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The Effect of Warming and Simulated Rainfall on Soil Microbial Community Structure and Function

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Abstract of Thesis

Soil respiration, from plant roots and soil microbes, accounts for 60 – 80 percent of total ecosystem respiration, with the microbial component contributing approximately 54 percent. Global climate trends resulting from CO_2 emissions include increased soil temperatures and changes in precipitation regimes resulting in less frequent, more intense rainfall events. Soil temperature and moisture availability drive soil respiration rates, but how they impact the microbial respiration is poorly qualified. I investigated how the soil microbial community responds to changes in temperature and moisture availability in a laboratory based experiment. Soils from a mixed hardwood forest under two thermal regimes received either a large or small simulated rainfall event. A large event corresponded with the highest recorded daily average rainfall event for a 30 year period and a small event was half that amount. Soil temperature, moisture, and respiration were measured at 30 minute intervals for the duration of the experiment. I used the following metrics to quantify microbial respiratory response: (1) maximum rate of soil microbial respiration (SMR_{max}); (2) the amount of time it took to reach SMR_{max} (T_{max}); (3) the amount of time it took to return to pre-rainfall rates of soil microbial respiration ($T_{duration}$); and the total CO_2 production in each mesocosm associated with rainfall (SMR_{total}). Temperature treatments positively influenced SMR_{max}, but had no impact on my other metrics. Rainfall event size positively impacted SMR_{max}, $T_{duration}$, and SMR_{total}. My research suggests that in temperate mixed hardwood forest soils moisture is a stronger driver of soil microbial respiration than temperature.

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The Effect of Warming and Simulated Rainfall on Soil Microbial Community Structure and Function

by

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Introduction

Widespread and accelerating anthropogenic climate change demands an improved understanding of terrestrial carbon cycling. Soil organic carbon represents a large pool in the terrestrial carbon cycle and respiration by plant roots and soil microbes accounts for 60 – 80 percent of total ecosystem respiration (Raich and Schlesinger 1992; Le Quere et al. 2009). Soil respiration rates are driven by complex and interacting controls that include exogenous (soil moisture and temperature) and endogenous (photosynthetic gain) factors (Barron-Gafford et al. 2014), but have consistently been shown to rapidly respond to changes in soil moisture, temperature, and substrate availability (Yuste et al. 2007; Guntiñas et al. 2013; Potts et al. 2014). A comprehensive understanding of how soil respiration is affected by changing temperature and precipitation regimes is therefore imperative to improving estimates of terrestrial carbon budgets (Figure 1).

Ever increasing greenhouse gas emissions are expected to raise global surface temperature considerably in the coming decades, resulting in changing precipitation regimes and longer growing seasons (IPCC, 2015). Global temperatures are projected to increase 0.3°C to 0.7°C by 2035 (IPCC, 2015) and recent climate models predict changes in the hydrological cycle resulting in less frequent, more intense rainfall events (Huntington 2006; Seneviratne et al. 2006; IPCC 2015). Increased temperatures are also responsible for early onset of spring, resulting in longer growing seasons (Linderholm 2006). Predicting the effects of warming temperatures and altered precipitation regimes on ecosystem function is a long-stated goal of ecology (Raich and Schlesinger 1992; Weltzin et al. 2003) and how these factors influence soil microbial

respiration through soil organic matter decomposition are highly relevant for global carbon budget estimates.

Pools of soil organic matter (SOM) are a result of inputs from both above-ground and below-ground carbon, primarily from leaf and root detritus, and outputs of CO_2 from plant roots and soil microbes (Davidson and Janssens 2006). Soil organic matter is composed of a combination of dead and living animal and plant material, but the main constituent of SOM is soil organic carbon (SOC), which makes up 58 percent of its total weight (Lal 2001). SOC is often conceptualized as consisting of two principle pools characterized by their availability to soil microbes and which vary in their residence time in the soil ranging from days to millennia (Schimel et al. 2005).

The labile pool of SOC is characterized by low molecular weight organic molecules which are readily available to soil microbes and have a short residence time in the soil. Conversely, the recalcitrant pool of SOC is characterized by high weight organic molecules which are very difficult for most soil microbes to metabolize and hence have a long residence time in the soil (McLauchlan and Hobbie 2004; Allison 2008). Soil CO_2 fluxes are largely dominated by the small, but highly bio-reactive transient labile pool, whereas long-term storage is determined by the abundance of the persistent recalcitrant fraction (Trumbore et al. 1990). As the labile pool is a direct reservoir of organic carbon readily available to soil heterotrophs, it has considerable control over ecosystem function. This pool, by impacting soil microbial activity, biomass, and rates of soil nutrient mineralization, has been shown to alter both ecosystem structure and productivity (Pastor and Post, 1986). The labile pool of carbon is therefore responsible for much of the CO_2 flux from soil to atmosphere and has been

shown to be sensitive to moisture and temperature alterations resulting from climate change (Zak et al. 1993; Trumbore et al. 1996).

Soil respiration combines the respiratory activity of plant roots and soil microbes (Ryan and Law 2005) and is an important ecosystem function in that it represents a principle pathway for the exchange of carbon between the land surface and the atmosphere (Houghton 2003). Soil microbial respiration (SMR), is estimated to contribute more than half of total soil respiration (about 54 percent annually), directly through cellular activity and indirectly through decomposition of carbon (Hanson et al. 2000). These estimates emphasize the considerable contribution of soil microbial communities to terrestrial CO_2 fluxes.

Whereas the soil microbial community is vast, research examining the link between community structure and function has focused primarily on the contributions of bacteria and fungi (Brandford and Fierer 2015). Due to the relative difficulty associated with isolating and identifying bacteria and fungi functionally and taxonomically, they are often considered functionally redundant (Allison and Martiney 2008). However, the fact that bacteria and fungi may differ in the preferences for particular organic carbon substrates challenges the notion of functional redundancy and may provide better understanding of how each of these microbial functional groups contributes to carbon cycling. Bacteria are known for their rapid utilization of readily available, easily decomposed, labile carbon (Coleman et al. 2004), whereas fungi utilize more recalcitrant organic matter, which is more resistant to decomposition (Carroll and Wicklow 1992).

