could have been mobilized during the water applications

(Borken et al. 2003, Miller et al. 2005). The potentially elevated pool of labile carbon

may have favored bacterial growth, as bacteria are better adapted for metabolizing labile

substrates. In the natural plots, normal decomposition and leaching of nutrients from the

litter layer should have maintained a relatively higher abundance of recalcitrant carbon,

favoring the growth of fungi (Figure 6), which possess a vast array of enzymes for the

decomposition of complex substrates (Sinsabaugh 2005).

The response of the bacteria:fungi ratio might have been affected by differences

in the size of ammonium pools, which averaged in 640 $\mu g*gOM^{-1}$ in drought plots and

144 $\mu g*gOM^{-1}$ in natural plots (Chapter 2). Because of the large ammonium

accumulation, the rewet of drought plots was essentially an ammonium fertilization.

Lucas et al. (2007) hypothesized that soil fungi are negatively impacted by ammonium

additions, which disrupts fungal exoenzyme production (Gallo et al. 2004). Their data,

based on samples removed 28 days following the treatment, did not support this

hypothesis. However, I did find a decrease in the relative abundance of fungal biomarkers

over a much shorter time scale (Figure 6). Fungal growth within natural plots (Figure 7)

may therefore have been inhibited by the high ammonium concentrations and unrelated to

soil carbon dynamics.

There was a significant decrease in the average Gram positive:Gram negative

bacteria ratio among all plots within only eight hours of the rewet event (Figure 8). This

was the opposite of what was predicted and does not support the hypothesis that the

Gram positive cell wall is more resistant to upshock stress. A more likely explanation is

that the water additions mobilized dissolved organic carbon, providing a flush of labile

energy for soil microbes. This would have favored the growth of Gram negative bacteria,

which are believed to be better adapted for rapid growth when resource availability is

high (Fierer et al. 2003b). Beginning with the sample event at one day, there was a shift

towards an increase in the Gram positive:Gram negative bacteria ratio. By this sampling

time, the labile carbon supply potentially mobilized by the water application may have

been consumed. This might have increased the relative abundance of recalcitrant carbon,

favoring the growth of Gram positive bacteria which are more abundant in regions with

low carbon availability (Fierer et al. 2003b).

Temporal dynamics

One important finding of this study is the impact that the temporal scale of

sampling can have on the results of an experimental manipulation of soil microbial

communities. When samples were removed more than 48 hours after a rewet event, no changes in microbial biomass and community composition were detected (Chapter 2). Much different results were found within hours of a rewet event. Similarly, Gordon et al. (2008) found an immediate increase in microbial biomass in response to a rewet event, but no differences with control treatments after eight days. These short-term and longer-term effects lead to different conclusions regarding the soil microbial community. The longer-term results point to a highly resilient and stable microbial community that is not affected by precipitation. However, the transient response that follows individual rain events shows a much more dynamic response. These short-term changes could have a cumulative effect on processes such as decomposition and nutrient cycling.

An example of how temporal dynamics of soil microbial communities can influence the outcome of a study comes from a comparison with that of Lucas et al. (2007). Coincidentally, one portion of this project to the test of the response of microbial communities to ammonium fertilizations to is in some ways remarkably similar to the rewet of drought plots in this study. Both experiments used similar soils from the New Jersey Pinelands, had the same response variable (PLFAs) and involved similar ammonium concentrations (5Kg/Ha vs. an estimated 4 to 6 Kg/Ha within drought plots of this study). Lucas et al. (2007) sampled the microbial community 28 days after ammonium fertilization and found no effect on fungal biomass. This is consistent with my findings after 21 days (Figure 6). However, I did observe a reduction in the relative abundance of fungi after three and eight hours and fungal biomass that was suppressed throughout most of the sampling period (Figure 7). These findings suggest that Lucas et

al. (2007) might have observed different results had they sampled on a finer temporal scale.

This study demonstrates that temporal dynamics limit the utility of using regression analysis to study the relationship between soil moisture and microbial biomass. Following the two day sampling event, a large decline in average soil moisture was observed within both treatments. The decline continued until the final sampling event, when average soil moisture was 30% of the value at day two (Figure 4). Over this same time period (two days to three weeks), average microbial biomass was mostly unchanged (Figure 5). As a result, soil moisture did not explain any of the variation in microbial biomass between two days and three weeks following the rewet event (Figure 9). In contrast, soil moisture explained 47% of the variation in microbial biomass over the first day of the experiment (Figure 10).

The energy source driving changes in the microbial community

There is an active debate in the literature regarding the source of the carbon that drives the microbial response to a rewet event. While it is clear that there are both microbial and non-microbial sources, there is uncertainty regarding the relative contribution of each. Xiang et al. (2008) hypothesized that the relative contribution of organic matter and microbially-derived C to the microbial response is influenced by the pool of mineralizable organic matter. Based on the large accumulation of litter biomass and the visual (but not quantified) observation of dead roots, the drought plots should have had the larger pool of labile carbon. The relatively higher bacteria:fungi ratio in drought plots after rewet is also indicative of a higher labile carbon pool. However, only

natural plots exhibited a rapid (3 hour) increase in microbial biomass. This would suggest

that microbes were more limited by microbially-derived carbon. Due to low summer

precipitation (Chapter 2) and low initial soil moisture values (Figure 4), the application of

water to natural plots can be seen as a more mild dry-wet event. With a potentially higher

population of metabolically active microbes, labile carbon could have been made

available in natural plots via the release of osmoregulatory solutes upon rewetting (Fierer

et al. 2003). This source of carbon was most likely less abundant in drought plots, as the

community appears to have been dominated by a less metabolically active community.

Additional evidence for a non-microbial energy source comes from the low

aggregate structure of the Pinelands soils. Studies that find an increase in the non-

biomass pool of organic matter usually involve aggregated soils (e.g., Wu and Brookes

2005, Xiang et al. 2008). Rewetting of aggregates causes slaking (Elliott 1986) which

physically disrupts the aggregate structure and exposes organic matter protected within

the intra macro-aggregate pore space. As a result, this organic matter becomes available

as an energy source. Because the soils measured in this study contain little aggregation,

this mechanism by which organic matter availability increases would not apply. This

reasoning was also employed by Xiang et al. (2008) to explain why more non-microbial

C was available in their aggregated soils, compared to the less aggregated soils used by

Mikha et al. (2005). Furthermore, van Gestel et al. (1991), found a higher release of

organic matter from the rewetting of well aggregated than less aggregated soil and, using

a loamy sand and non aggregated soil, Magid et al. (1999) found no effect of a rewet on

organic matter turnover.

Adaptation to drought and moisture stress

Several investigators have hypothesized that soil microbes exposed to frequent drying and wetting are better adapted to dry-wet cycles. Kieft et al. (1987) used this reasoning to explain why microbes from coarse soils were more sensitive to dry-wet cycles than microbes from finer textured soils. Fierer et al. (2003a) used similar reasoning to explain why microbes from forested, but not grassland soils were affected by dry-wet cycles. As opposed to many forest soils in the northeastern U.S., the relatively high sand content of the organic pinelands soils make them more susceptible to drying. Additionally, because the organic horizon is fairly small, and lies above a very sandy mineral soil, water passing through the organic horizon drains rapidly, and is less likely to rise from capillary action. Furthermore, soil aggregation is low in pineland soils, reducing the number of pores spaces that could remain water-filled when soil is dry. Therefore, relative to other forest soils, it is likely that organic soils in the pinelands experience more drying and wetting, suggesting that, like grassland soils, microbes within these soils are well adapted to the stress of dry-wet cycles.

The lack of a long-term effect (Chapter 2 and Figures 5-8) supports the hypothesis that microbial communities in the pinelands are well adapted to drought stress and even the most severe dry-wet cycles. On the other hand, a more detailed analysis of the microbial community with more recently developed molecular techniques might reveal more subtle long-term changes. As researchers continue to improve the functional interpretation of these results (Fierer et al. 2007), they should be utilized to improve the analysis of the microbial response to precipitation and climate change.

Conclusions

This study demonstrates that rainfall can alter soil microbial biomass and community composition in the New Jersey Pinelands within hours, but that the treatment effects of a single rain event are undetectable on the scale of weeks. Similar results have been found using a diverse range of soil types (ex: Bottner 1985, Bloem et al. 1992, Scheu and Parkinson 1994), suggesting that this is a universal property of soil microbial communities.

There was a rapid and large increase in fungal biomass within natural plots that was not seen in drought plots. This could have been due to high ammonium concentrations in drought plots that inhibited fungal exoenzyme production. The response of microbial biomass in natural plots is also consistent with a hypothesized fast growing microbial community that quickly responded to the rewet event, followed by a slower, less metabolically active community that gradually increased over one week. The fast growing microbial community appears to have been reduced within drought plots, with the response dominated by a slower growing microbial community. A review of studies into the effects of dry-wet cycles suggests that, in soils with low aggregation, the increase in microbial biomass following rewet is of microbial origin. This might help to explain the less dramatic increase in microbial biomass within drought plots. The hypothesized reduction in the active microbial community may have prevented the release of osmoregulatory solutes that would have stimulated microbial growth.

These results have important implications for experimental manipulations of soil microbial communities. Although a two year drought is unlikely to occur, the microbial community within drought plots appears to be resilient to even this extreme and

prolonged disturbance (Chapter 2). The fact that microbial communities within drought and natural plots were similar only three weeks after a single rewet event supports this conclusion. Functionally, the transient response of bacteria and fungi to individual rain events could have a cumulative effect on soil nutrient cycling. Furthermore, if the drought treatment did in fact alter the relative abundance of microbes with higher vs. low metabolic activity, then effects on nutrient cycling might be sustained for days or weeks.

The response of microbial communities to rewet events under field conditions are an integrative response to many factors that are simultaneously altered in response to drying. Two such factors were identified here. A large buildup of ammonium pools potentially influenced the fungal response in drought plots, and a doubling of the litter biomass may have increased the labile carbon supply, but also could have buffered against a more rapid increase in microbial biomass. These results highlight the need for more field studies of soil microbial communities, as such studies will help to reveal many of the complex interactions occurring under natural conditions. As these factors are identified, they may provide the basis for improved laboratory studies that identify important mechanisms.

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Table 1. List of fatty acids identified by the MIDI protocol and confirmed by GC-MS (group assignments based on Frostegård and Bååth 1996, O'Leary and Wilkinson 1988, Wilkinson 1988 and Zelles 1999).

Biomarker for	Fatty Acid
Gram positive bacteria	i15:0, a15:0, i16:0, i17:0, a17:0
Gram negative bacteria	16:1 ω7c, i16:1 G, i16:1 I, 17:1 ω8c, cy17:0, 18:1 ω7c, cy19:0ω8c
Bacteria	All above, plus 16:0 10Me, 17:0 10Me, 18:0 10Me
Fungi	18:2 ω6, 9c
Microbial Biomass	All above, plus 18:1 ω9c, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0

Table 2. Response of average microbial biomass, the bacteria:fungi ratio (B:F) and the Gram positive:Gram negative bacteria ratio (±SE) to the rewetting of drought and natural plots. Letters ([a] and [b]) denote significant differences among treatment means (Tukey's studentized range test, $p < 0.05$).

Time	Treatment	% SM	Biomass $\mu*gOM^{-1}$	B:F	Gram+:Gram-
Init	Drought	0.02 (0.002)[a]	74 (14)	5.3 (0.5)	0.6 (0.1)
	Natural	0.10 (0.01)[b]	99 (14)	6.3 (0.5)	0.6 (0.04)
3Hr	Drought	0.10 (0.02)[a]	103 (17)[a]	7.5 (0.6)[a]	0.6 (0.1)
	Natural	0.19 (0.01)[b]	172 (16)[b]	5.4 (0.3)[b]	0.5 (0.1)
8Hr	Drought	0.10 (0.01)[a]	81 (19)[a]	6.9 (0.6)[a]	0.5 (0.1)
	Natural	0.17 (0.02)[b]	180 (17)[b]	6.1 (0.03)[b]	0.5 (0.1)
1Day	Drought	0.13 (0.02)	104 (10)	6.0 (1.2)	0.6 (0.04)
	Natural	0.18 (0.01)	161 (30)	6.3 (0.3)	0.6 (0.1)
2Day	Drought	0.15 (0.03)	130 (34)	7.0 (0.7)	0.7 (0.1)
	Natural	0.17 (0.004)	182 (40)	5.9 (0.2)	0.6 (0.1)
1Wk	Drought	0.07 (0.01)	126 (11)[a]	6.9 (0.7)[a]	0.8 (0.03)
	Natural	0.11 (0.02)	214 (14)[b]	5.5 (0.4)[b]	0.78 (0.02)
3Wk	Drought	0.04 (0.01)	143 (20)	5.9 (0.1)	0.8 (0.1)
	Natural	0.05 (0.003)	190 (6.7)	5.7 (1.1)	0.6 (0.03)

Table 3. Results of the analysis of variance of contrast variables for the response of microbial biomass, the bacteria:fungi ratio and the Gram positive:Gram negative bacteria ratio. The F statistic (F) and corresponding p-value (p) are shown for the contrast of all post-rewet sampling events with the initial sampling event. Values in red indicate differences in means and values in blue represent treatment effects. 3Hr = three hours, 8Hr = eight hours, 1Day = one day, 2Day = two days, 1Wk = one week and 3Wks = three weeks following the rewet event.