In addition to substrate utilization, fungi and bacteria have physiological differences that influence carbon cycling in soils. Fungi not only can utilize more lignified carbon, but also have hyphae which permit them access to surface litter (Holland and Coleman 1987). The cell walls of fungi are composed of polymers of melanin and chitin, which are much more resistant to degradation than the readily-decomposable, energy-rich phospholipid walls of bacteria (Guttenberger et al. 1999). As a consequence of these physiological differences, soil microbial communities dominated by fungi have slower carbon turnover rates because they incorporate more carbon into biomass than bacteria, have more recalcitrant cell walls, and facilitate carbon stabilization and protection by enhancing aggregation of soils (Six et al. 2006).

Soil temperature is a principle factor regulating microbial respiration (Raich and Schlesinger 1992) and accounts for the majority of seasonal and diel variation in CO_2 flux (Davidson et al. 1998). Soil temperature controls SMR directly with increased temperature resulting in increased metabolic activities of the soil microbes, and indirectly through increased photosynthetic activity and the corresponding release of root exudates (Bertin et al. 2003). Tower-based measurements of ecosystem respiration which integrate respiratory efflux of CO_2 from both above- and belowground sources correlated positively with both photosynthetic rates and site productivity, illustrating the important contribution of plants' physiological performance to SMR (Craine et al. 1999; Janssens et al. 2001). Root respiration does not contribute to the microbial component of soil respiration however, the increased release of root exudates which is correlated with increased photosynthetic activity, provides more readily assessable labile carbon to

microbial communities resulting in increased microbial respiration rates (Hogberg and Read 2006).

The positive influence of temperature on soil respiration rates is widely documented (Rustad et al. 2000) however, the pattern is not universal, suggesting that other biotic or abiotic factors may influence SMR. For example, in a boreal forest greenhouse study, Allison and Treseder (2008) found that experimental soil warming resulted in an initial increase in soil respiration but a then steady decline toward the end of the growing season. The authors suggested that the decline in respiration was associated with decreased soil moisture resulting from the temperature treatment. This observation is supported by a growing body of research which demonstrates that soil temperature sensitivity is closely tied to soil moisture content, (Kirschbaum, 1995; Curiel Yuste et al., 2003; Lavigne et al., 2004), and that studies of soil temperature alone are incomplete and soil respiration fluxes cannot be accounted for solely on this factor.

The relationship between soil water content and microbial respiration is complex, but is consistently demonstrated with a decrease in microbial activity with decreased moisture (Or et al. 2007), and ceasing entirely at extreme lows (Schimel et. al.1999). This decrease in metabolic activity is caused by both physical and physiological processes. As soil drying occurs, available water in pores becomes disconnected, slowing down diffusion of solutes and limiting substrate availability resulting in a decline in nutrient flow to microbes (Schjonning et al. 2003). Increased soil respiration with water addition is a well-documented relationship (Liu et al. 2008; Yuste et al. 2003), but perhaps more interesting is how microbial communities respond to episodic rainfall events and how event magnitude interacts with temperature to drive respiration rates

Resource availability is an obvious factor that limits metabolic activity, and water is necessary for microbial metabolic processes. Stress to organisms can be defined as any disturbance that results in a community shift in resource use from growth to physiological maintenance (Odum 1985). In this sense, the drying-rewetting of soils associated with episodic rainfall events may represent a considerable stress on soil microbes. Additionally, pulses of activity as a result of intermittently available resources are common (Yang et al. 2010). The down regulation of microbial activity associated with drying and subsequent pulse of metabolic activity following wetting is a well-documented phenomenon (Birch 1958; Fierer and Schimel 2003; Huxman et al. 2004).

Projected changes in precipitation regime associated with global climate change include increased frequency in extreme events (Heinmann and Reichstein 2008), coupled with heat spells (Gangley et. al 2009) and longer periods of drought (Meehl et al. 2007). Soils in most ecosystems experience periods of extended drying interspersed with rapid rewetting events (Fierer et. al 2003), but these trends present a scenario in which temperate soils could experience longer periods of drying similar to arid and semi-arid ecosystems and therefore be more sensitive to discrete rainfall events. For example, an experimental rainfall redistribution experiment in a temperate grassland demonstrates the metabolic sensitivity of soil microbial communities and associated carbon cycling consequences of soil wetting and drying associated with isolated rainfall events in an otherwise mesic ecosystem (Fay et al. 2008).

In water limited systems, long periods of drying occur, followed by discrete precipitation events (Loik et al. 2004). Because water is required for soil microbes to acquire and utilize carbon, the magnitude and duration of these pulses of water

availability are strong drivers of microbial activity (Huxman et al. 2004). The positive relationship between pulse driven moisture availability and soil respiration has been thoroughly demonstrated in these systems (Potts et al. 2006; Jenerette and Chatterjee 2012,), but it is possible, given warming temperatures and changing precipitation regimes that similar relationships may be also exist in temperate ecosystem soils.

Temperate forest ecosystems are historically less susceptible to fluctuations in moisture availability due to larger quantities of rainfall, but also the ability of the plant community to redistribute water from wetter, deeper soils (Borken et al. 2006). This results in less stress to microbial communities, potentially driving a milder response to pulse moisture availability than in arid ecosystems. This resilience could be at risk however, due to predicted changes in precipitation regime for temperate systems. For example, Borken et al. (2006) demonstrated that prolonged summer drought in a temperate forest resulted in a decrease in soil respiration, supporting the idea that less frequent rainfall has respiratory consequences in temperate systems. Certainly, temperate microbial communities will exhibit a positive respiratory response to water addition (Lee et. al 2004), but the magnitude and duration of this response, as well as interactive effects with increased temperatures in temperate soils is unclear.

The objective of this research was to examine how rainfall event size, coupled with increased temperature, influences dynamic shifts in soil microbial community structure and function. My goal was to quantify shifts in soil microbial community structure with concurrent measurements of SMR in the context of a laboratory-based full factorial soil moisture and temperature manipulation experiment. Due to difficulties associated with PCR inhibition, I was unable to collect community structure data.

Predictions, methods, and discussion of soil microbial community structure can be found in appendix A.

Soil Microbial Community Function Hypotheses:

I predicted that discreet rainfall events, regardless of their magnitude, would result in increased SMR. Further, I predicted that rainfall event size will not influence maximum rates of SMR (Huxman et al. 2004). Rather, I predicted that increasing rainfall event magnitude would increase the duration of SMR and, in turn, increase the total amount of C respired in response to rainfall. Conversely, I predicted that soil warming would positively influence maximum rates of SMR but cause a decline in the duration of SMR as a result of warmer temperatures' effect on increased soil evaporation. Finally, I predicted that the influence of warming on the duration of SMR would decline with increased rainfall magnitude due to increased soil moisture residence time resulting in decreased rates of evaporation.

Methods:

Site Description and Soil Collection

I collected soils from a representative stand of second growth mixed hardwood forest on the property of the Beaver Meadow Audubon Center (BMAC), a 324-acre wildlife preserve established in 1951 (42°40'27.1"N 78°22'42.1"W, 4756 – 4920 meters) (Figure. 3). At the site, soils are classified as Howard-Madrid gravely loam, a deep, well

drained, medium textured alkaline outwash soil with considerable quantities of gravel (National Resources Conservation Service 2010, Soil Conservation Service, 1956).

On July 28, 2014 I collected mineral soil from the O and shallow A horizons to a depth of approximately 20 cm. Working along two parallel 800 meter transects located 200 meters apart and using a spade, I first removed the litter layer and then collected soils. Soils were transported back to the lab, bench dried, passed through a 2 mm sieve to remove large stones, roots, and macro fauna, homogenized, and stored at 4°C.

Soil mesocosms were exposed to two temperature regimes consisting of an ambient temperature control (20-21°C), and an experimentally increased temperature (ambient+5°C), which was accomplished by placing seed heating mats under the planting tray which contained the soil mesocosm. In addition to the temperature manipulation, mesocosms were randomly assigned to one of two types of simulated rainfall events. I used average daily rainfall data for the period 1981-2010 recorded at the nearest climate monitoring site (Wales, NY) to estimate average growing season daily rainfall. The largest average rainfall event on record for the aforementioned period was used as a large rainfall event (90 mm) and half that amount for a small rainfall (45 mm). Water was applied to soils using a horticultural-style hand pumped sprayer set to apply a heavy mist to the entire soil surface. This method allowed a steady, consistent, and accurate application of water to the mesocosm.

Experimental Design and Soil Mesocosm Construction

The experiment was designed as a factorial experiment which examined the interactive effects of soil warming and simulated rainfall event size on soil microbial community structure and function. Two replicates of each of three experimental

treatments and a control were randomly assigned to the 8 available mesocosms. Upon completion of the experiment, each mesocosm was refilled with new soil following the protocol outlined above. The complete experiment was repeated two additional times. For statistical purposes, subsequent repetition of the experiment was treated as a block in my statistical models of the effect of soil warming and rainfall magnitude.

Soil mesocosms consisted of a 12” PVC collar resting on a seed planting tray, which allowed for drainage. Soil temperature and volumetric soil water content were measured with soil moisture and temperature probes (model EC-5 and 8150-203 respectively, Decagon Devices, Pullman, WA) positioned horizontally, approximately 5 cm deep in the soil. Soil temperature and moisture were recorded at 30-minute intervals for the duration of the experiment. SMR was measured using an automated soil respiration monitoring system (model LI-8100, Licor Environmental, Lincoln NE, USA) configured to take respiration measurements at 30-minute intervals. I quantified SMR with the following metrics: (1) maximum rate of SMR (SMR_{max}); (2) the amount of time each mesocosm took to reach SMR_{max} (T_{max}); (3) the amount of time each mesocosm took to return to pre-rainfall rates of SMR ($T_{duration}$); and the total CO_2 production in each mesocosm associated with rainfall (SMR_{total}; Figure 4). These data were assessed for normality and statistical analyses of the effects of soil warming, rainfall magnitude and their interactions was conducted using a two-way analysis of variance (JMP 7, SAS Institute, Cary, NC USA).

In preparation for respiration measurements, soils were brought to room temperature by placing on bench in the laboratory for 48 hours. Soils were then homogenized a second time and loaded into PVC collars. Preliminary experiments

suggested that loading the soil into mesocosms resulted in artificially high respiration rates. As such, mesocosms were allowed to acclimate for 5 days before beginning the experiment.

Results

Ambient (19.6 °C ± 0.01, n = 1949) temperature treatments were significantly different from increased (24.9 °C ± 0.02, n = 1949) treatments, such that warmed soils were consistently an average of 5°C warmer than ambient soils (Figure 5). Large rainfall treatments (127 mm) resulted in a similarly sharp spike in volumetric water content, followed by a decline, with warmed soils drying more rapidly than ambient (Figure 6). Small rainfall treatments (64mm) did not both reach a similar peak moisture rate as warmed soils lost much water to evaporation almost immediately and prior to saturating to probe depth (Figure 6).

Soil wetting strongly influenced rates of SMR in our experimental mesocosms (Figure 7). Baseline SMR rates were very low in both ambient and elevated temperature treatments, but within minutes of soil wetting, SMR increased rapidly for several hours before reaching SMR_{max}. However, the period of maximum metabolic activity was short-lived as SMR soon began a pattern of decline over a period of several days before returning to baseline. In all temperature and rainfall treatments, SMR_{max} was reached within the first several hours following soil wetting. Contrary to our prediction, temperature (ANOVA, *F = 1.47, df = 5,23, P = 0.24*; Figure 8A) and rainfall (ANOVA, *F = 2.57, df = 5,23, p = 0.13*; Figure 8A) did not influence T_{max}. However, warmed treatments had greater SMR_{max} than ambient temperature controls (ANOVA, *F = 28.20,*

df = 5,23, p < 0.0001; Figure 8B). Similarly, large rainfall treatments had greater SMR_{max} than small rainfall treatments (ANOVA, *F = 8.73, df = 5,23, p = 0.0085*; Figure 8B).

Increasing simulated rainfall event size had a strong, positive influence on $T_{duration}$ (ANOVA, *F = 81.01, df = 5,23, p < 0.0001*; Figure 8C), such that small events (6.4 ± 0.4 days) returned to pre-pulse conditions approximately four days prior to large events (10.6 ± 0.6 days). By doubling the amount of simulated rainfall I observed a 36 percent longer $T_{duration,}$ with increased heat treatments returning to pre-pulse rates an average of a day earlier than ambient treatments (Figure 8C).