Time	Contrast	Biomass		Bacteria:Fungi		Gram+:Gram-	
		F	p	F	p	F	p
3Hr	Mean	31.7	0.001	1.4	0.28	2.0	0.20
	Treatment	5.8	0.05	24.6	0.003	0.00	0.99
8Hr	Mean	6.5	0.04	1.7	0.24	6.1	0.05
	Treatment	4.6	0.08	14.1	0.01	0.25	0.63
1Day	Mean	7.2	0.04	0.04	0.84	0.03	0.88
	Treatment	0.81	0.40	1.4	0.29	0.16	0.70
2Day	Mean	7.5	0.03	4.9	0.07	0.01	0.91
	Treatment	0.31	0.60	17.4	0.006	0.23	0.65
1Wk	Mean	22.7	0.003	2.9	0.14	8.2	0.03
	Treatment	3.2	0.13	14.1	0.009	2.2	0.20
3Wk	Mean	21.7	0.004	0.09	0.78	1.7	0.24
	Treatment	0.37	0.56	3.0	0.13	0.17	0.69
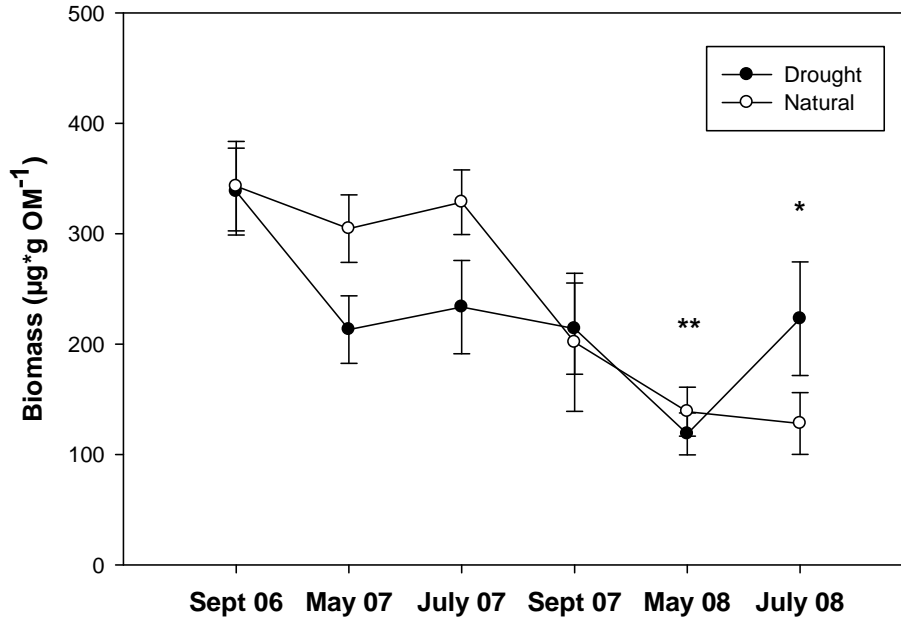
Figure 1. Response of average microbial biomass (±SE) over time during the two year rainout shelter study.
Stars (*) denote significant differences between mean values of the September 2006 and subsequent
sampling events. * $p < 0.05$, ** $p < 0.001$.
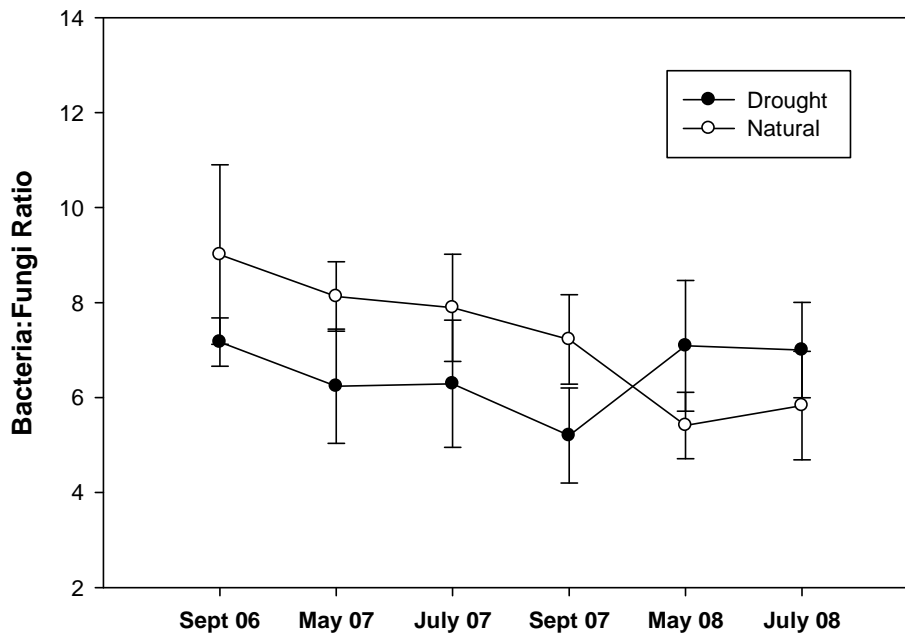
Figure 2. Response of the average bacteria:fungi ratio (±SE) over time during the two year rainout shelter
study.
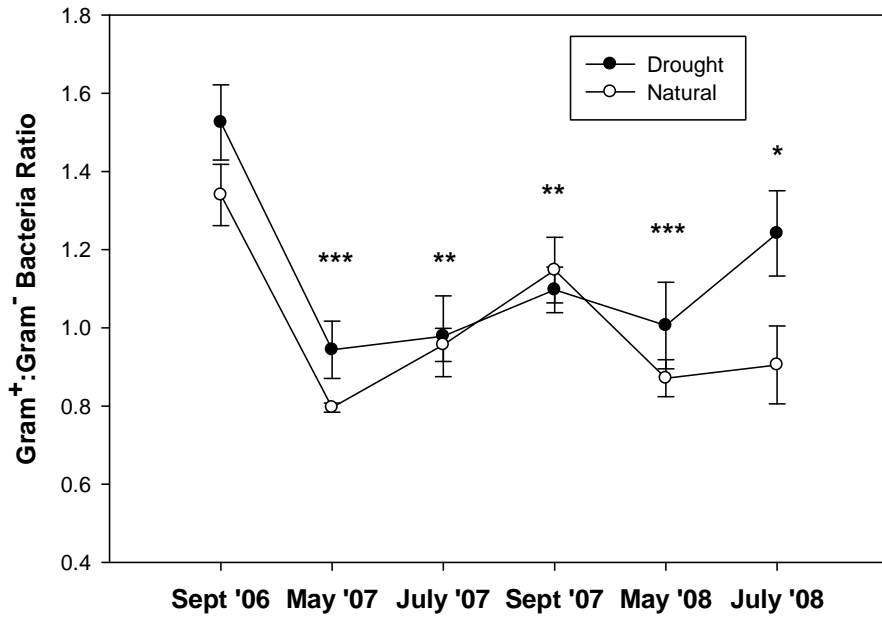
Figure 3. Response of the average Gram positive to Gram negative bacteria ratio (±SE) over time during

the two year rainout shelter study. Stars (*) denote significant differences in the average ratio between the

September 2006 and subsequent sampling events. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$
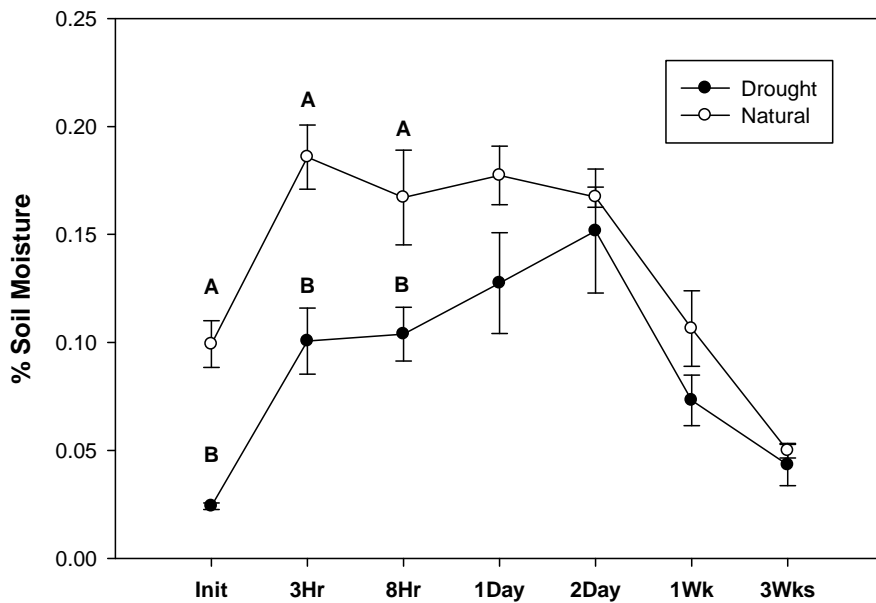
Figure 4. Response of average soil moisture (±SE) to the rewetting of drought and natural plots. Letters (A

and B) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$).
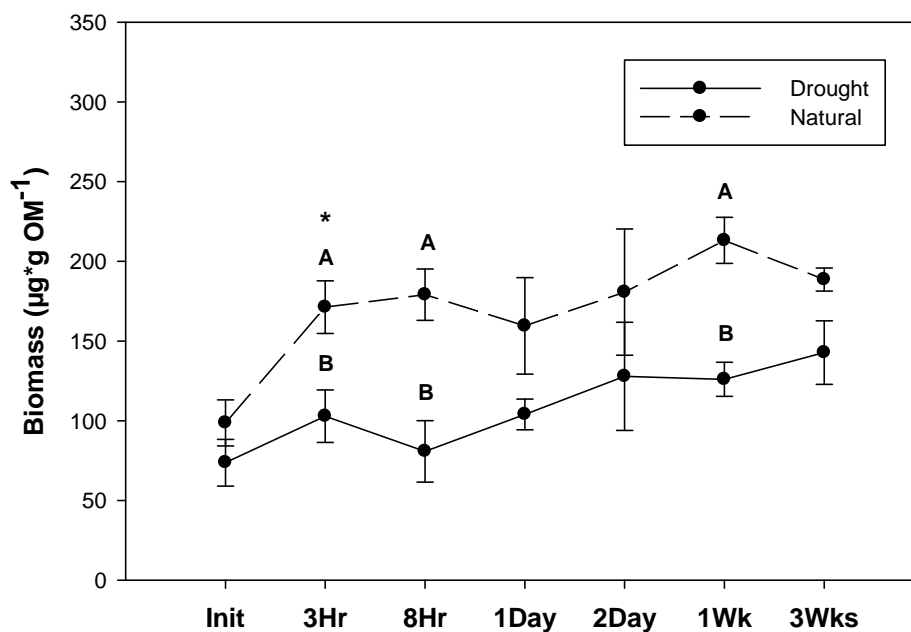
Figure 5. Response of average microbial biomass (±SE) to the rewetting of drought and natural plots. The initial (Init) value represents average microbial biomass before the water application and the response was measured three hours (3Hr), eight hours (8Hr), one day (1Day), two days (2Day), one week (1Wk) and three weeks (3Wks) following the rewet event. Star (*) represents a significant ($p = 0.05$) time by treatment interaction between the initial and 3Hr sampling event (analysis of variance of contrast variables).
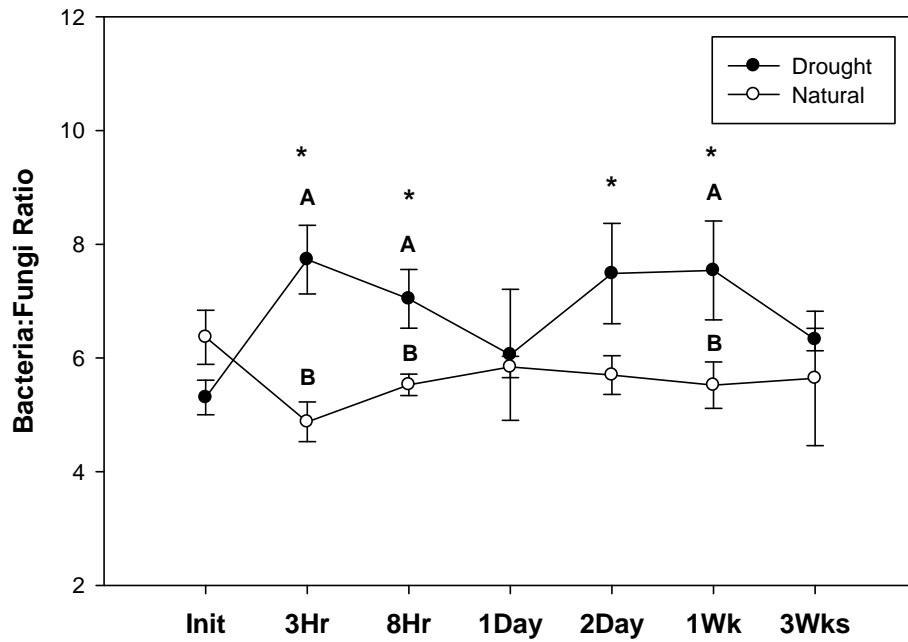
Figure 6. Response of the average bacteria:fungi ratio (±SE) to the rewetting of drought and natural plots. Letters (A and B) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$). Stars (*) represent significant time by treatment effects between the initial and subsequent sampling events ($p < 0.01$, analysis of variance of contrast variables).
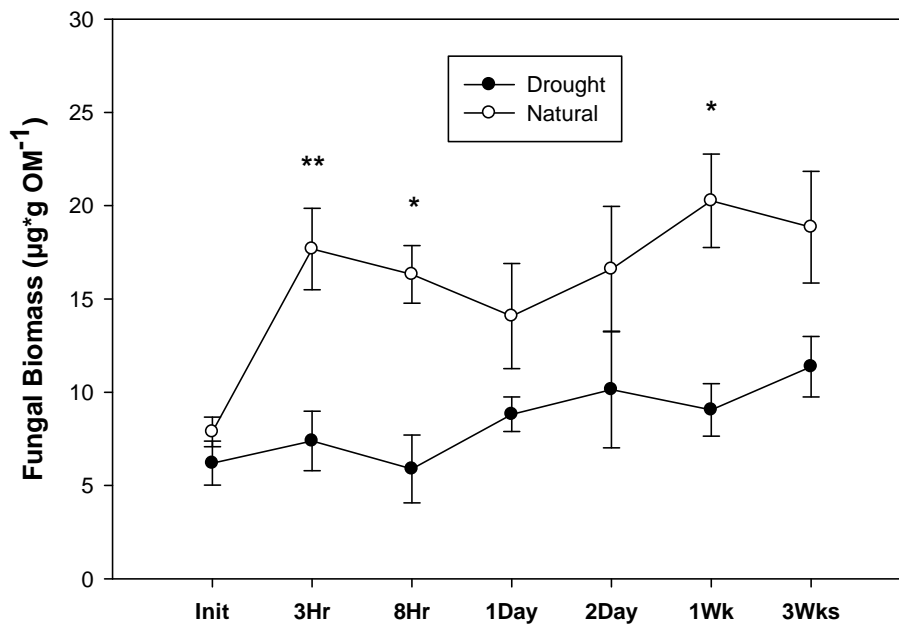
Figure 7. Response of average fungal biomass (±SE) to the rewetting of drought and natural plots. Stars (*) show significant time by treatment effects between the initial and subsequent sampling events (analysis of variance of contrast variables. * p < 0.05, ** p < 0.01.
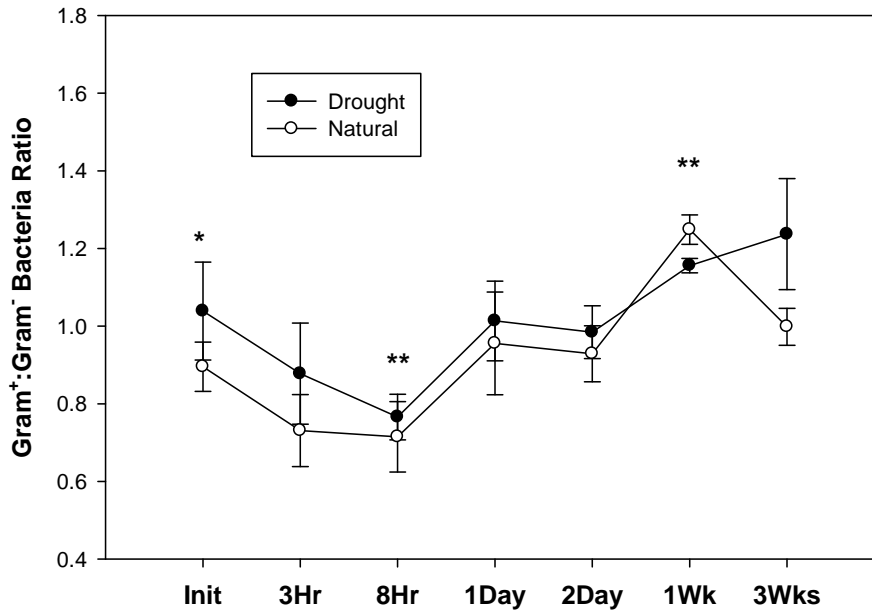
Figure 8. Response of the average Gram positive to Gram negative bacteria ratio (±SE) to the rewetting of drought and natural plots. Stars (*) denotes significant differences between the initial (*) and subsequent (**) sampling events (analysis of variance of contrast variables, $p < 0.05$)
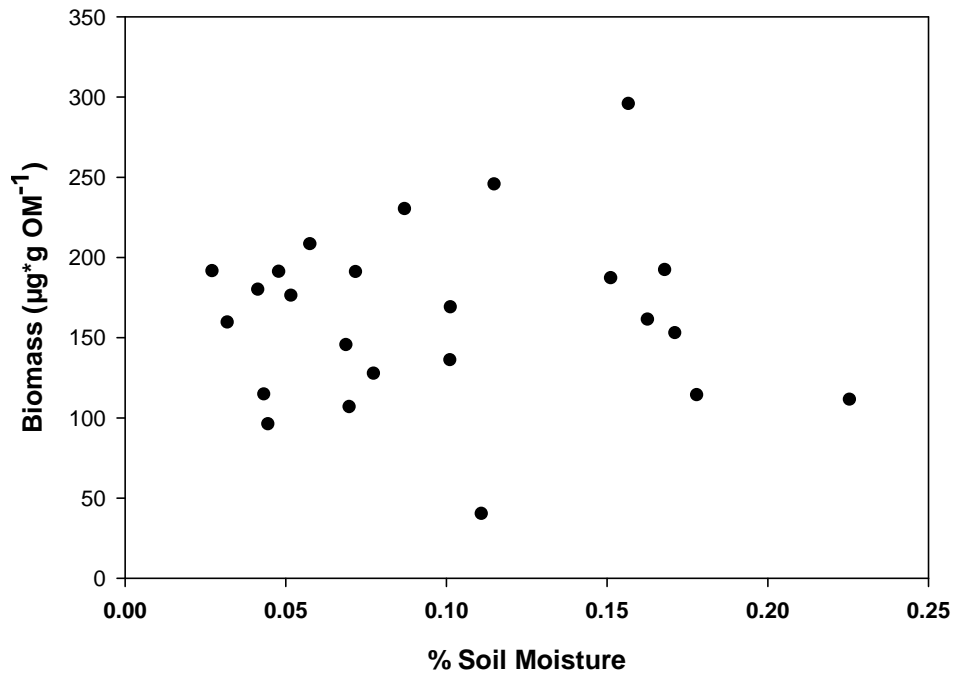
Figure 9. Relationship between soil moisture and microbial biomass, using samples removed between two days and three weeks following the rewet event.
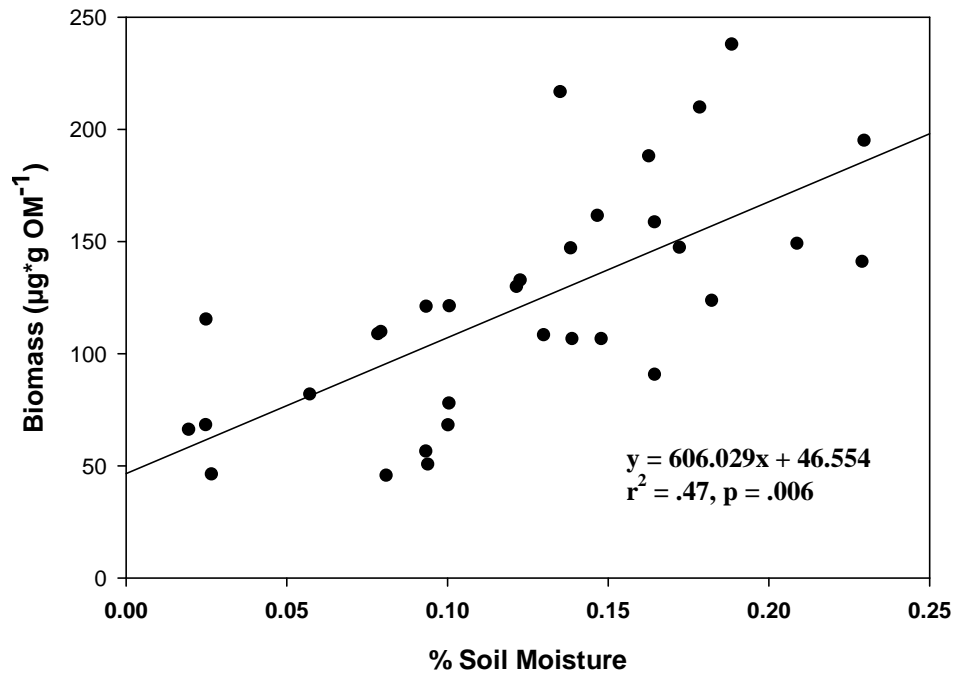
Figure 10. Linear regression of the relationship: soil moisture x microbial biomass using initial samples and samples removed three hours, eight hours and one day following the rewet event.
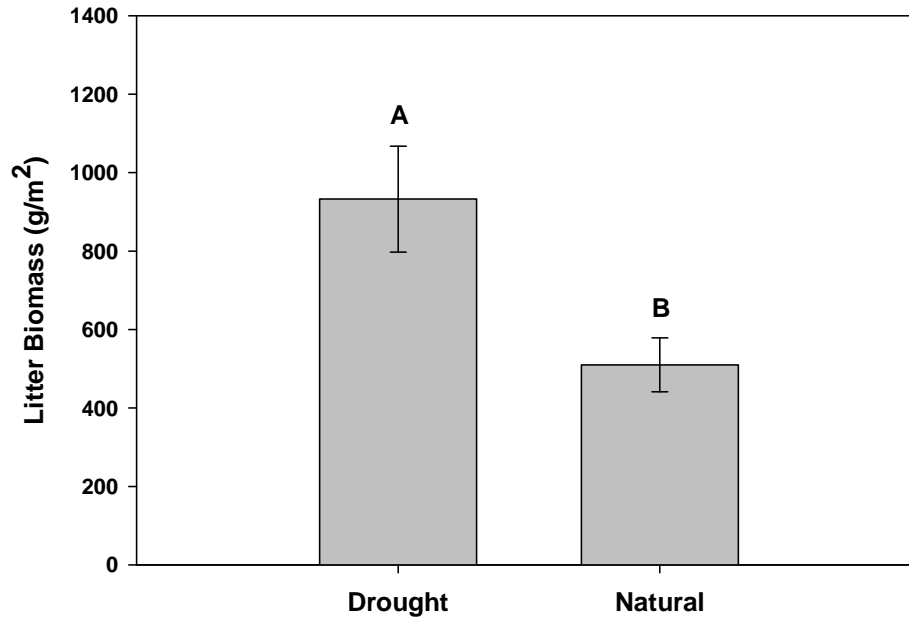
Figure 11. Average litter biomass ($\pm$SE) in drought and natural plots measured at the conclusion of the rewet experiment. The values within drought treatments are the result of a two year rainfall exclusion (July 2006 – August 2008). Letters (A and B) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$).

CHAPTER 4

**Soil nematode community composition, but not density, is resilient to long-term**

changes in precipitation in the New Jersey Pinelands

INTRODUCTION

The relationship between soil microbial community structure and nitrogen cycling is usually considered without concern for concurrent effects on microbial grazers, despite the fact that nitrogen mineralization is often stimulated by top-down interactions (Clarholm 1985, Bonkowski and Brandt 2002). The contribution of microbivores to nitrogen mineralization can be a significant portion (~30%) of overall nitrogen cycling rates (Hunt et al. 1987, Moore et al. 1988, Verhoef and Brussaard 1990), and soil nematodes are one of the most important groups of microbivores responsible for stimulating nitrogen mineralization (Woods et al. 1982, Ingham et al. 1985, Fu et al. 2005) and amino acid production (Anderson et al. 1983). Bacterivorous nematodes are generally responsible for much higher rates of nitrogen cycling than fungivorous nematodes (Ingham et al. 1985, Chen and Ferris 1999). Therefore, climatic changes that alter the bacteria:fungi ratio or the nematode bacterivore:fungivore ratio may have important implications for the soil nitrogen cycle. For example, a shift in precipitation amount may alter nitrogen cycling rates by disrupting grazer-microbe interactions (Moore et al. 2003) because both soil nematodes and their microbial prey are sensitive to changes in soil moisture.

As a result of atmospheric warming, a change in precipitation patterns is expected to occur in the northeastern U.S. (Baede et al. 2001), with climate models forecasting an increase in average annual precipitation amount (Baede et al. 2001, Hayhoe et al. 2006).

This, combined with reduced stomatal conductance due to elevated atmospheric CO_2 concentrations (Field et al. 1995), may increase soil moisture in northeastern forests. However, models also predict an increase in rainfall intensity (Allan and Soden 2008). The concentration of rainfall into fewer, more extreme events may increase the total number of dry days in a year. Combined with warmer air temperatures, higher rates of evapotranspiration and potentially less water infiltration due to runoff (Trenberth et al. 2003), greater precipitation intensity may increase soil drying.

Soil moisture impacts the habitat quality for bacteria and fungi by altering the number of air and water-filled pore spaces in soil (Elliott and Coleman 1988). Bacteria require water for movement, nutrient acquisition, and diffusion of waste products (Harris 1981) and should respond positively to an increase in soil moisture (Wilson and Griffin 1974). Most fungi, on the other hand, are aerobic organisms, and water saturation may inhibit their growth and activity. In dry soil, fungi can extend hyphae through air-filled pore spaces to access moisture and nutrients and can translocate these resources to water and nutrient-limited cells within their mycelial network (Jennings 1990, Jennings 1976). Fungi are tolerant of extremely dry conditions (Griffin 1969) and can quickly resume activity when favorable conditions return (Zak et al. 1995). If soil moisture decreases, fungi may gain a relative advantage over bacteria with respect to decomposer activity. The shift towards a greater dominance of the fungal or bacterial decomposition pathway will impact nutrient cycling rates in soil (Coleman et al. 1983, Moore et al. 2003).

Nematode grazers of microorganisms will also be impacted by climatic changes that influence soil moisture. These impacts will be direct, via changes in soil properties, and indirect, via changes in the abundance of bacterial or fungal prey. Directly, soil

structure interacts with soil moisture to create habitable pore space for nematodes and can influence the response of nematode density to soil moisture manipulations. Under drought stress in aggregated soil, nematodes may thrive within a thin film of water that does not drain. Because these pores are surrounded by dry soil, nematodes cannot leave the pore space and instead become concentrated with their prey (Görres et al. 1999). Many studies on nematode community dynamics use aggregated soils and the extent to which these effects apply to less aggregated soils is unclear.

Nematodes are indirectly influenced by moisture-dependent shifts in the microbial community. Increased soil moisture may favor a greater dominance of bacteria over fungi. This should stimulate nutrient cycling rates by increasing the relative abundance of bacterivorous nematodes. Because their C:N ratio is higher than that of their prey, bacterivorous nematodes often excrete excess ammonium or amino acids as a result of grazing activity (Woods et al. 1982, Anderson et al. 1983, Ingham et al. 1985). Bacterivorous grazing also stimulates nitrogen mineralization by increasing microbial growth and activity (Ingham et al. 1985). On the other hand, if a reduction in precipitation amount increases the dominance of the fungal decomposition pathway, the relative abundance of fungivorous nematodes may increase. Fungivorous nematodes have less of a stimulatory effect on nitrogen mineralization rates (Ingham et al. 1985, Chen and Ferris 1999). This is because the C:N ratio of their prey is much higher than that of bacteria. During grazing, a higher proportion of the fungal nitrogen is assimilated into nematode biomass.

To better understand how changes in precipitation patterns will impact soil microbial communities, their grazers and nitrogen cycling, more information is needed,

especially from under natural field conditions. The objective of this study was to measure the response of the bacteria:fungi ratio, the bacterivore:fungivore ratio of nematode communities, and nitrogen cycling to long-term field manipulations of precipitation (increased and decreased). I hypothesized that an increase in precipitation amount would increase the bacteria:fungi ratio, the nematode bacterivore:fungivore ratio and rates of potential net nitrogen mineralization and amino acid production. I predicted that a decrease in precipitation would have the opposite effect: a decrease in the bacteria:fungi ratio, a decrease in the nematode bacterivore:fungivore ratio and lower nitrogen mineralization and amino acid production rates.

This project was a collaborative effort with Amy Treonis at the University of Richmond. Dr. Treonis performed the nematode identification and calculation of community indices.

METHODS

Experimental design

Twelve rainout shelters (l x w x h: 2.47m x 1.91m x 0.7m) were established within an upland oak-pine stand of the Rutgers Pinelands Field Station (Brendan Byrne State Forest). Each shelter was covered with clear PVC panels and pitched such that rainwater was diverted into 208 liter tanks adjacent to the shelters. The shelters were distributed so that shrub density was similar below the shelters. Shelters were randomly assigned one of three treatments: "drought" (no rain fall), "ambient" (100% rain) or "high" (200% of rain). A "natural" treatment was established by delineating four

additional plots (1.4m x 0.8m) which received no manipulation. Plots were established in July and August of 2006 with a total of four replicates per treatment.

Water collected in the 208 liter tanks was usually applied to plots within two days and usually never more than one week following a rain event. Using a battery-operated pump attached to a garden hose, rain water was simultaneously removed from tanks and applied to the plots. The ambient treatment received the contents of one tank, the high treatment plots received the contents of two tanks and no rain was ever applied to the drought treatments. During winter, snow and ice were removed from drought shelters and applied to the high treatments. Snow and ice falling on ambient shelters were applied to ambient plots.

Sampling procedure

Soil cores (5cm diameter) were collected in September 2007, 14 months after the start of the manipulations. Samples were removed from the well-defined organic horizon, approximately 3-5 cm in depth. Below this depth, the soils rapidly transition into sand. Due to the higher water holding capacity and closer proximity to the soil surface, the organic horizon should be more impacted by rainfall events and especially small rain events that do not penetrate into deeper layers (Borken et al. 2003). At the same time, the defined organic horizon is a region of high biological activity that makes a large contribution to nitrogen cycling. Litter and loose sand were removed and the remaining organic material from the three subsamples was pooled and retained for analysis of microbial community composition, inorganic nitrogen (NO_3^--N and NH_4^+-N) and amino acid-N pools. These samples were also used to measure soil moisture and percent organic

matter. Three additional cores were removed immediately adjacent to each initial sample. These cores were placed in plastic bags, buried and removed after 30 days. These *in situ* incubation cores were analyzed for inorganic nitrogen (NO_3^- and NH_4^+-N) and amino acid-N pools. Potential net nitrogen mineralization and amino acid production were calculated as the difference between the incubated and initial cores. For a detailed description of soil processing procedures, see Chapter 2.

Percent soil moisture was measured on the initial and incubated cores by weighing fresh soil into aluminum containers and drying in a 70°C oven for 48 hours. Soil moisture ($SM_{PERCENT}$) was calculated as the difference between the fresh weight ($Soil_{FW}$) and dry weight ($Soil_{DW}$) of the subsample, per unit dry weight soil: $SM_{PERCENT} = [(Soil_{FW}) - (Soil_{DW})]/(Soil_{DW})$. The percent organic matter content of samples was determined by loss on ignition at 550°C for 12 hours.

KCl extractions were performed on the initial and incubated cores. These extractions were used to measure inorganic nitrogen pools (NO_3-N and NH_4^+-N). Concentrations of ammonium-N and nitrate-N in the KCl extracts were measured on an Astoria Pacific auto analyzer. Nitrates were analyzed by the hydrazine method and ammonia by the phenate method. Ammonium and nitrate pools were calculated on a per grams *dry weight organic matter* ($\mu g NH_4^+$-N*g OM^{-1} and $\mu g NO_3$-N*g OM^{-1}) basis (Chapter 2). Potential net nitrogen mineralization per day was calculated as the difference between inorganic nitrogen concentrations measured on the incubated and initial samples, using the following equation: $N_{MIN} = [(\mu g*g^{-1} NO_3^-$-$N_{INCUB} + \mu g*g^{-1} NH_4^+$-$N_{INCUB})$ to $(\mu g*g^{-1} NO_3^-$-$N_{INIT} + \mu g*g^{-1} NH_4^+$-$N_{INIT})]/$ # of days of incubation (Robertson et al. 1999).

The KCl extractions were used to measure amino acid pools and potential net amino acid production (Berthrong and Finzi 2006). The concentration of amino acid-N was determined colorimetrically through the reaction of KCl extracts with a 2% ninhydrin solution (ninhydrin and hydrantin in dimethyl sulfoxide and lithium acetate buffer, pH 5.2, Sigma-Aldrich, Inc.). During this reaction, ninhydrin forms a complex with ammonium and the α-amino groups of amino acids, resulting in a deep purple color (Joergensen 1995). KCl extracts were mixed with the ninhydrin reagent (2:1 v/v), heated in boiling water for 30 minutes and diluted in an ethanol-water mixture (1:1 v/v) (Joergensen 1995). Absorbance was read at 570 nm on a Spectronic 20 Genesys spectrophotometer. See Chapter 2 for a detailed description of the calculation of amino acid concentrations.

Phospholipid fatty acid analysis

Microbial biomass and the bacteria:fungi ratio were analyzed with phospholipid fatty acid (PLFA) analysis following the methods of (White and Ringelberg 1998). The PLFA profiles of soils was determined on 3 g subsamples that had been stored at -20°C. For a detailed description of the procedure and calculations, see Chapter 2.

Microbial biomass was calculated on a per grams *dry weight organic matter* (μgPLFA*g OM^{-1}) basis (Chapter 2). Bacterial biomass was estimated as the sum of all branched and monounsaturated fatty acids (Zelles 1999), with the exception of 16:1ω5c, and all 10-Methyl fatty acids. Saprotrophic and ectomycorrhizal fungal biomass was estimated from the biomass of linoleic acid (18:2ω6) (Frostegård and Bååth 1996). The bacteria:fungi ratio was calculated using these bacterial and fungal biomarkers.

Nematode community analysis

On 25 September 2007, eight days after the sampling event for microbial community and nitrogen analysis, additional cores were removed, using the same procedure described above, for the extraction and trophic-level identification of nematodes. Nematodes were extracted from 50 g samples using a modified Cobb's decanting and sieving method (s'Jacob and van Bezooijen 1984). This was accomplished by submerging samples in de-ionized water, which released nematodes from soil and into the water solution. The nematode + water mixture was decanted onto a 1 mm sieve and the nematodes trapped on the sieve were backwashed into a container. The water passing through the sieve was collected, re-mixed with the soil sample, and the process was repeated using successively smaller sieves (422 µm, 125 µm, 75 µm and 43µm). The collected nematodes in water were poured over a cotton milk filter and submerged in water just above the filter. This caused the nematodes to travel through the pore spaces of the filter and into clean, deionized water. All nematodes that traveled through the milk filter after 48 hours were collected.

The nematodes were concentrated into 100 mL of water and all individuals in a 10 mL subsample were counted with a dissecting microscope. A minimum of 250 individual nematodes were identified to the family-level (genus where possible) using a Zeiss Axiovert 40 CFL inverted microscope. The exception was sample one, a drought treatment, for which only 15 individuals were counted in the entire sample. Nematode density per gram dry soil was calculated as follows: [(# individuals)*10]*g soil^{-1}. Following counting, nematodes were concentrated into 10 mL of water and mixed with

10% hot formalin. Nematodes were identified to family level by Amy Treonis at the University of Richmond.