SMR_{total} was positively influenced by increased rainfall (ANOVA, *F = 52.05, df = 5,23, p < 0.0001*; Figure 8D) but there was no effect of temperature (ANOVA, *F = 1.84, df = 5,23, p = 0.19*; Figure 8D) nor did the influence of simulated rainfall on SMR_{total} depend on temperature (ANOVA, *F = 0.002, df = 5,23, p = 0.97*; Figure 8D). Large rainfall treatments had greater SMR_{total} than small rainfall treatment (2.46 mol CO^2 ± 0.2, 1.2 mol CO^2 ± 0.07 respectively).

Discussion

The role of soil temperature

In agreement with my prediction, I found that soil warming resulted in a significant increase in SMR_{peak} over ambient temperature controls. According to kinetic theory, SOM decomposition rates should increase with increasing temperatures

(Arrhenius 1889). Microbes degrade SOM using extracellular enzymes through oxidative or hydrolytic processes and enzyme production has been shown to sharply increase with an increase in temperatures (Kirschbaum 2006, Wallenstein et al. 2010). Increased SMR respiration in response to increased temperatures may therefore simply be the result of increased enzymatic activity.

In addition to increased enzymatic activity, increased temperatures could also influence substrate availability for the soil microbes. In the soil, minerals regularly bind to organic matter making it unavailable to the SMC (Tisdall and Oades 1982, Six et al. 2002). Turnover times for compounds bound to soil minerals can be orders of magnitude longer than for bio-available compounds (Sorensen 1972). However, SOM and soil mineral sorption-desorption processes can be described as reversible equilibrium reactions and are therefore subject to Le Chatelier's principle (1985) which states that in endothermic reactions (i.e. desorption) temperature increases should result in a shift to more product being produced. This trend of an increase in desorption rates has been observed in experimental studies (Kalbitz et al. 2000) supporting the idea of increased substrate availability resulting in increased SMC respiration rates.

Whereas kinetic theory supports my observation that increased soil temperature results in a short-term increase in SMR, this trend is not supported in the results of my other longer-term metrics of soil microbial community function. Contrary to my prediction, I did not find a significant impact of temperature on $T_{duration}$ or on SMR_{total}. These metrics are closely linked, as a longer period of respiratory activity following soil wetting would, all other things being equal, result in greater total CO_2 production. These

results suggest that temperature increases may impact SMC respiration rates shortly after warm up, but may lose strength as a driver of SMR over time.

Despite the support for increased substrate availability of more recalcitrant compounds with increased temperatures, SMR_{total} in response to experimental rainfall was not influenced by increased temperature (Figure 8D). This suggests that the significantly greater SMR_{max} observed in response to temperature are perhaps due to increased utilization of labile SOC and not a result of an increase in the availability of recalcitrant SOC. Support for this can be found in studies which have examined the temperature sensitivity of carbon stocks in soils. While there are difficulties associated with differentiating between carbon pools, studies have reported that the labile pool is more sensitive to temperature changes than the recalcitrant pool (Liski et al. 1999, Melillo et al. 2002).

Another reason for inaccurate temperature response predictions may be a result of the nature of studying soil respiration. For example, many field studies are limited in their ability to differentiate between the contributions of autotrophs and heterotrophs to soil respiration. Therefore published results of temperature dependent soil respiration responses may not give an accurate description of the microbial community responses. For example, Boone et al. (1998) found that roots exert a strong influence on the temperature dependence of soil respiration, but the microbial community was less responsive to changes in temperature. Other studies have also credited the strong dependence of soil respiration to temperature changes to root activity (Atkin et al. 2000).

The role of soil moisture

Since first observed by Birch (1958), the rapid respiratory pulse response of soil biota to drying and rewetting has been well documented (Manzoni et al. 2012). Large and rapid metabolic response to episodic moisture availability are observed in semi-arid ecosystems (Potts et al. 2006), Mediterranean (Carbone et al. 2011), and more mesic ecosystems (Daly et al. 2009). Similar to these studies, I also observed a rapid response of soil microbial community function to soil wetting (Figure 7).

Based on the rational and hypotheses presented by Huxman et al. (2004), I predicted small and large rainfall events would cause similar increases in shallow soil moisture and therefore would not have significantly different effects on SMR_{peak}. Contrary to this prediction, I found that rainfall event size did have a significant impact on peak rate, such that large events resulted in significantly higher rates than small events. My results are likely a result of the physical changes in the soil complex associated with drying and rewetting. During dry periods, soil water content declines and the water in soils pores becomes increasingly disconnected resulting in a decrease in diffusion of solutes and a limitation of substrates to the soil microbial community (Schjonning et al. 2003). Upon rewetting diffusion is no longer restricted, and labile carbon sources become displaced as water infiltrates pore spaces, making them available to microbes and facilitating diffusion (Hungate et al 2007). Water has also been shown to break up soil aggregates releasing older labile carbon that would otherwise not be assessable to microbes (Appel 1998). Assuming saturation did not occur, it stands to reason that my large rainfall event provided access to more substrate resulting in increased peak microbial respiration rates.

The accessibility of substrate to the soil microbial community could also explain results for my other measurement metrics. In agreement with my predictions, both $T_{duration}$ and SMC_{pulse} were significantly impacted by rainfall event size, such that large events resulted in both a longer duration and more total carbon evolved. While increased access to labile carbon plays an obvious role in these metrics, physiological factors associated with drying-rewetting cycles in soils can also help improve our understanding.

Osmotic regulation is important for the soil microbial community as they have semi-permeable membranes and are both small, and in close contact with soil water. As soil drying occurs microbial cells must accumulate solutes to avoid dying from dehydration (Harris 1981). Accumulating solutes to produce osmolytes is energetically costly and results in a shift in carbon utilization, resulting in a decline in respiration rates during drying. This relationship could help explain the shortened duration and SMC_{pulse} from small rainfall treatments as the microbial community shifts carbon allocation from growth (resulting in respiration) to water stress adaptation. As osmolytes can be metabolized upon rewetting (Fierer and Schimel 2003), the subsequent release of these osmoregulatory substances can also help explain the pulse respiratory response.

Conclusions

My research suggests that in forest soils the soil microbial communities' functional response is more dependent on moisture availability than temperature. Increased temperature positively influenced peak respiration rate, but I did not see an effect on my other measurement metrics. In contrast, soil moisture strongly influenced

all facets of microbial community function. This could have important implications for global carbon budgets. Increased temperatures are likely to impact global CO_2 emissions, but, shifting rainfall patterns associated with climate change may have greater impact (Weltzin et al. 2003). Further research should include similar studies on different soil types and an improved methodologies for collecting corresponding community structure data.

Tables:

TABLE 1. A description of the group-specific primers used for the qPCR assays[a]

Domain	Target group	Primer sequence (5′-3′)	Primer name	Reference
Bacteria	All groups	ACT CCT ACG GGA GGC AGC AG	Eub338	13
	All groups	ATT ACC GCG GCT GCT GG	Eub518	18
	α-*Proteobacteria*[b]	TCT ACG RAT TTC ACC YCT AC	Alf685	13
	β-*Proteobacteria*[b]	TCA CTG CTA CAC GYG	Bet680	21
	Actinobacteria[c]	CGC GGC CTA TCA GCT TGT TG	Actino235	26
	Firmicutes[d]	GCA GTA GGG AAT CTT CCG	Lgc353	17
	Bacteroidetes[e]	GTA CTG AGA CAC GGA CCA	Cfb319	15
	Acidobacteria	GAT CCT GGC TCA GAA TC	Acid31	2
Eucarya (*Fungi*)	All groups	TCC GTA GGT GAA CCT GCG G	ITS1f	7
	All groups	CGC TGC GTT CTT CAT CG	5.8s	31
	Basidiomycota	CAG GAG ACT TGT ACA CGG TCC AG	ITS4b	7
	Basidiomycota	TCG ATG AAG AAC GCA GCG	5.8sr	31

[a] The bacterial taxonomy follows that described in the latest edition of *Bergey's Manual of Systematic Bacteriology*.
[b] This class is within the phylum *Proteobacteria*.
[c] Commonly labeled the Actinomycete or high-GC-content gram-positive group.
[d] Commonly labeled the low-GC-content gram-positive group.
[e] Commonly labeled the *Cytophaga-Flavobacteria* or *Cytophaga-Flexibacteria-Bacteroides* group.

Table 1. Group specific primers with sequence and target group (from Fierer et al. 2005).

TABLE 2. Primers used for qPCR assays, annealing temperatures, target regions, and the specificity of the amplicons cloned from qPCR assays with soil DNA[a]

Target group	Forward primer	Reverse primer	Approximate amplicon length (bp)	Annealing temp (°C)	% of soil clones belonging to the target group[c]
All *Bacteria*	Eub338	Eub518	200	53	100
α-*Proteobacteria*	Eub338	Alf685	365	60	75
β-*Proteobacteria*	Eub338	Bet680	360	60	96
Actinobacteria	Actino235	Eub518	300	60	60
Firmicutes	Lgc353	Eub518	180	60	100
Bacteroidetes	Cfb319	Eub518	220	65	100
Acidobacteria	Acid31	Eub518	500	50	100
All *Fungi*	5.8s	ITS1f	300[b]	53	100
Basidiomycota	ITS4b	5.8sr	500[b]	55	100

[a] The bacterial qPCR assays target 16S rRNA genes, while the fungal assays target the internal transcribed spacer region found in rRNA genes.
[b] The length of the targeted ITS region can vary significantly between different fungal strains.
[c] The percentage includes only those clones that were nonchimeric and exceeded the 80% confidence threshold for taxonomic assignment using the RDP classifier program.

Table 2. Forward and reverse primers, amplicon length, and target annealing temperatures (from Fierer et al. 2005).

Figures:

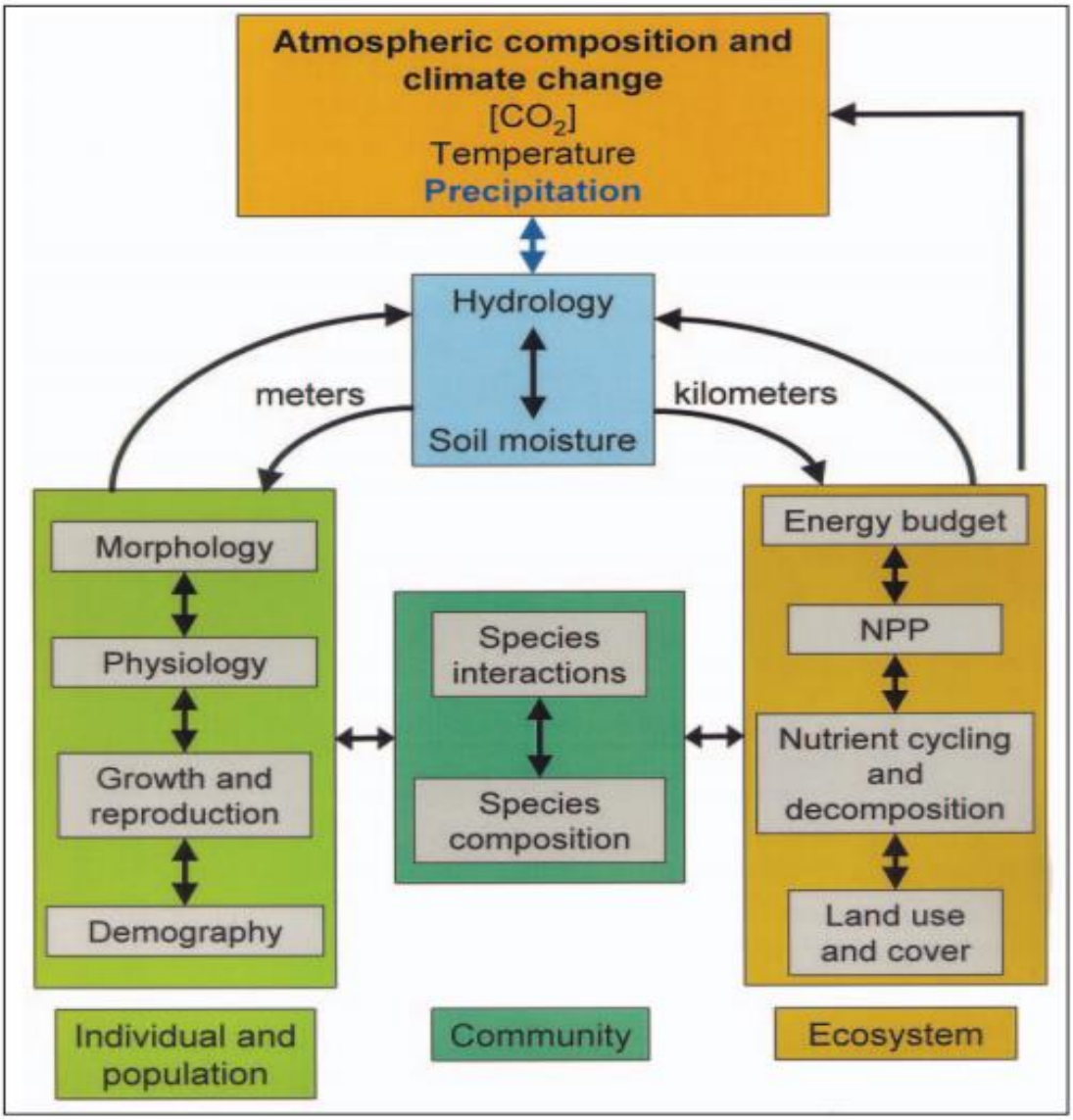

Figure 1. Conceptual model illustrating soil moisture's mediating influence on biological responses to anthropogenic climate change (Weltzin et al 2003).

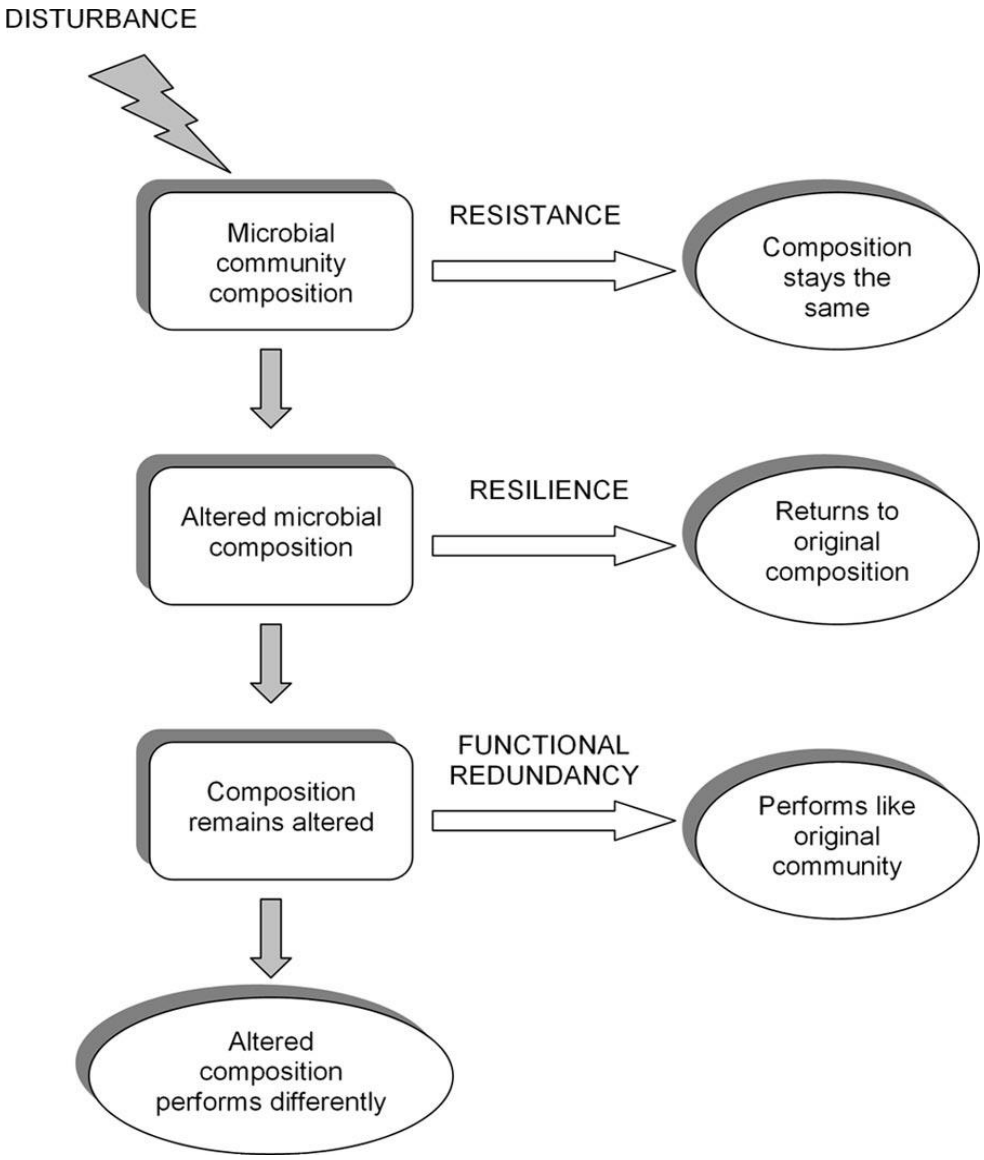

Figure 2. A schematic of how a rainfall event (labeled here as “disturbance”) could impact soil microbial composition, supporting multiple competing hypotheses proposed for changes in community structure (Allison and Martiney 2008).