Nematode community composition was assessed by assigning families to trophic groups based on Yeates et al. (1993), allowing for an assessment of the relative abundance of bacterial-feeders (BF), fungal-feeders (FF), plant-parasites (PP) and omnivores (OM) (Table 1). These assignments were determined by Amy Treonis of the University of Richmond. There is uncertainty with respect to the feeding behavior of some groups of nematodes, especially for the Tylenchidae and Aphelenchoididae, species of which may be fungivores, plant-parasites or both (Yeates et al. 1993). For the purposes of this analysis, all nematodes from these families were assumed to be fungivores (FF). This was done because fungi are the dominant soil microbes in the New Jersey Pinelands. Feeding behavior of omnivores is also ambiguous, but is believed to include a wide diversity of species from multiple trophic levels (Yeates et al. 1993).

Nematode community structure was also characterized using the Maturity Index (Bongers 1990). The Index is a weighted mean of families based on functional and life history characteristics. Families are assigned a score, one through five, based on their response to disturbance. Species from families that are tolerant of disturbance, the "colonizers", are assigned a score of 1. These species can quickly increase in abundance following recovery and are considered to be *r*-strategists. Higher scores are assigned to "persisters", species from families that are sensitive to disturbance, have long life cycles and are never present in large numbers (i.e., *k*-strategists). Species within the same family are given the same *c-p* value. The Maturity Index (MI) is calculated as follows:

$$MI = \sum_{i=1}^{n} v(i)*f(i)$$

where $v(i)$ is the c-p value of a family and $f(i)$ is the frequency of the family in the

community (Bongers 1990).

Plant-parasitic nematodes are dependant upon plant communities and may have

different life strategies from other nematodes. Therefore, specific maturity indices were

calculated separately for free-living plant parasites (PP), free-living nematodes (FL) and

both plant parasites and free-living nematodes combined (Table 1).

Meteorological data

Daily precipitation and soil temperature values were obtained from data loggers

installed by the U.S. Forest Service approximately 100 meters from the study site. Soil

temperature was measured by Campbell Scientific CS107 sensors buried to a depth of

5cm. Precipitation was measured with a Campbell Scientific TE525-LC tipping bucket

rain gauge. Meteorological data was recorded on a Campbell Scientific CR23X

micrologger. Soil temperature is reported as cumulative thermal warming days. One

warming day was calculated by taking the average half-hourly soil temperature values of

the sensors and summing these values over a 24 our period.

Statistical analyses

The response of soil moisture, microbial biomass, the bacteria:fungi ratio, and

nitrogen pools were analyzed with repeated measures analysis of variance. The repeated

measures model was used so that results are consistent with the two-year rainout shelter study (Chapter 2). Significant differences between treatment means were based on the Tukey's studentized range test. The response of nitrogen mineralization and amino acid production rates were analyzed with a one way analysis of variance. During the two-year study (Chapter 2), the repeated measures model was not used for these variables due to missing data points. Nematode density was analyzed with a one-way analysis of variance followed by Tukey's studentized range test. All statistical analyses were performed using SAS 9.2 and graphed in Sigma Plot.

RESULTS

Soil moisture and meteorological data

There were no significant differences in soil moisture among treatments during the September 2007 sampling event. Average soil moisture in drought, ambient and high rainfall plots ranged from 6 to 9% (Table 2). In natural plots, soil moisture values were higher (17%), but this difference was not statistically significant. On 23 September, two days before nematode communities were sampled, 8 mm and 16 mm of rain water were applied to ambient and high rainfall plots respectively. This water had been collected during a series of small rain events immediately before and after the sampling event for microbial community and nitrogen analyses. As a result of the natural rain and water applications, there was a small (but non-significant) change in soil moisture in ambient, high and natural plots (to 11%, 14% and 9% respectively). There was no change in soil moisture within drought plots (6% soil moisture).

With the exception of drought plots, from which all rain was excluded for fourteen months, these soil moisture values were low relative to other sampling dates (Chapter 2). This is partly explained by the fact that, during the two months leading up to the sampling event, there was a total of 166 mm of natural rainfall, which was 70 mm lower than the previous year. Furthermore, only 8 mm of rain fell in the 26 days prior to the sampling events performed for this study. Total thermal warming, measured as the sum of average daily soil temperatures in the two months leading up to the sampling event, was 1390°C. These warm temperatures, which were comparable to thermal warming in the summer of 2006 (1407°C), drive the rapid loss of soil water due to evapotranspiration.

Microbial communities

Microbial biomass ranged from 202 $\mu g*gOM^{-1}$ in natural plots to 281 $\mu g*gOM^{-1}$ in high rainfall plots (Table 2). The bacteria:fungi ratio ranged from 5.20 in drought plots to 7.22 in natural plots. Neither variable was significantly different among treatments.

Nitrogen pools and flux

Ammonium pools within drought treatments (243 $\mu g*gOM^{-1}$) were more than five times the value in the ambient, high and natural plots, all of which were similar (44 to 47 $\mu g*gOM^{-1}$) (Table 2). There were no significant differences between treatments for nitrates (4.9 to 5.9 $\mu g*gOM^{-1}$), amino acids (44 to 87 $\mu g*gOM^{-1}$), nitrogen mineralization (0.14 to 1.00 $\mu g*g^{-1}*d^{-1}$) or amino acid production (-0.12 to -0.0004 $\mu g*g^{-1}*d^{-1}$) (Table 2).

Nematode communities

Overall, there was a positive relationship between precipitation amount and nematode densities. The lowest average densities were observed in drought plots, the highest densities were found in high rainfall plots and ambient plots were of intermediate value (Figure 1). Nematode densities within drought treatments (533 $*g^{-1}$ soil) were significantly lower than densities in high (1002 $*g^{-1}$ soil) and natural (1058 $*g^{-1}$ soil) plots ($P = 0.0006$, Figure 1). Densities were significantly lower in ambient vs. natural plots. Although the average value in ambient plots was more than four times the value of drought plots, this different was not statistically significant. The values within natural and high rainfall plots were similar and not statistically different. A total of 14 families were identified, of which 6 comprised 97% of the community (Table 1). These were Tylenchidae, Criconematidae, Aphelenchoididae, Cephalobidae, Plectidae, and Qudsianematidae. The majority of species within these families are bacterial and fungal feeders. The most commonly encountered morphospecies across the samples were an Aphelenchoides sp. (23 to 62% of the community) and a *Wilsonema* sp. (0.3 to 34% of the community) (data not shown).

The effect of the treatments were not uniform across the families (Table 3). Tylenchidae, Aphelenchoididae, and Plectidae were all negatively impacted by drought as compared to high rainfall plots (p < 0.03 for each, Figure 2). Aphelenchoididae also were negatively affected by the drought treatment as compared to natural plots. Plectidae density was highest in the high rainfall treatment as compared to the ambient and drought treatments, although not significantly higher than the natural plots. The densities of Criconematidae, Cephalobidae, and Qudsianematidae were not significantly affected by

treatment (p > 0.1 for each). Neither the density of total nematodes nor the densities of any specific nematode family were affected by the high treatment, as compared to the natural plots (p > 0.05 for each). A *Teratocephalus* sp. and a *Prismatolaimus* sp. were routinely encountered in very low numbers (< 3% of the total community for each), but these two species were completely absent from the drought treatment (data not shown).

Overall, the proportional representation of the various families in the community was unaffected by treatment (Table 4). This was true for Tylenchidae and Aphelenchoididae, despite the fact that their densities were significantly impacted by the treatments (compare Figure 2 to Figure 3). For Plectidae, proportions were significantly reduced in drought treatments (p = 0.002, Figure 3). With the exception of the Plectidae, the high treatment did not affect the proportional representation of the nematode families (p > 0.05).

There was no treatment effect on nematode community composition, as measured by the trophic classifications (Table 5). The Maturity Indices were not significantly different among the treatments (Table 6).

DISCUSSION

Rainfall manipulations had no effect on average microbial biomass, the bacteria:fungi ratio or the relative abundance of bacterivorous and fungivorous nematodes. These findings are consistent with Neher et al. (1999) who found no change in the bacterivore:fungivore ratio of nematodes in response to manipulations of soil matric potential. This would suggest that climate-induced changes in precipitation patterns will not alter the relative dominance of the bacterial or fungal energy channels in

soil. The response of nematode and microbial communities to soil moisture will likely depend on many factors, including nematode and prey densities (Fu et al. 2005) and life history traits (Demeure et al. 1979, Woods et al. 1982). Soil characteristics such as soil structure (Görres et al. 1999) and soil texture (Elliott et al. 1980) will also influence nematode community dynamics. Therefore, these results cannot be generalized to other sites, different depths or times of year. More studies are necessary before definitive conclusions can be reached.

The complex biological interactions that occur under natural field conditions may have contributed to the lack of a shift in the bacterivore:fungivore ratio among treatments. Fungivorous collembola and microarthropods could have competed with nematodes for fungal biomass, especially in dry soil where these aerobic organisms may have a competitive advantage over nematodes. Similarly, protozoa could have competed with nematodes for bacterial prey. Because they are much smaller organisms (2 to 160 μm), protozoa may have been able to access remaining water-filled pores, most of which might have been restricted to pore spaces too small for nematodes (0.16 to 2 mm) (Elliott and Coleman 1988). The expected increase in the relative abundance of bacterivores due to elevated soil moisture may have been offset by an increase in fungivore biomass resulting from higher root or ectomycorrhizal biomass (Keith et al. 2009). However, root biomass measurements were not taken and the PLFA data does not show an increase in fungal biomass. While not a complete list of potential interactions, these examples highlight the need for caution when interpreting field results.

No changes in nematode community composition were observed in response to drought, ambient or high rainfall treatments (Tables 5-6). These findings are similar to

those of Savin et al. (2001) and Neher et al. (1999) who also found no change in community structure due to manipulation of matric potential. It appears that the impact of drought on the bacterial-feeders *Wilsonema* sp., *Teratocephalus* sp., and *Prismatolaimus* sp. did not affect the abundance of bacterial-feeders overall. Species of Cephalobidae (*Eucephalobus* sp., *Cervidellus* sp., *Acrobeloides* sp.) were most abundant in the drought treatment, although not significantly so. This suggests that there could be functional redundancy within the bacterial-feeding nematodes that can provide a degree of resiliency to the soil food web (Ettema 1998). Species-specific responses of nematodes within a trophic group to soil moisture were also found by Neher et al. (1999).

One of the most striking results was a sharp decrease in nematode density in response to the drought treatment. This is in contrast with other experiments, in which nematode density was no different between high and low soil moisture treatments (Görres et al. 1999, Neher et al. 1999, Savin et al. 2001). In these studies, water-filled soil aggregates may have provided habitable pore spaces in which nematodes were concentrated with their prey, prolonging survival (the "enclosure hypothesis"). The reduction in nematode density within drought plots in the current study is consistent with the "exclusion hypothesis" which predicts that nematode survival decreases with decreasing soil moisture due to restricted movement and inability to access prey (Görres et al. 1999). The exclusion hypothesis explanation for the reduction in nematode densities is supported by the fact that aggregation, while not directly measured, should be very low in Pinelands soil. These soils contain only very small quantities of clay (Douglas and Trela 1998), which are centers of aggregate formation (Jastrow et al. 2007). With fewer water stable aggregates, the drought plots should have contained few water-filled pore

spaces large enough to contain nematodes. Nematode movement and access to prey would therefore be restricted, resulting in the observed decline in densities.

The three families most affected by drought were the Tylenchidae (fungal feeders), the Aphelenchoididae (fungal feeders) and the Plectidae (bacterial feeders) (Figure 2). Together, they represent two thirds of all nematodes sampled (Table 1). For the Tylenchidae and Aphelenchoididae, the decline in densities within drought plots did not alter their proportions. Only the Plectidae experienced a significant reduction in their relative abundances within drought plots (Figures 2 and 3). This may be a reflection of the general trend of reduced bacterial abundances under very low soil moisture conditions (Chapter 2), but cannot be confirmed because the overall bacterivore abundance in drought plots was unchanged.

The duration of the manipulation (one year) far exceeds the 21 day incubation in the Savin et al. (2001) and Görres et al. (1999) studies. There is limited interpretive value to the drought manipulations in this study, because a one year drought is unlikely to occur. More research is needed to understand the temporal (days to weeks) response of nematode densities to drought as this is a more likely scenario. On the other hand, after one year there was no significant difference in nematode densities between ambient, high and natural rainfall treatments. This provides insight into the resiliency of nematode populations to large, long-term changes in precipitation amount.

I predicted that high rainfall treatments would result in higher nitrogen mineralization rates, resulting from an increase in the relative dominance of bacteria and bacterivorous nematodes. Instead, there was a sharp increase in ammonium pools as well as elevated (but not significant) nitrogen mineralization rates in response to the drought

treatment (Table 2). Given the severely reduced nematode populations in drought plots, it is unlikely that this increase in ammonium production was the result of nematode grazing. Instead, the production of ammonium could have been due to sustained microbial activity (Table 2 and Chapter 2) and a reduced population of ammonium oxidizing bacteria (Hastings et al. 2000). Due to their smaller size and ability to access the smallest pore spaces in soil, it is also possible that protozoan grazing contributed to the accumulation of ammonium. The very dry soil conditions in drought plots over most of the year would have greatly reduced diffusion rates, preventing plant and microbial immobilization, and resulting in the observed accumulation of ammonium (Chapter 2).

Conclusions

Neher et al. (1999) observed that the relative abundance of fungivores increases in response to a decrease in soil matric potential, in some but not all months. Because the current study involved a single sampling event, caution must be exercised in the interpretation of the results. Furthermore, while the results pertaining to the bacterivore:fungivore ratio are contingent upon the accurate assignment of nematodes to trophic group, there is uncertainty with respect to the feeding behavior of nematodes. The trophic assignments used are representative of most species in a given family but there are species-specific differences (Yeates et al. 1993). At the same time, feeding behavior may change with life stage and patterns observed under laboratory conditions may differ from field conditions.

Overall, the findings indicate that nematode density in the organic horizon of Pinelands soil are sensitive to long-term drought, but not a doubling of rainfall. The

minimal aggregation in these soils might play an important role in determining the impact of precipitation patterns on nematode density. There was no effect of precipitation amount on the relative importance of the bacterial or fungal energy channel, and while nitrogen cycling was altered in drought plots, this was most likely unrelated to nematode grazing but instead the result of sustained microbial activity, a reduced population of nitrifying bacteria and reduced plant uptake. The response of nematode community composition indicates that nematode families possess similar adaptations to changes in soil moisture.

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Table 1. Nematode families grouped by trophic assignment. Proportions represent the relative abundances of families among all samples.

Trophic Group	Family	Proportion
Fungal Feeders	Tylenchidae	13.5%
	Aphelenchoididae	39.5%
Plant Parasites	Hoplolaimidae	0.4%
	Pratylenchidae	0.6%
	Criconematidae	6.4%
Bacterial Feeders	Bunonematidae	0.02%
	Cephalobidae	16.7%
	Teratocephalidae	1.1%
	Monhysteridae	0.2%
	Plectidae	13.2%
	Prismatolaimidae	0.8%
Omnivores	Chrysonematidae	0.2%
	Thornematidae	0.1%
	Qudsianematidae	7.5%

Table 2. Summary of the response (± SE) of soil moisture, microbial biomass, the bacteria:fungi ratio, nitrogen pools and flux to rainfall manipulations. Letters ([a] and [b]) denote significant differences (Tukey's studentized range, $p < 0.05$) between treatment means. Nmin = Nitrogen mineralization, AA Prod = Amino Acid production

Response	Drought	Ambient	High	Natural
Soil Moisture	0.1 (.01)	0.1 (.01)	0.1 (.01)	0.2 (.1)
Biomass ($\mu g*gOM^{-1}$)	214 (41)	263 (30)	281 (46)	202 (63)
Bacteria:Fungi	5.2 (1.0)	6.7 (.6)	6.1 (.1)	7.2 (.9)
NO_3^--N ($\mu g*gOM^{-1}$)	5.9 (.5)	5.9 (.4)	4.9 (.8)	6.4 (1.2)
NH_4^+-N ($\mu g*gOM^{-1}$)	243 (70)[a]	48 (8)[b]	45 (4)[b]	44 (4)[b]
AA-N ($\mu g*gOM^{-1}$)	87 (20)	44 (5)	54 (5)	57 (6)
Nmin ($\mu g*g^{-1}*d^{-1}$)	0.1 (.8)	0.2 (.1)	0.1 (.2)	0.2 (.03)
AA Prod ($\mu g*g^{-1}*d^{-1}$)	-0.008 (.2)	-0.01 (.04)	-0.1 (.2)	-0.004 (.04)

Table 3. Response of average nematode densities ($\mu g*g$ soil^{-1}, ± SE) grouped by family. Letters ([a] and [b]) denote significant differences among treatment means (Tukey's studentized range, $p < 0.05$). Only the six most abundant families, representing >97% of all species identified, are listed.

Family	Drought	Ambient	High	Natural	F	P
Tylenchidae	581 (257)[a]	9802 (3404)[ab]	14304 (2565)[b]	12011 (3645)[ab]	4.6	0.02
Criconematidae	2305 (1983)	2445 (1033)	4906 (1775)	8058 (3202)	1.6	0.25
Aphelenchoididae	6250 (4001)[a]	20765 (3015)[ab]	37134 (12677)[b]	46045 (5119)[b]	5.9	0.01
Cephalobidae	2393 (1729)	5403 (1534)	8087 (2560)	12971 (5653)	1.8	0.19
Plectidae	73 (30)[a]	7090 (3796)[a]	26182 (6357)[b]	14936 (777)[ab]	9.1	0.002
Qudsianematidae	1630 (1285)	6058 (2454)	5989 (2121)	5724 (1743)	1.2	0.35

Table 4. Response of average nematode proportions (±SE) grouped by family. Letters (a and b) denote significant differences among treatment means (Tukey's studentized range, p < 0.05). Only the six most abundant families, representing >97% of all species identified, are included.

Family	Drought	Ambient	High	Natural	F	P
Tylenchidae	0.1 (0.03)	0.2 (0.1)	0.2 (0.1)	0.1 (0.03)	0.9	0.5
Criconematidae	0.1 (0.1)	0.1 (0.02)	0.1 (0.02)	0.1 (0.03)	0.3	0.9
Aphelenchoididae	0.4 (0.1)	0.4 (0.01)	0.4 (0.1)	0.4 (0.02)	0.2	0.9
Cephalobidae	0.3 (0.2)	0.1 (0.1)	0.1 (0.02)	0.1 (0.1)	1.6	0.2
Plectidae	0.01 (0.01)[b]	0.1 (0.1)[ab]	0.3 (0.04)[a]	0.2 (0.02)[a]	9.5	0.002
Qudsianematidae	0.1 (0.03)	0.1 (0.04)	0.1 (0.03)	0.1 (0.01)	0.6	0.6

Table 5. Average response of nematode functional groups (proportions, ±SE) to rainfall manipulations.

Trophic Group	Drought	Ambient	High	Natural
Bacterial Feeders	0.4 (0.2)	0.3 (0.1)	0.4 (0.1)	0.3 (0.1)
Fungal Feeders	0.5 (0.1)	0.6 (0.1)	0.5 (0.2)	0.6 (0.03)
Bacterivores:Fungivores	1.7 (1.4)	0.5 (0.2)	0.7 (0.2)	0.6 (0.1)
Omnivores	0.1 (0.03)	0.1 (0.04)	0.1 (0.03)	0.1 (0.01)
Plant Parasites	0.1 (0.1)	0.1 (0.02)	0.1 (0.02)	0.1 (0.03)

Table 6. Response of Bonger's Maturity Indices (BMI) (±SE) to rainfall manipulations. PPN = plant parasites only

Index	Drought	Ambient	High	Natural
BMI (w/PPN)	2.2 (0.1)	2.3 (0.1)	2.2 (0.1)	2.2 (0.04)
BMI (free-living only)	1.8 (0.03)	1.8 (0.2)	1.7 (0.1)	1.8 (0.1)
PPI	0.4 (0.1)	0.5 (0.1)	0.5 (0.2)	0.5 (0.1)
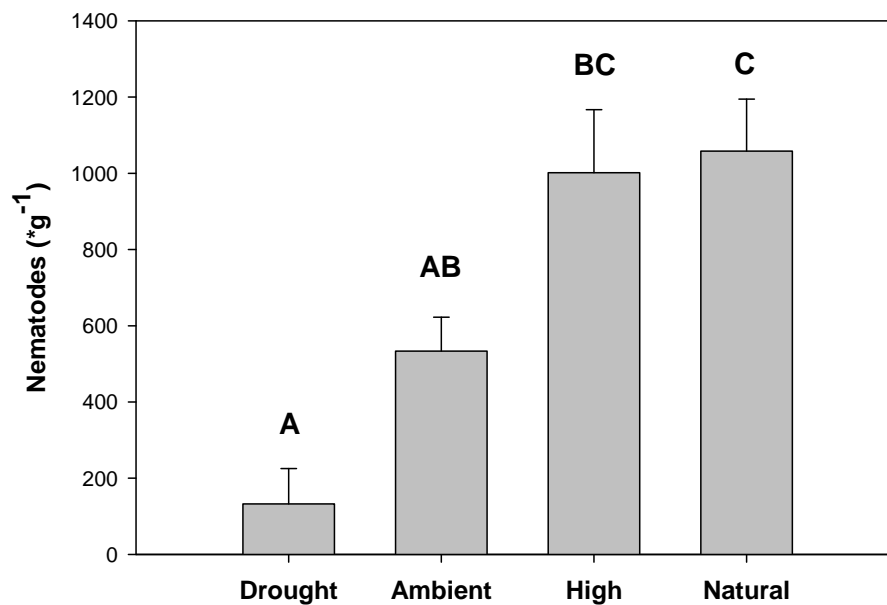
Figure 1. Response of average nematode densities (±SE) to precipitation treatments. Letters (A, B and C)

denote significant differences between treatment means (Tukey's studentized range test).

Figure 2. Response of average nematode densities (±SE) by family to the precipitation treatments. Letters

(A and B) denote significant differences between treatment means (Tukey's studentized range test, $p <$

0.05). Results are displayed for the families with a significant response to the treatments and whose

abundances represented more than 3% of the total community.

Figure 3. Response of average nematode proportions (±SE) by family to the precipitation treatments.

Letters (A and B) denote significant differences between treatment means (Tukey's studentized range test,

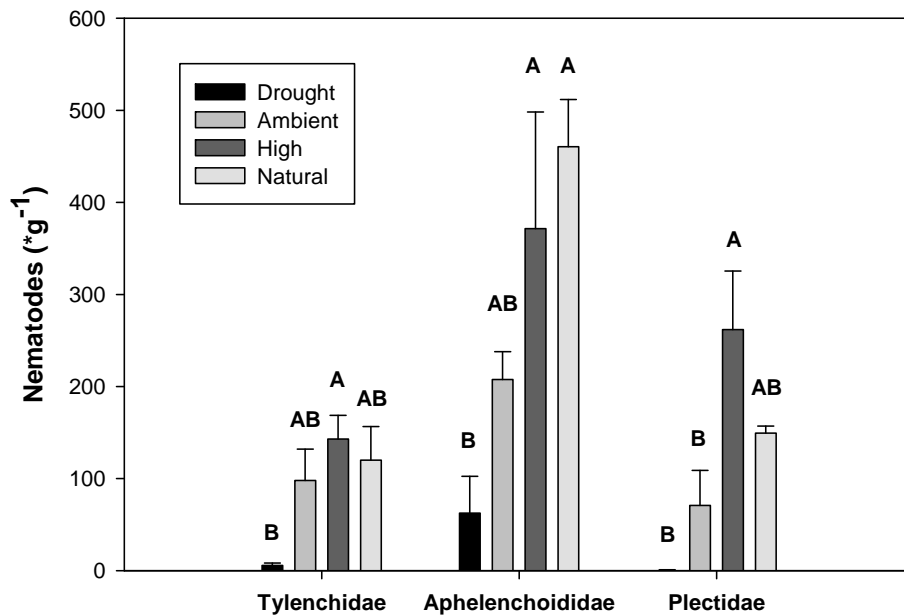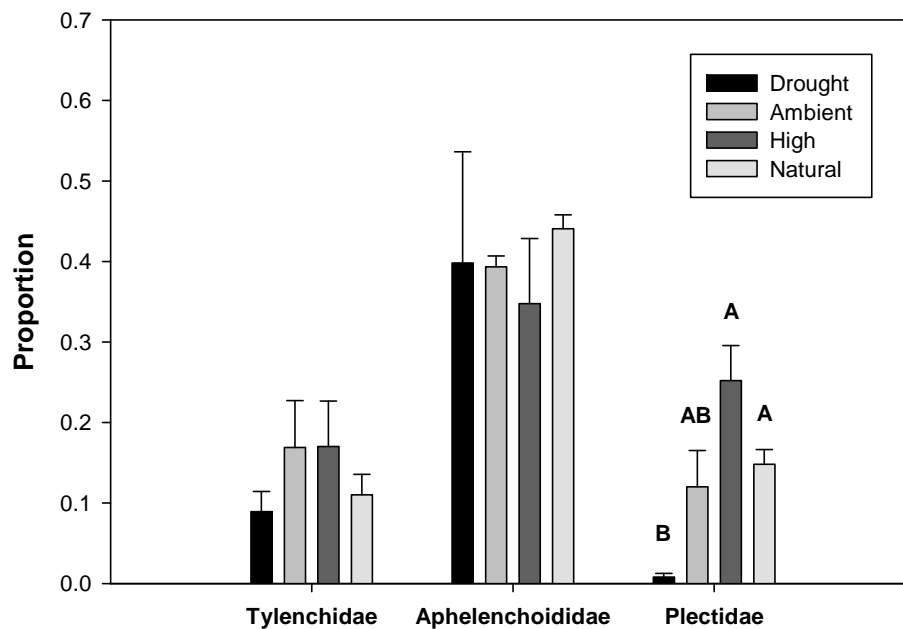$p < 0.05$). Results are displayed for the families represented in Figure 2.

CHAPTER 5

Potential effects of elevated winter rainfall on soil microbial communities and

nitrogen cycling in the New Jersey Pinelands

INTRODUCTION

Winter is an important time for microbial activity and carbon and nitrogen cycling in soils. In the temperate forests of the northeastern U.S., up to 15% of annual nitrogen mineralization occurs during winter (Groffman et al. 2001) and at more extreme latitudes microbial activity and nutrient cycling are much higher (Clein and Schimel 1995, Williams et al. 1998, Schimel et al. 2004). In cold ecosystems, continuous winter snow cover insulates the soil allowing it to maintain stable, elevated soil temperatures and higher rates of microbial activity (Brooks and Schmidt 1997, Williams et al. 1998, Schimel et al. 2004). During spring thaw, as snow melts and plants become active, the inorganic nitrogen produced during winter becomes an important source of plant nitrogen (Brooks et al. 1998, Schimel et al. 2004).

Rising surface air temperatures due to anthropogenic warming (Baede et al. 2001) are likely to disrupt the timing and rates of nitrogen cycling during winter. Warmer winter temperatures are expected to reduce winter snow cover or delay snow pack formation. This will result in soil freezing (Groffman et al. 2001), which causes lysis of bacterial and fungal cells, (Biederbeck and Campbell 1971, Skogland et al. 1988, Schimel and Clein 1996), disruption of soil aggregates (Steinweg et al. 2008) and root damage (Tierney et al. 2001). The exposure to more variable soil temperatures will lead to a higher frequency of freeze-thaw cycles. As soils freeze and thaw throughout the winter,

microbial activity is stimulated by pools of labile carbon originating from lysed microbial cells and disruption of organic matter. As a result of freeze-thaw cycles, a pulse of carbon and nitrogen mineralization are commonly observed (Schimel and Clein 1996).

The stimulation of microbial activity due to freeze-thaw cycles represents a disruption in the nitrogen cycle. It leads to the production of inorganic nitrogen at a time when it cannot be used by plants. This disruption in the timing of nutrient cycling could have negative consequences for environmental quality as it can lead to greater nitrogen leaching (Brooks et al. 1999). Additionally, winter nitrogen loss reduces plant productivity by decreasing nitrogen availability in the spring. At the same time, soil freezing damages plant roots (Fitzhugh et al. 2001), which further inhibits plant nitrogen uptake and increases nitrogen leaching into waterways.

Most investigations into winter processes occur at northern latitudes or high elevations. Little attention has been paid to lower latitudes, where winters are cold enough to freeze soil, but not cold enough to sustain snow pack for more than a few days. Because these soils are not insulated from cold winter temperatures, plants and soil microbes are continually exposed to freeze-thaw cycles. If winter precipitation increases, as predicted by climate models (Hayhoe et al. 2006), elevated soil moisture may increase the severity of these freeze-thaw cycles by creating additional ice lenses that damage even more microbial cells and plant roots. This could result in a reduction in microbial biomass or altered microbial community composition during winter, as more cells are damaged by freeze-thaw events.

In the New Jersey pinelands, freeze-thaw cycles should have the greatest negative impact on ectomycorrhizal fungi, which are abundant in these forests, and especially

vulnerable to freeze damage (Fitzhugh et al. 2001). At the same time, an increase in the labile carbon pool due to freezing should favor the growth and reproduction of bacteria. If more severe freeze-thaw cycles increase the bacteria:fungi ratio during winter, this should lead to higher nitrogen cycling rates (Coleman et al. 1983) at the onset of spring. Higher nitrogen cycling rates, combined with freeze damage to root and ectomycorrhizal fungi, could accelerate nitrogen losses during spring. The sandy soils of the New Jersey Pinelands may be especially susceptible to such losses.

Studies of nitrogen cycling typically focus on inorganic forms of nitrogen (NO_3^- and NH_4^+) however it is now widely accepted that organic nitrogen is an important component of plant nutrition in many systems (Schimel and Chapin 1996, Näsholm et al. 2000). It is suggested by studies with similar characteristics that amino acids are likely an important source of nitrogen in the New Jersey Pinelands. For example, it was demonstrated that pine trees and ericaceous shrubs can take up amino acids under field conditions (Nasholm et al. 1998) and that amino acid production is an important part of the nitrogen cycle in temperate forests (Berthrong and Finzi 2006). Furthermore, recent ecological theory predicts that, in the most highly nitrogen-limited systems, microbial production of amino acids is the dominant source of plant nitrogen (Schimel and Bennett 2004).

The purpose of this study was to measure the response of soil microbial communities and nitrogen cycling to an increase in winter precipitation. I hypothesized that elevated winter soil moisture will interact with mild freeze-thaw events to reduce microbial biomass during early spring. I predicted that, because freezing should be most damaging to ectomycorrhizal cells and favor bacterial growth by increasing the labile

carbon pool, elevated winter rainfall will increase the bacteria:fungi ratio of soil. I predicted that a higher bacteria:fungi ratio would lead to higher rates of nitrogen mineralization and amino acid production at the start of spring.

METHODS

Experiment 1: Weekly water additions

The effect of elevated winter precipitation on soil microbial community structure and nitrogen cycling was studied during a two year water addition experiment in the New Jersey Pinelands. From early January to mid-March of 2007 and 2008, five experimental plots (1.4m x 0.8m) received weekly water additions by applying 18.9L of well water from the underlying aquifer (the equivalent of 17 mm of rainfall). Water was added once per week for nine weeks and natural rainfall was not excluded from experimental plots. Five control plots were exposed to natural conditions and received no manipulation.

The response of soil microbial communities and nitrogen cycling rates were measured in mid-March, one week after the final water additions. Three cores from randomly selected points within each plot were removed to a depth of 3cm and pooled. These samples were analyzed for microbial biomass, community composition and pools of inorganic nitrogen (NO_3^--N and NH_4^+-N) and amino acid-N (AA-N). Three additional cores were removed adjacent to the three initial cores, placed into plastic bags and immediately buried. These cores were removed from the ground after one month and analyzed for NO_3^--N, NH_4^+-N and AA-N. Potential net nitrogen mineralization was estimated as the difference between NO_3^--N and NH_4^+-N in incubated and initial cores (Robertson et al. 1999). Potential net amino acid production was estimated as the

difference between the amino acid concentrations of the incubated and initial cores

(Berthrong and Finzi 2006).

Soil processing

Soil was processed immediately after sampling. The three pooled cores from each

plot were broken apart by hand and homogenized through a 4.75 mm and a 2 mm sieve.

All visible roots were separated from the samples. Homogenized samples were divided

into subsamples for soil moisture and percent organic matter determination (5g) and KCl

extractions (10g). The remaining sample was stored at -20°C in plastic bags. Subsamples

were removed from the freezer on a later date for microbial community analysis by the

phospholipid fatty acid (PLFA) method. See Chapter 2 for more details on soil

processing.

Soil moisture was measured by weighing fresh soil into aluminum containers and

drying at 70°C for 48 hours. Soil moisture ($SM_{PERCENT}$) was calculated as the difference

between the fresh weight ($Soil_{FW}$) and dry weight ($Soil_{DW}$) of the subsample, per unit dry

weight soil: $SM_{PERCENT} = [(Soil_{FW}) - (Soil_{DW})]/(Soil_{DW})$. The percent organic matter

content of the dried soil was determined by loss on ignition at 550°C.