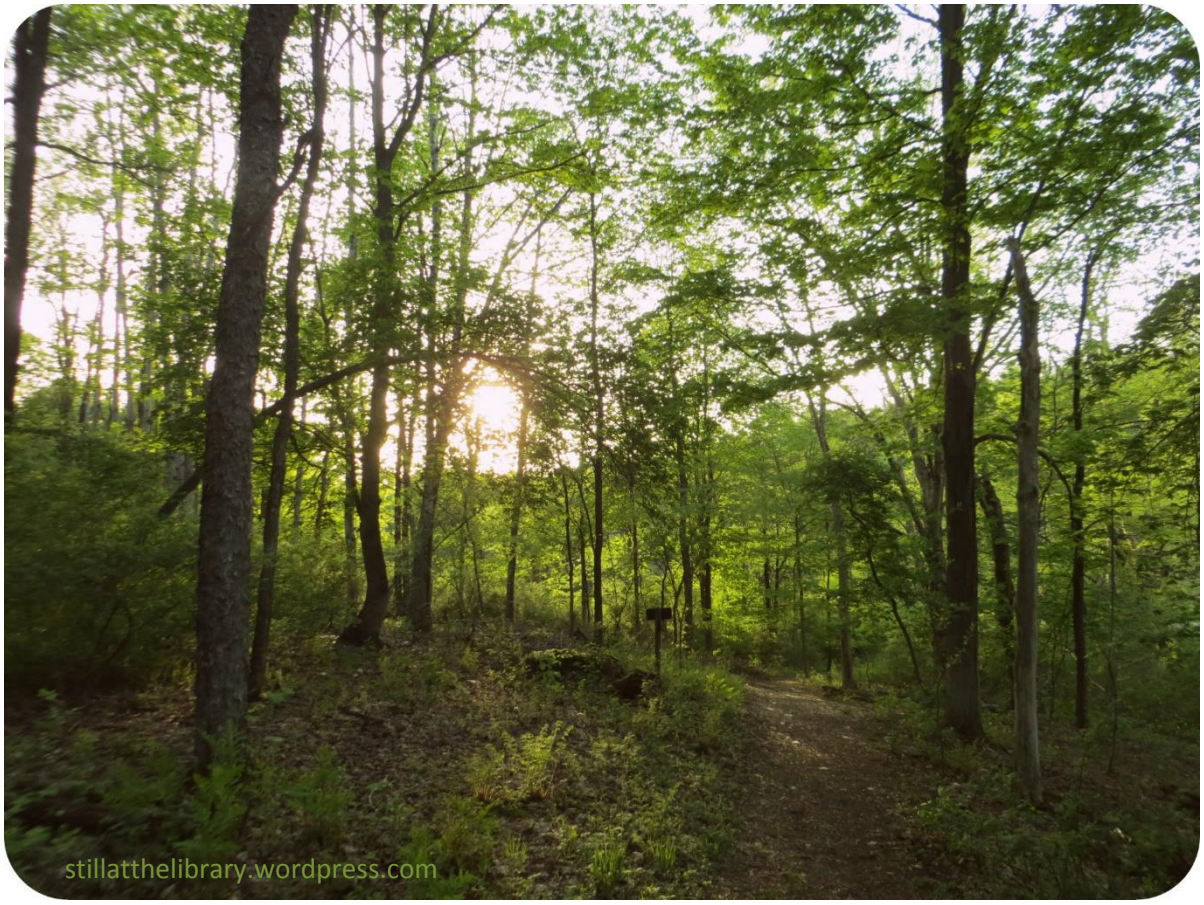

Figure 3. Soils were collected from a second growth mixed hardwood forest near Java, NY during July, 2014.

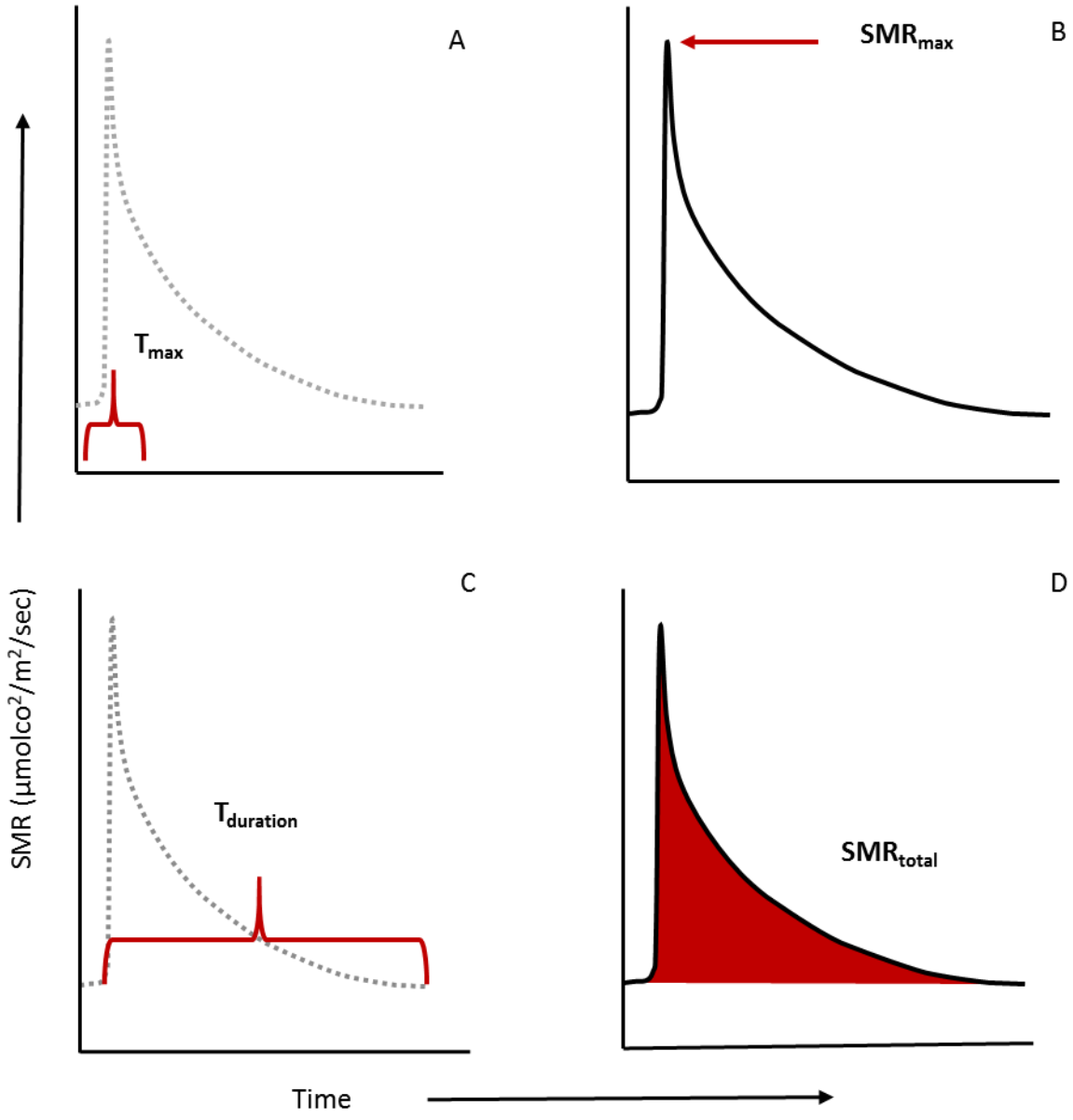

Figure 4. Visualization of metrics used to quantify soil microbial functional responses to experimental warming and simulated rainfall. A) T_{max} was the time it took to reach peak respiration rate. B) SMR_{max} was measured as the highest single recorded respiration rate post rainfall addition. C) $T_{duration}$ was the time it took for 24-hour average respiration rates to return to pre pulse 24-hour average respiration. D) SMR_{total} was calculated by integrating SMR with respect to time.