Inorganic nitrogen and amino acids were extracted from soil by mixing 10g

subsamples with 30mL of 2M KCl. The soil-KCl mixture was placed on a shaker for one

hour and filtered through cellulose filter paper (8μm). The extracts were stored in a -20°C

freezer. Concentrations of NO_3^{-}-N and NH_4^{+}-N in the KCl extracts were measured on an

Astoria Pacific AP-3 auto analyzer (Astoria Pacific International, Clackamas, OR). The

concentration of amino acid-N was determined colorimetrically through the reaction of

KCl extracts with a 2% ninhydrin solution (Joergensen 1995). Absorbance was read at 570 nm on a Spectronic 20 Genesys spectrophotometer. Amino acids were only measured during the winter of 2007 while nitrogen mineralization rates were measured during 2007 and 2008. For a detailed description of the analysis of nitrates, ammonium and amino acids, see Chapter 2.

Microbial biomass and microbial community composition were analyzed with the phospholipid fatty acid analysis (PLFA). Following the methods of White and Ringelberg (1998), samples were saturated in a one phase solvent of chloroform, methanol and phosphate buffer (1:2:0.8 ratio). The phospholipids were separated on a silicic acid column by sequentially eluting with organic solvents of increasing polarity: chloroform (5mL), acetone (10mL) and methanol (5mL). The phospholipids, dissolved in methanol and amended with a nonadecanoic acid standard (100 µl), were saponified and methylated (MIDI 1995), forming fatty acid methyl esters (FAMEs). The identity of individual FAMEs were determined based on their retention time on a gas chromatograph and in conjunction with the MIDI Sherlock Microbial Identification System (MIDI 1995). Peak identification was confirmed by a combination of gas chromatography and mass spectroscopy. For a detailed description of the PLFA analysis, see Chapter 2.

The biomass of Gram positive bacteria was estimated as the sum of all iso and anteiso branch chain fatty acids (O'Leary and Wilkinson 1988) and the sum of all mono-unsaturated and cyclopropane fatty acids were used to estimate Gram negative bacterial biomass (Wilkinson 1988, Zelles 1999) (Table 1). Linoleic acid (18:2ω6) was used as an estimate of saprotrophic and ectomycorrhizal fungal biomass (Frostegård and Bååth 1996). Total bacterial biomass was estimated as the sum of Gram positive and Gram

negative biomarkers. Fatty acids with a methyl group on the 10th carbon are believed to be biomarkers for actinomycetes and were also used in the calculation of bacterial biomass. However, because actinomycetes are functionally distinct from other Gram positive bacteria, they were not used in the calculation of the Gram positive to Gram negative bacteria ratio.

The conversion of mono-unsaturated to cyclopropane fatty acids was used as an indicator of physiological stress (Grogan and Cronan 1997, Pinkart et al. 2002). "Stress Index 1" was defined as the sum of 16 and 18 carbon mono-unsaturated fatty acids divided by the sum of 17 and 19 carbon cyclopropane fatty acids. This index is based on the assumption that microbes convert mono-unsaturated fatty acids to cyclopropane fatty acids when under physiological stress (Kaur et al. 2005). A high value of the index means that microbial communities are less stressed, and lower values indicate greater physiological stress of the microbial community. The analysis of the stress index was repeated individually for each pair of fatty acids (Table 1).

Meteorological Data

Soil temperature and precipitation data were acquired from sensors installed by the U.S. forest service, approximately 100m from the study site. Soil temperature was measured with probes buried to a depth of 5 cm and recorded on a CR23X data logger. The depth of soil temperature probes was close to the 3cm sampling depth used during this study. The soil temperature data was used to determine the timing and intensity of soil freezing, under natural conditions, during the two year study. Additionally, soil thermal warming was calculated as the sum of average daily soil temperatures for the

three months prior to the mid-March sampling date. Continuous soil moisture data was not collected during this study. Instead, daily precipitation data, collected by the U.S. Forest Service with a Campbell Scientific TE525-LC tipping bucket rain gauge, was used *in lieu* of soil moisture. This data was used to calculate total precipitation for the three months leading up to the mid-March sampling date.

Experiment 2: Short-term effect of elevated soil moisture on cold soil

The objective of Experiment 2 was to assess the potential for elevated soil moisture to interact with a single freeze-thaw cycle to alter soil microbial community structure. For this experiment, soil temperature sensors were installed to a depth of 3cm in one of the experimental plots and measurements were recorded every two hours on a HOBO data logger (Onset Computer Corporation). These sensors recorded slightly lower soil temperature values than the U.S. Forest service probes. Because these probes were not in place during 2007, they could not be used to compare soil temperature between years. A comparison between treatments was not possible because sensors were not installed in control plots.

During January of 2008, air temperatures fell below $0^{o}C$ for 58 consecutive hours. I assumed that this would result in an extended period of soil freezing, but this did not occur. Instead, soil temperature, which was only measured in one experimental plot, fell below $1^{o}C$ for 44 consecutive hours and slightly below $0^{o}C$ (to $-0.16^{o}C$), for less than four hours. Therefore, this study measured the short-term effect of the interaction of elevated soil moisture and cold, but not frozen, soil on microbial community biomass and microbial community composition.

Immediately prior to the rapid decline in air temperatures, 18L of well water was applied to five field plots (1.4m x 0.8m). The five control plots, which received no manipulation, were the same plots used as the control for Experiment 1 (see above). Samples were removed from experimental and control plots on January 19, immediately prior to the water applications. Four days later, after soil temperatures returned close to pre-water application values, additional samples were removed from all plots. Only trace amounts of rainfall (.25mm) were recorded during the four day experiment. The response of microbial biomass, the bacteria:fungi ratio, the Gram positive:Gram negative bacteria ratio and stress indices 1-3 before and after the water applications was quantified. Percent soil moisture and percent organic matter were measured during the pre and post water application sampling events.

Statistical Analyses

The effect of the weekly water additions on microbial biomass, community composition and nitrogen mineralization were analyzed with repeated measures analysis of variance. Significant differences between treatment means was based on the Tukey's studentized range test ($p < 0.05$) and between-year mean differences were based on the test for the effect of time. The effect of the weekly water additions on amino acid production rates was assessed with a one way analysis of variance followed by means separation (Tukey's studentized range test, $p < 0.05$). The extent to which the weekly water additions impacted stress indices 1 though 3 was analyzed using both repeated measures analysis of variance.

The effect of the water addition on microbial biomass, the bacteria:fungi ratio and the Gram positive:Gram negative bacteria ratio during experiment 2 was analyzed with repeated measures analysis of variance. Significant differences between treatment means was based on the Tukey's studentized range test ($p < 0.05$). A significant difference in the response of microbial communities over time was based on the test for a time by treatment effect.

RESULTS

Experiment 1 – Weekly water additions

Average air temperature over the study period was $0.33^{o}C$ in 2007 and $2.77^{o}C$ in 2008. The colder air temperatures in 2007 resulted in an average soil temperature over the study period that was $1.31^{o}C$ lower than soil temperatures in 2008. Over the course of the nine week water additions, average soil temperature under natural conditions was $3.15^{o}C$ in 2007 and $4.46^{o}C$ in 2008. During 2007, there were five freeze-thaw events over a period of five days (Figures 1-2). The fluctuations ranged from $+0.6^{o}C$ to $-0.5^{o}C$ below zero and the timing corresponded approximately with day and night. In 2008, there were two occasions (January 21 to 22 and January 25 to 26) during which soil temperatures fell below $1^{o}C$ overnight (Figure 1). However, soil freezing was not observed with the U.S. Forest Service sensors during the winter of 2008.

In un-manipulated plots, there was a large decrease in microbial biomass between years, from 300 $\mu g*gOM^{-1}$ in 2007 to 152 $\mu g*gOM^{-1}$ in 2008 (Table 2). There was also a significant decrease in microbial biomass among all plots between years ($p = 0.002$, Figure 3). Average microbial biomass was 311 $\mu g*gOM^{-1}$ during March 2007 and 177

µg*gOM^{-1} in March 2008. Although average soil moisture decreased (but not significantly so) between years, winter rainfall and soil thermal warming increased (Table 2).

The water additions did not result in a difference between soil moisture in experimental and control plots during mid-March, one week after the final water additions, in either year (Table 3). During March 2007, after nine weeks of water additions, soil moisture in experimental and control plots were both 56%. Average soil moisture among all plots declined to 45% during March of 2008 but was not significantly lower.

The water applications did not have an affect on microbial biomass during either year. During March of 2007, average microbial biomass in experimental plots was 322 µg*gOM^{-1} and in control plots was 300 µg*gOM^{-1} (Table 3). Average microbial biomass in March of 2008 was 152 µg*gOM^{-1} and 202 µg*gOM^{-1} in experimental and control plots respectively.

There was no change in the bacteria:fungi ratio or the Gram positive:Gram negative ratio during either year as a result of the treatments (Table 3). The average bacteria:fungi ratio between 2007 and 2008 was similar while there was a significant decrease in the Gram positive:Gram negative ratio from 2007 to 2008 ($p = 0.003$). The Gram positive:Gram negative ratio declined from 1.02 in 2007 to 0.82 in 2008 (Table 3).

There was no treatment effect on stress indices during either year. However, the mean of stress index 1 was significantly different between years ($p = 0.0001$, Figure 4). The values were lower during 2008, indicating that microbial communities experienced greater physiological stress during that year. There was a small decrease in stress index 2

between years that was suggestive of a significant difference (p = 0.07, Table 4) and a much larger and highly significant difference between years for the mean of stress index 3 (p = 0.0002, Table 4). This demonstrates that the change in stress index 1 was primarily due to a decrease in stress index 3.

During 2007, average ammonium-N concentrations in experimental plots (71 $\mu g*gOM^{-1}$) were significantly lower than ammonium-N concentrations in control plots (212 $\mu g*gOM^{-1}$) (Figure 5). Average ammonium-N pools in control plots declined to 54 $\mu g*gOM^{-1}$ in 2008 (Figure 5) and there was no significant difference between treatments during that year. There were no significant differences between treatments for nitrate pools in either year, and NO_3^--N pools were extremely low (Table 5).

Treatment means for nitrogen mineralization rates were significantly different in 2007 but not in 2008. From mid-March to mid-April of 2007, there was a small amount of nitrogen mineralization (0.28 $\mu g*g^{-1}*d^{-1}$) in the experimental plots and immobilization (-0.94 $\mu g*g^{-1}*d^{-1}$) in the control plots (Figure 6). In 2008, average nitrogen mineralization rates between experimental and control plots were close to zero (-.19 $\mu g*g^{-1}*d^{-1}$ and -.15 $\mu g*g^{-1}*d^{-1}$ respectively) and not significantly different. The change in nitrogen mineralization rates between years for control plots, but not experimental plots, resulted in a significant time*treatment effect (p = 0.03). No net nitrification was observed in any plots during either year (Table 5).

The average amino acid-N pools among all plots during March of 2007 was 57.4 $\mu g*gOM^{-1}$. These values are comparable with amino acid pools measured in May 2007 (45 $\mu g*gOM^{-1}$, Chapter 2). The water additions did not result in a significant difference in amino acid production rates between treatment means during 2007, the only year in

which this variable was measured. Average amino acid production rates were 0.29 $\mu g*g^{-1}*d^{-1}$ in experimental plots and 0.03 $\mu g*gOM^{-1}$ in control plots (Table 5). These values are similar to rates observed during May of 2007 (0.12 $\mu g*g^{-1}*d^{-1}$ among all treatments, Chapter 2).

Experiment 2 – Elevated precipitation followed by cold soil

From January 20 to 22, 2008, air temperatures were below $0^{\circ}C$ for 48 hours. This resulted in the coldest soil temperatures of the winter. At the time of the pre-treatment sampling event, on January 19, soil temperature at a depth of 3cm was $3.74^{\circ}C$. Over the next 40 hours, soil temperature declined to $0.87^{\circ}C$ and remained below $1^{\circ}C$ for 44 hours (Figure 7). On one occasion, a soil temperature value of $-0.16^{\circ}C$ was recorded. Because readings were recorded every four hours, this means that soil temperatures could not have been below $0^{\circ}C$ for more than 4 hours. At the time of the post-freeze sampling event, soil temperature was $1.74^{\circ}C$.

Prior to the water applications, average soil moisture was 37% in experimental plots and 45% in control plots (Table 6). Four days after the water applications, average soil moisture in experimental plots was 54% and 43% in control plots. This corresponded to a 17% increase and a 3% decrease in soil moisture in experimental and control plots respectively. However, these changes in soil moisture values were not statistically significant (time*treatment effect, $p = 0.33$).

Before water was added, average microbial biomass was 145 $\mu g*gOM^{-1}$ in experimental plots and 236 $\mu g*gOM^{-1}$ in control plots (Figure 8). This was a statistically significant difference (Tukey's studentized range test, $p < 0.05$). After four days, there

was no significant difference in average microbial biomass between drought and natural

plots. This was due to a decrease of over 100 μg in control plots and no change in

microbial biomass in experimental plots (Figure 8) and resulted in a significant

time*treatment effect (p = 0.02). The Gram positive:Gram negative and bacteria:fungi

ratios were similar between experimental and control plots prior to the start of the

experiment and the water applications did not alter the ratios between experimental and

control plots (Table 6). The treatment did not result in significant changes in any of the

stress indices (data not shown).

DISCUSSION

The positive association between air and soil temperatures during this study is in

contrast with northern forests, where air and soil temperatures were inversely correlated

(Groffman 2001). The difference in the relationship is due to the presence of a winter

snow cover at northern latitudes. When air temperatures are sufficiently cold, a persistent

winter snow pack insulates soil, but when temperatures are warmer, the snow pack is

reduced or forms later, causing soil freezing. While soil temperatures in the New Jersey

Pinelands in 2007 were colder than values recorded in 2006 and 2008, they were still not

cold enough to create a continuous winter snow cover, which requires sustained sub

freezing temperatures (Groffman et al. 2001).

Soil moisture values were much higher during mid- and late-winter than values

recorded in May, July and September (Chapter 2). The weekly water additions during

Experiment 1 had no effect on soil moisture at the end of winter, one week following the

last water application. On a finer temporal scale (Table 6) during mid-winter, there was

little effect of the water applications on soil moisture. It is therefore unlikely that the

weekly water applications during Experiment 1 resulted in even short-term (days) effects

on soil moisture throughout the winter. The high winter soil moisture is probably the

cumulative effect of winter precipitation and reduced evapotranspiration rates. I conclude

that winter precipitation in the New Jersey Pinelands leads to saturation of soils, and that

any increases in precipitation in the future will not significantly alter winter soil moisture.

Experiment 1 – Microbial Communities

The water additions had no effect on microbial biomass or community

composition during either year. Given that the water additions had no effect on soil

moisture, these results are not surprising and indicate that soil moisture is not limiting to

microbial communities during winter. These results are consistent with the two year

rainout shelter study (Chapter 2), during which there was no long-term effect of elevated

rainfall on soil microbial communities. The results were similar to the findings of Chapter

3, which found a short-term (hours to days), but no long-term (days to weeks) effect of

precipitation on microbial biomass.

Between March 2007 and March 2008, there was a 43% decrease in average

microbial biomass among all plots. A decline in microbial biomass was also observed

between May 2007-2008 and July 2007-2008 (Chapter 2), but this could not be fully

explained by inter-annual variability in soil temperature or precipitation. Similarly, the

decline in microbial biomass in this study cannot be explained by rainfall patterns

because total winter precipitation was highest 2008 (Table 2). The higher winter soil

temperatures in 2008 may have increased microbial activity, causing greater carbon

release via respiration, however this should not come at the expense of microbial biomass.

A gypsy moth (*Lymantria dispar*) outbreak may have contributed to the decline in microbial biomass between years. During the summer of 2007, gypsy moths were present in the study site in large numbers within the canopy, on tree stems and in the shrub layer. A visual inspection of the canopy revealed approximately 50% defoliation due to gypsy moth herbivory. Inefficiencies in gypsy moth feeding resulted in an early input of leaf litter during the summer of 2007 and a reduced input of litter system during the fall of 2007. At the same time, the gypsy moth frass may have increased the pool of recalcitrant carbon (see Chapter 2). Herbivory may have reduced rhizodeposition by decreasing the total amount of plant photosynthate (Cardon et al. 2002), further limiting the availability of labile carbon to soil microbes. A potentially reduced labile carbon pool due to the gypsy moth outbreak may explain the reduction in microbial biomass that was first observed during September of 2007 (Chapter 2). Cold winter soil temperatures in 2007, may have sustained the reduced levels of microbial biomass until March of 2008.

Gram negative bacteria have been found to be more dominant in bulk soil (Marilley and Aragno 1999) and at depth (Fierer et al. 2003), while Gram positive bacteria are dominant in surface soils (Fierer et al. 2003) and in the rhizosphere (Marilley and Aragno 1999). These patterns are most likely related to differences in substrate quality, with surface and rhizosphere soils containing a greater abundance of labile carbon substrates. The fact that the Gram positive:Gram negative bacteria ratio decreased between years suggests that there was a relatively higher labile carbon pool in 2008. The source of the carbon pool may have been rhizodeposition which, due to the higher soil

temperatures, should have been greater in 2008 relative to 2007. Greater root activity in 2008 is further supported by the lower soil moisture values (Table 2), which is indicative of greater plant water uptake. Therefore, while the overall carbon pool may have been lower in 2008 as a result of the gypsy moth outbreak, the higher soil temperatures appear to have resulted in a higher *relative abundance* of labile carbon during 2008, explaining the higher relative abundance of Gram negative bacteria.

Past studies have found that freeze-thaw cycles can stress microbial communities (Schimel and Clein 1996, Schimel et al. 2007), but in this case the communities were more stressed following the warmer winter with more mild freeze-thaw cycles. During the two year rainout shelter study (Chapter 2), drought conditions increased physiological stress relative to high rainfall treatments. It is unlikely that the reduction in stress indices observed in this study was due to the lower soil moisture values because in both years soil moisture was close to saturation. The gypsy moth outbreak, by reducing litter biomass during the fall of 2007, provides the most likely explanation for the reduction in microbial biomass, and consequently the reduction in stress indices between March 2007 and March 2008. However, this hypothesis cannot be confirmed with the existing data.

Experiment 1 - Nitrogen Cycling

During March of 2007, ammonium pools in control plots were three times higher than ammonium pools in experimental plots and almost four times higher than the values in control plots in 2008 (Figure 5). The accumulation of ammonium in control plots was followed by net nitrogen immobilization between mid-March and mid-April 2007 (Figure 6). These results are consistent with the findings of (Groffman et al. 2001) who found

that freezing increased inorganic nitrogen pools, but that the increase was not due to higher mineralization rates. Other studies suggest that ammonium can be released from freezing-induced disruption of litter or soil aggregates (Fitzhugh et al. 2001, Steinweg et al. 2008). Soil aggregates as the source of ammonium can be ruled out because soil aggregation in these soils is low. While no sampling was performed in the litter layer, it is possible that ammonium produced in the litter layer was translocated to the organic horizon during watering events. The most likely source of the ammonium was reduced plant uptake due to damaged roots (Groffman et al. 2001). Ectomycorrhizae may have also been damaged by freezing (Fitzhugh et al. 2001). A reduction in ectomycorrhizal biomass could not be confirmed by the PLFA data.

Amino acid production rates were negligible and similar to values recorded during other months of the same year (Chapter 2). The use of a buried bag for the *in situ* measurement of amino acid production severs plant root connections, potentially inhibiting exoenzyme production by mycorrhizal fungi. The low amino acid production rates could also be related to the low soil pH, which may reduce amino acid cycling (Sinsabaugh et al. 2008). On the other hand, the enzyme ß-N-acetylglucosaminidase, important in the degradation of chitin, is most active in acidic soils (Sinsabaugh et al. 2008). There is an abundant supply of chitin in pinelands soils, originating from fungal cells and the exoskeletons of arthropods. If ammonification was primarily the result of chitin metabolism, this would explain the weak relationship between amino acid production and nitrogen mineralization.

Experiment 2

The period of cold to but not freezing to soil temperatures in 2008 resulted in a large reduction in microbial biomass in control plots that was detected in a matter of days (Figures 8). This is consistent with Schimel and Clein (1996), who found that freeze-thaw cycles alter soil microbial communities. However, it is in contrast with (Lipson et al. 2000), who found no effect of freeze-thaw cycles on microbial biomass when a realistic freeze-thaw regime was used. Because the Lipson et al. (2000) study used soil from an alpine system, the difference in the effect of freeze-thaw cycles may be due to the fact that microbes from the colder alpine soils are better adapted to cold stress.

The mild freeze during Experiment 1 did not alter microbial biomass at the end of winter, but the cold mid-January temperatures during 2008 did decrease microbial biomass by 39% within four days to an average value of 145 $\mu g * gOM^{-1}$. By the end of winter, 55 days after the end of the cold period, microbial biomass more than doubled to 300 $\mu g * gOM^{-1}$. These counter-intuitive responses of microbial biomass to freezing in 2007 and cold (but not frozen) soil in 2008 can be explained by the temporal scale of sampling and highlights the resiliency of microbial communities to freezing stress. It is difficult to assess the impact of the temporal scale of sampling in other studies because few report the timing of sampling in relation to the freeze-thaw events. However, Groffman et al. (2001) hypothesized that more intensive temporal sampling may have revealed differences in microbial biomass as a result of their snow removals.

I predicted that elevated soil moisture would increase the severity of freeze-thaw cycles by forming a larger number of damaging ice lenses that lyse microbial cells and plant roots. Instead, the opposite effect was observed: the water additions appear to have

protected soil from freeze damage, presumably by elevating soil temperature (Willis et al. 1961). At cold temperatures, microbes are very sensitive to even small changes in temperature (Brooks and Schmidt 1997, Mikan et al. 2002). In Experiment 2, the decrease in soil temperature negatively impacted soil microbial biomass, while water applications in experimental plots probably insulated microbes from cold stress. Further evidence for a protective role of water from freeze damage comes from Experiment 1. During this study, ammonium pools increased in control plots, presumably due to freeze damage. The lower ammonium concentrations in experimental plots could have been the result of an insulating effect on plant roots.

Conclusions

In the northeastern U.S., frost free dates are starting sooner (Cooter and LeDuc 1995) and the number of extremely cold days are decreasing (DeGaetano 1996). There is concern that global warming may alter winter nitrogen cycling patterns in forests and lead to greater nitrogen export into waterways (Brooks et al. 1999, Groffman et al. 2001). The findings of this study suggest that natural variability in winter soil temperatures may influence the magnitude of ammonium accumulation in soils of the New Jersey Pinelands. While an increase in winter rainfall is unlikely to affect soil moisture or increase nitrogen leaching, it may buffer against these higher rates of ammonium accumulation by increasing soil temperature.

Climate models predict that both air temperatures and precipitation amount will increase during winter (Baede et al. 2001). If warming continues, the frequency of the mild freeze-thaw events observed in 2007 will decrease and ammonium pools at the start

of spring will be lower. It must be emphasized that the effects of climate on winter processes in the New Jersey Pinelands are fundamentally different from what occurs at northern altitudes and higher elevations. In these colder regions, winter snow cover results in a large pulse of water and dissolved nutrients during spring thaw. Such a defined period of water and nutrient inputs do not exist in the Pinelands, where precipitation is relatively constant throughout the year. The increase in ammonium pools observed during March of 2007 may have only a minor impact on plant and microbial communities. More detailed investigations into the effects of altered winter nitrogen cycling on plant and microbial communities in the New Jersey Pinelands are warranted.

The results of Experiment 2 demonstrate that reductions in microbial biomass can occur in response to cold, but not freezing, soil conditions. Lipson et al. (2000) found that winter pools of soil carbon exerted a stronger control on microbial biomass than freezing soil temperatures. In the Pinelands, if microbial communities were indeed under carbon stress due to the gypsy moth outbreak, this may have made them more sensitive to cold stress. Future studies should investigate a potential interaction of cold stress and carbon limitation on microbial communities during winter.

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Table 1. List of fatty acids identified by the MIDI protocol and confirmed by GC-MS (group assignments based on Frostegård and Bååth 1996, O'Leary and Wilkinson 1988, Wilkinson 1988 and Zelles 1999).

Biomarker for	Fatty Acid
Gram positive bacteria	i15:0, a15:0, i16:0, i17:0, a17:0
Gram negative bacteria	16:1 ω7c, i16:1 G, i16:1 I, 17:1 ω8c, cy17:0, 18:1 ω7c, cy19:0ω8c
Bacteria	All above, plus 16:0 10Me, 17:0 10Me, 18:0 10Me
Fungi	18:2 ω6, 9c
Microbial Biomass	All above, plus 18:1 ω9c, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0
Stress Index 1	$(16:1 \ \omega7c + 18:1 \ \omega7c) \div (cy17:0 + cy19:0\omega8c)$
Stress Index 2	$16:1 \ \omega7c \div cy17:0$
Stress Index 3	$18:1 \ \omega7c \div cy19:0\omega8c$

Table 2. Average microbial biomass (±SE) in natural plots in relation to total winter precipitation (Precip), soil moisture (SM), winter soil thermal warming ($Temp^1$) and average soil temperature on the day of sampling ($Temp^2$).

Year	Biomass $\mu g*gOM^{-1}$	Precip (mm)	SM	$Temp^1$ (^oC)	$Temp^2$ (^oC)
2007	300 (30)	246	0.6	375	7.0
2008	152 (32)	278	0.5	422	5.4

Table 3. Response of average (±SE) soil moisture (SM), microbial biomass, the bacteria:fungi ratio (B:F) and the Gram positive:Gram negative bacteria ratio (G^+:G^-) to water additions during March 2007 and March 2008. E = water additions, C = control. Stars (*) denote significant differences between mean values (test for effect of time). * ($p < 0.01$), ** ($p < 0.001$)

Year	Treatment	SM	Biomass ($\mu g*gOM^{-1}$)	B:F	G^+:G^-
2007	E	0.56 (0.1)	323 (36)	6.8 (0.5)	1.0 (0.02)
	C	0.56 (0.1)	300 (30)	7.4 (0.9)	1.0 (0.03)
	Mean	0.56 (0.1)	311 (22)*	7.1 (0.5)	1.0 (0.02)**
2008	E	0.44 (0.1)	152 (25)	6.8 (1.0)	0.8 (0.1)
	C	0.45 (0.1)	202 (32)	7.1 (0.3)	0.8 (0.03)
	Mean	0.45 (0.1)	177 (21)*	7.0 (0.5)	0.8 (0.03)**

Table 4. Response of average (±SE) stress indices 1-3 to water additions during March 2007 and March 2008. E = water additions, C = control. Stars (*) denote significant differences between mean values between years (test for effect of time). *($p < 0.1$), ** ($p < 0.001$)

Year	Treatment	Stress Index1	Stress Index2	Stress Index3
2007	E	1.6 (0.1)	4.9 (0.3)	0.7 (0.1)
	C	1.6 (0.1)	5.5 (0.3)	0.7 (0.1)
	Mean	1.6 (0.04)**	5.2 (0.2)*	0.7 (0.03)**
2008	E	1.0 (0.1)	4.6 (0.7)	0.4 (0.03)
	C	1.0 (0.1)	4.2 (0.2)	0.4 (0.03)
	Mean	1.0 (0.1)**	4.4 (0.3)*	0.4 (0.02)**

Table 5. Response of average (± SE) nitrate-N (NO_3^-N), ammonium-N (NH_4^+-N) and amino acid-N (AA-N) pools ($\mu g*gOM^{-1}$) and potential net nitrification (Nitrif), nitrogen mineralization (Nmin) and amino acid production (AA Prod) ($\mu g*g^{-1}* d^{-1}$) to weekly water additions. E = water additions, C = control. Letters ([a] and [b]) denote significant differences between treatment means (Tukey's studentized range, $p < 0.05$). Standard error for Nitrif was not included because the values were close to zero.

	2007		2008	
Response	E	C	E	C
NO_3^--N	0.8 (.1)	0.9 (.1)	0.8 (.1)	0.8 (.1)
NH_4^+-N	71 (27)[a]	212 (33)[b]	50 (11)	54 (17)
AA-N	53 (8)	62 (9)	-	-
Nitrif	0.001	0.0001	-0.001	0.01
Nmin	0.3 (.4)[a]	-0.9 (.3)[b]	-0.2 (.1)	-0.2 (.1)
AA Prod	.3 (.2)	.02 (.1)	-	-

Table 6. Response of average (±SE) soil moisture, microbial biomass, the Gram positive:Gram negative bacteria and bacteria:fungi ratios before and after a two day period of very cold soil temperatures. E = water addition, C = control. Letters ([a] and [b]) denote significant differences (Tukey's studentized range, $p < 0.05$) between treatment means.

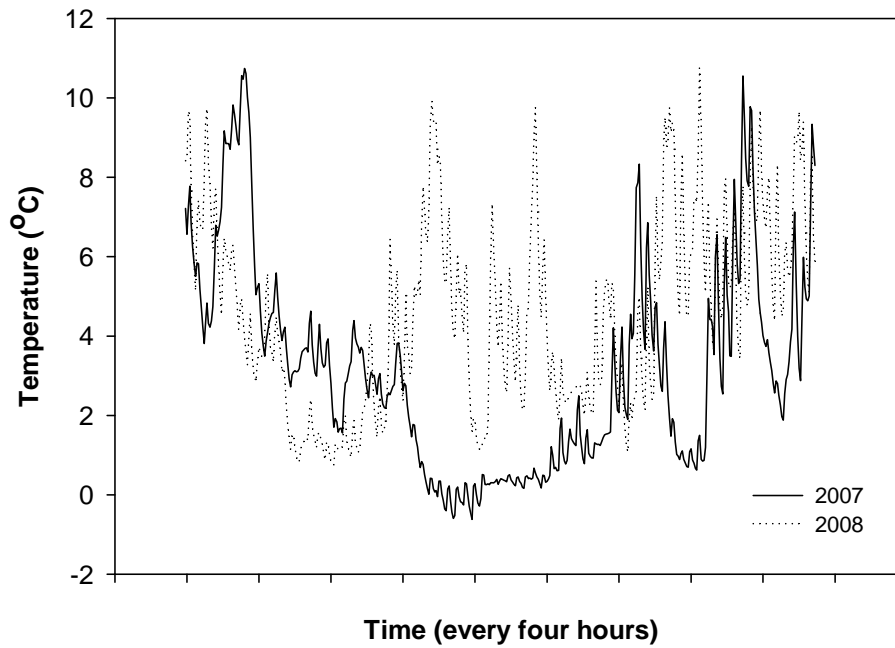| Response	Treatment	Initial	Final	Difference
% Soil Moisture	E	0.37 (.1)	0.54 (.1)	0.17 (.2)
	C	0.45 (.1)	0.43 (.03)	-0.03 (.1)
Biomass ($\mu g*gOM^{-1}$)	E	145[a] (24)	143 (10)	-1.9[a] (17)
	C	236[b] (21)	133 (15)	-102[b] (31)
Gram$^+$:Gram$^-$	E	0.9 (.1)	0.9 (.1)	-0.002 (.2)
	C	0.9 (.03)	0.8 (.1)	-0.04 (.1)
Bacteria:Fungi	E	9.2 (.3)	8.5 (.8)	-0.7 (.8)
	0C	8.4 (.4)	7.9 (1.0)	-0.6 (1.2)

Figure 1. Soil temperature (^{o}C) at a depth of 3cm during the study period (January 9 to March 22 of 2007 and 2008).
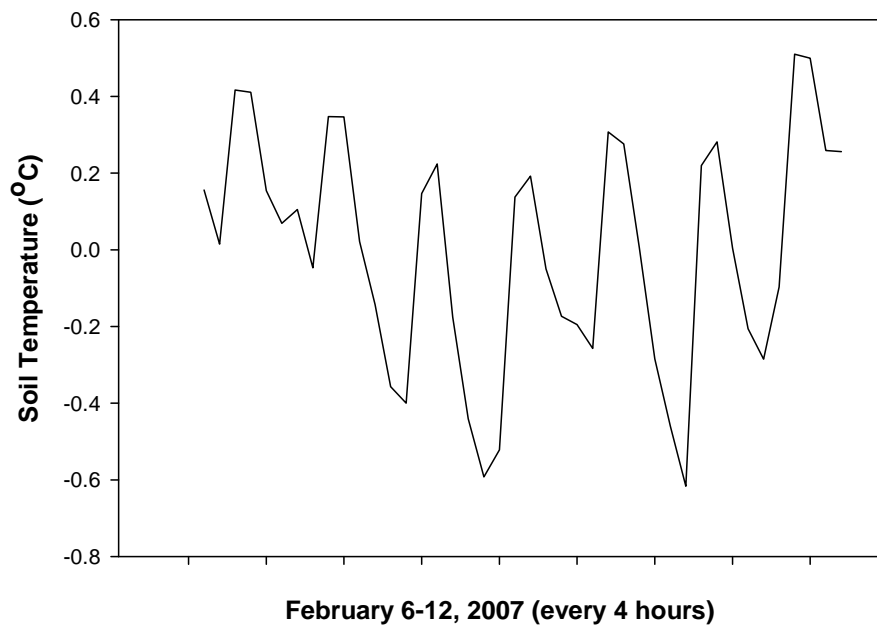

Figure 2. Soil temperatures during a series of mild freeze-thaw events during the winter of 2007. The cycles correspond approximately with day and night.
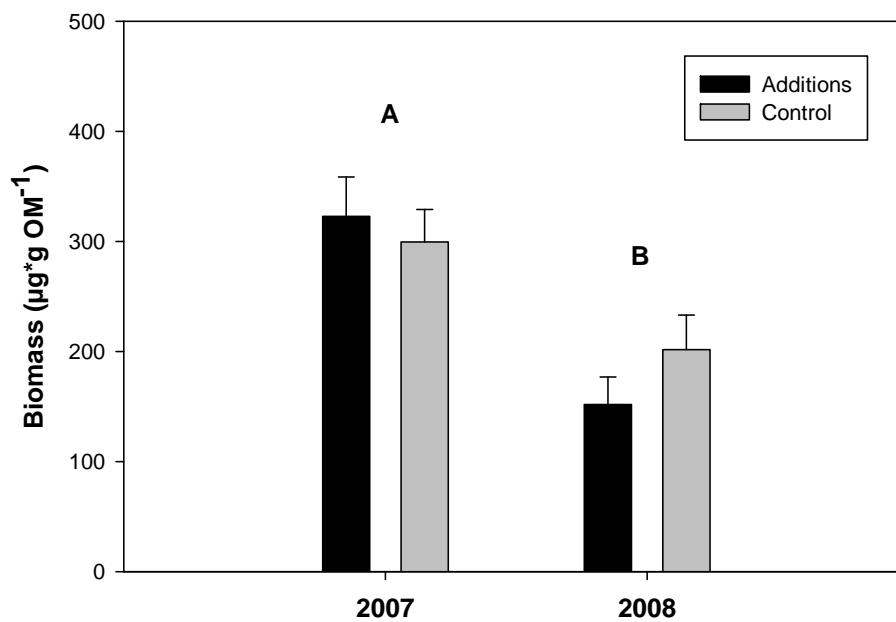
Figure 3. Response of average microbial biomass (±SE) to the water additions in March 2007 and March 2008. Letters (A and B) denote significant differences in average microbial biomass between years (p = 0.002).
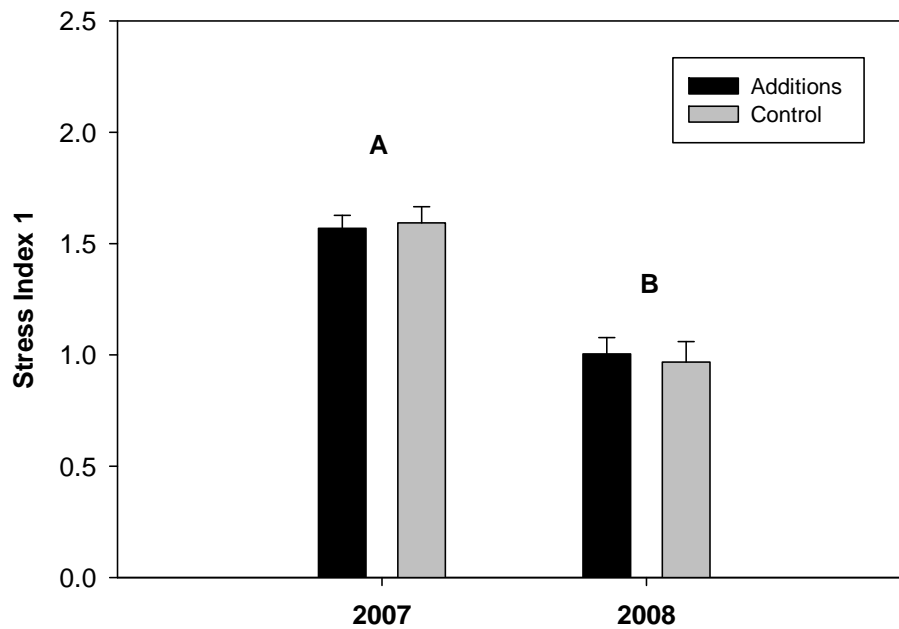

Figure 4. Response of stress index 1 to weekly water applications (Experiment 1). Letters (A and B) denote significant differences between mean values (p = 0.0001).
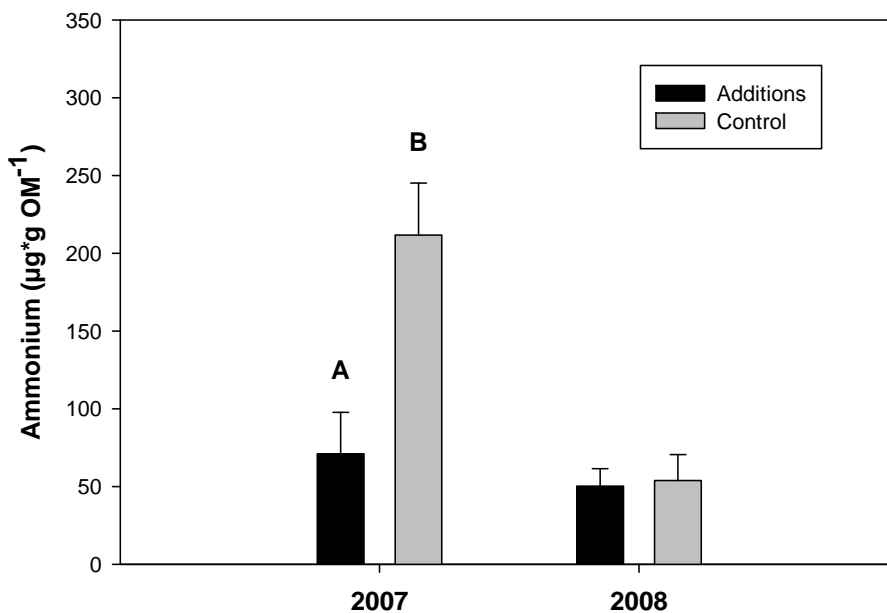
Figure 5. Response of average ammonium pools (±SE) to the weekly water Additions in March 2007 and March 2008. Letters (A and B) denote significant Differences between treatment means (Tukey's studentized range test, $p < 0.05$).

Figure 6. Response of average potential net nitrogen mineralization (±SE) to the weekly water additions in March 2007 and March 2008. Letters (A and B) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$).
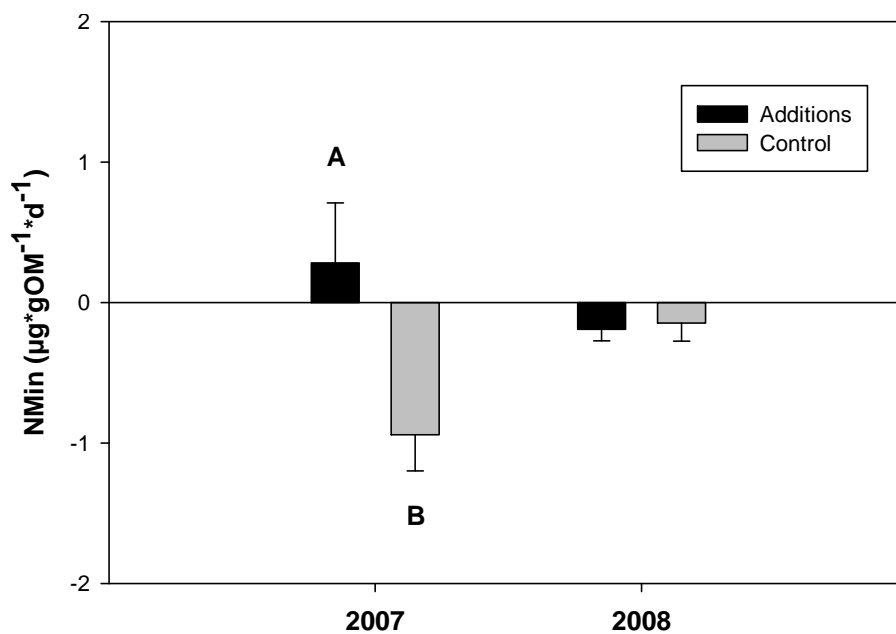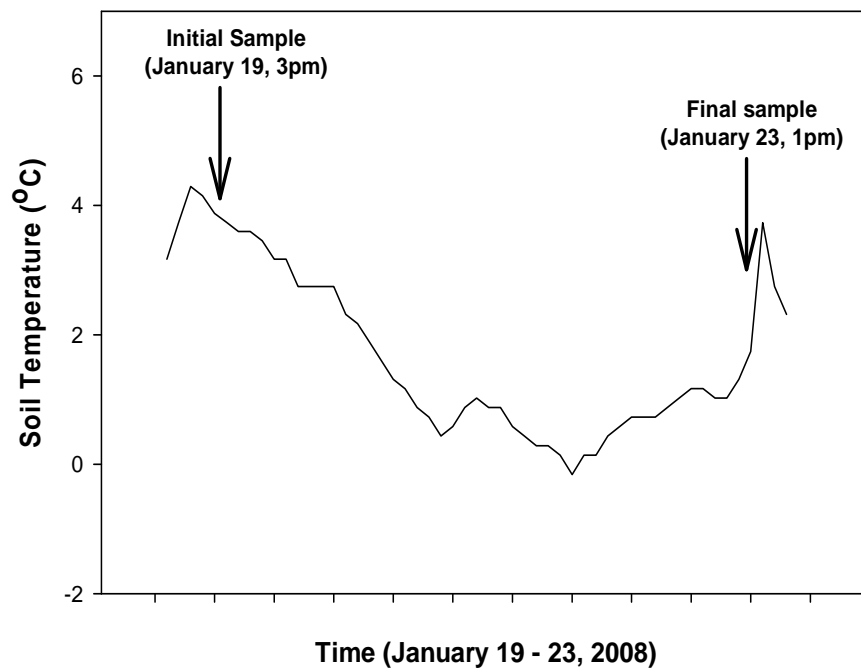
Figure 7. Soil temperature (recorded every four hours) during Experiment 2. Arrows indicate initial and final sampling events. Water was applied immediately after the initial samples were removed.
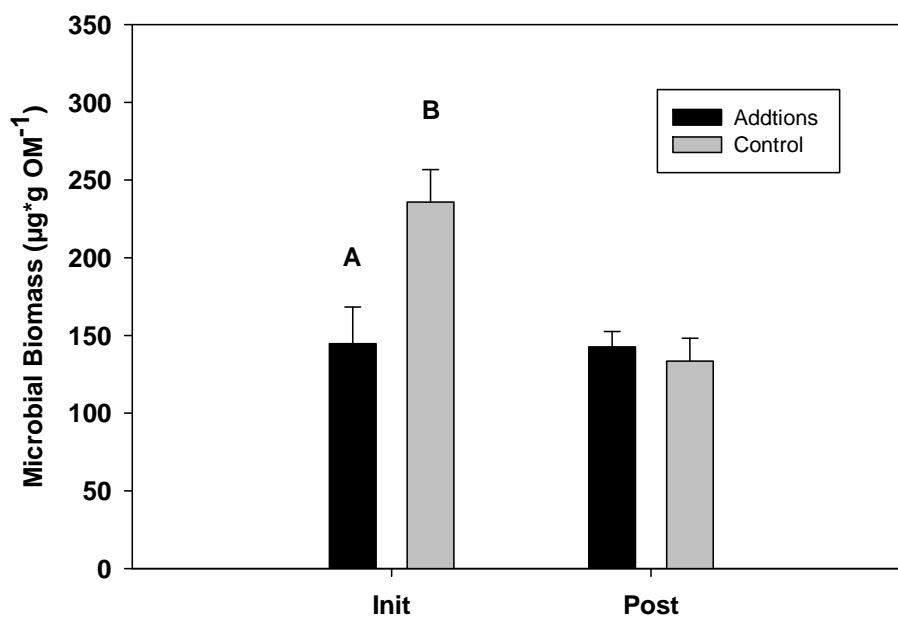

Figure 8. Response of average microbial biomass (±SE) before and after two days of cold soil (Experiment 2). Letters (A and B) denote significant differences between treatment means (Tukey's studentized range test, $p < 0.05$).

CHAPTER 6

Conclusions and synthesis

During this study I demonstrated that soil microbial communities are highly resistant to extreme drought conditions and insensitive to a doubling of rainfall. In response to the drought treatment in Chapter 2, I detected a change in the fatty acid composition of the microbial community that was indicative of a survival response. Because nematode densities responded positively to elevated precipitation, I concluded that microbivorous grazing may have placed an upper limited on the increase in microbial biomass. Therefore, a combination of physiological modifications and biological interactions may have prevented a more dramatic response of the soil microbial community to the rainfall manipulations.

The results from chapters three and five demonstrate that the response to soil drying, rainfall and cold soil temperatures is rapid. For example, the dry conditions between the July 2008 sampling event and the start of the rewet experiment resulted in a 23% drop in microbial biomass within natural plots in only 25 days. Following the rewet event, this biomass was recovered within three hours. In response to the cold – but not freezing – conditions in January 2008, microbial biomass declined by 43% within four days. This biomass was mostly recovered by the end of winter. These temporal dynamics provided further insight into the microbial response observed in Chapter 2.

Taken together, the results of Chapters two, three and five demonstrate that neither precipitation nor soil temperature patterns result in a large and sustained reduction in microbial biomass or change in community composition. However, a substantial and prolonged reduction in microbial biomass and change in community composition was

observed in my study site. The large gypsy moth outbreak provides the best possible explanation for these changes. While no specific mechanism can be demonstrated, it seems plausible that the reduction in photosynthate would have altered the microbial community by reducing litter biomass and/or the quality or quantity of rhizodeposition.

Assuming that the gypsy moth outbreak reduced microbial biomass via changes to the soil carbon pool, this leads to somewhat of a paradox, because carbon limitation should not have resulted in such a large amount of cell lysis. This is demonstrated by the two year drought treatment which, due to the reduction in diffusion rates, can also be seen as a carbon reduction treatment. Instead of a reduction in biomass, the microbial community responded by modifying their cell membrane composition. Processes that lyse microbial cells include dry-wet cycles, freeze-thaw cycles and grazing. The results from Chapters three and five indicate that neither freeze-thaw cycles nor the most extreme drying and rewetting event result in a large and sustained reduction in microbial biomass. I did not quantify microbivorous grazing and therefore this cannot be ruled out as having contributed to the reduction in microbial biomass and change in community composition. One possible hypothesis is that there was an interaction between the gypsy moth outbreak and grazing activity. For example, if the gypsy moth outbreak reduced soil carbon availability, the microbial community may have responded by investing less energy in anti-predation defenses (Jousset et al. 2008). This could have led to greater grazing intensity and reductions in microbial biomass, the bacteria:fungi and the Gram positive:Gram negative bacteria ratio.

I found that both soil drying and soil freezing can result in a large accumulation of ammonium in pinelands soils. This buildup was the result of sustained microbial activity

across a broad range of soil conditions, including the driest and coldest soils that are likely to occur in the New Jersey Pinelands. A reduced population of nitrifying bacteria (Hastings et al. 2000) and root damage caused by both drying (Chapter 2) and soil freezing (Chapter 5) appears to have prevented the consumption of this ammonium. Because ectomycorrhizal fungi are comprised of a very thin and delicate layer of cells, it seems likely that these cells should have been damaged by freezing or drought (Fitzhugh et al. 2001), contributing to reduced plant nitrogen uptake. However, a reduction of ectomycorrhizal fungi could not be confirmed by the PLFA data. Because fungi can extend hyphae outside the experimental plots to access nutrients and translocate nutrients within the mycelial network (Jennings 1976, Jennings 1990), cell death among ectomycorrhizae may have been limited.

To the best of my knowledge, no previous studies have measured amino acid production in the pinelands. However, research within sites with similar characteristics suggests that amino acid uptake should be an important source of plant nitrogen in the pinelands (Nasholm et al. 1998, Berthrong and Finzi 2006). I detected only very low rates of amino acid production during this study. This could be due to the fact that amino acid cycling is very low in acidic soils (Sinsabaugh et al. 2008), or could be the result of a tight coupling of amino acids between mycorrhizal fungi and plants (Nasholm et al. 1998). An important assumption I made, that appears to be false, is that ammonification is closely linked to amino acid cycling in the organic horizon of Pinelands soils. Instead, based on what is known about enzyme activity in acidic soils (Sinsabaugh et al. 2008), it is more likely that chitin metabolism was the primary source of ammonification.

Implications for climate change

The results of this study suggest that changing precipitation patterns will not directly impact soil microbial biomass or community composition in the New Jersey Pinelands over the long-term. Because I did not find a long-term change in microbial community composition in response to manipulations of rainfall, I conclude that there is no correlation between the bacteria:fungi ratio or the Gram positive:Gram negative bacteria ratio and nitrogen mineralization rates. Changing precipitation patterns are more likely to alter nitrogen mineralization rates via the impact on physiological stress of the microbial community and altered rates of nitrogen diffusion. Biological interactions are difficult to quantify in soil, but the sensitivity of nematode densities to precipitation is a strong indication that the effects of changing precipitation patterns may be mediated via the grazer community. As our ability to measure grazer-microbe interactions improves, this will be a critical area for future research into soil microbial ecology and climate change.

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