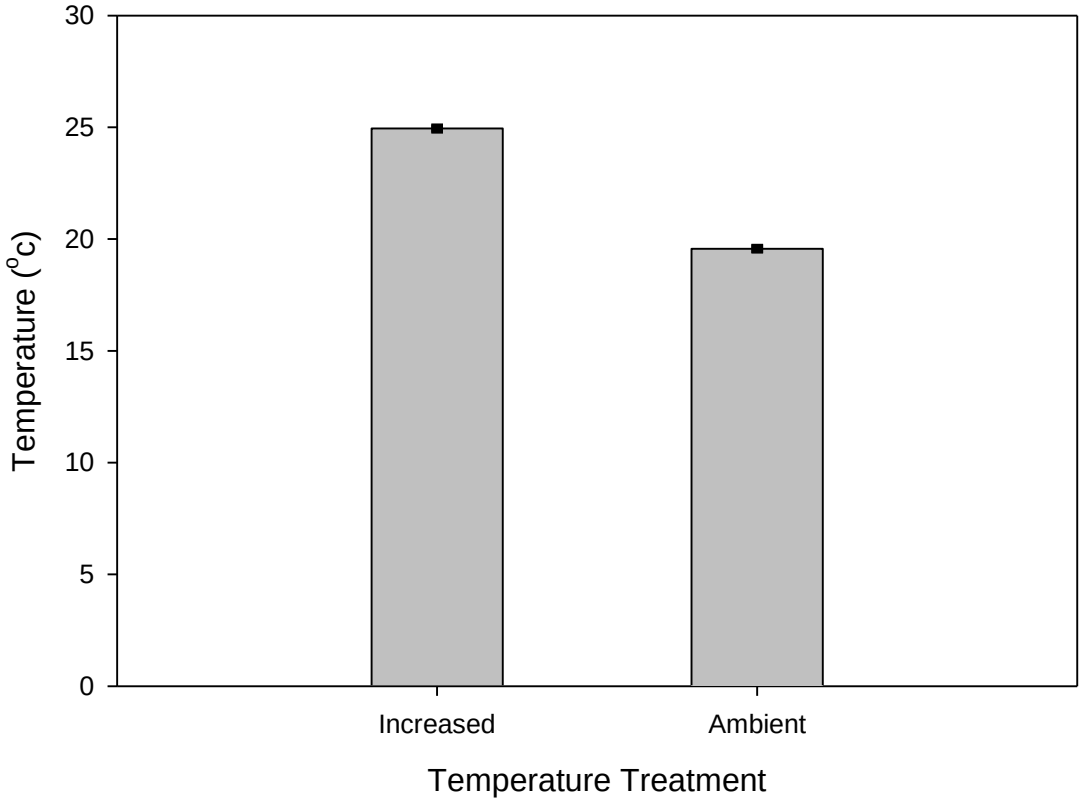

Figure 5. Difference in mean ± SE of increased vs. ambient temperature treatments in laboratory incubation of forest soils. Ambient treatment was maintained at room temperature of the laboratory and increased treatment (+5°C) was accomplished by placing soil mesocosms on seed heating mats. Measurements were taken twice hourly and maintained for the duration of the experiment.

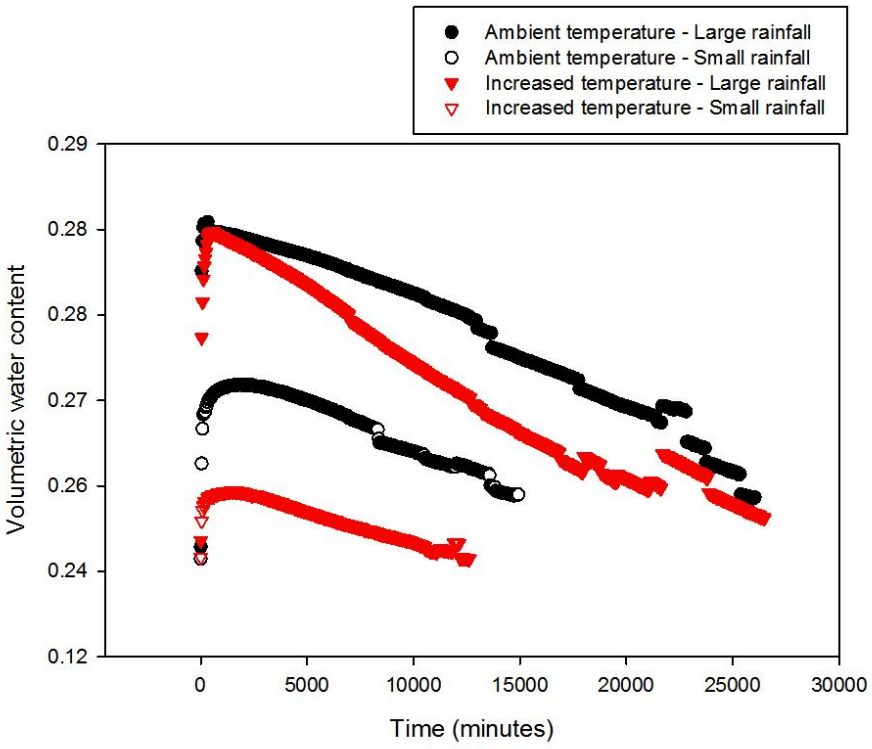

Figure 6. Time course of volumetric water content (VWC) for duration of simulated rainfall pulse response in forest soils. Black lines indicate warmed (24.9 °C ± 0.02) and red lines ambient (19.6 °C ± 0.01) temperature treatments. A large rainfall event (solid) was 785 ml of water, equivalent to the largest rainfall event on record in the area for a 30 year period, and a small event (outline) was 392.5 ml, half of the large event. Measurements were taken every half hour and logged for duration of experiment.

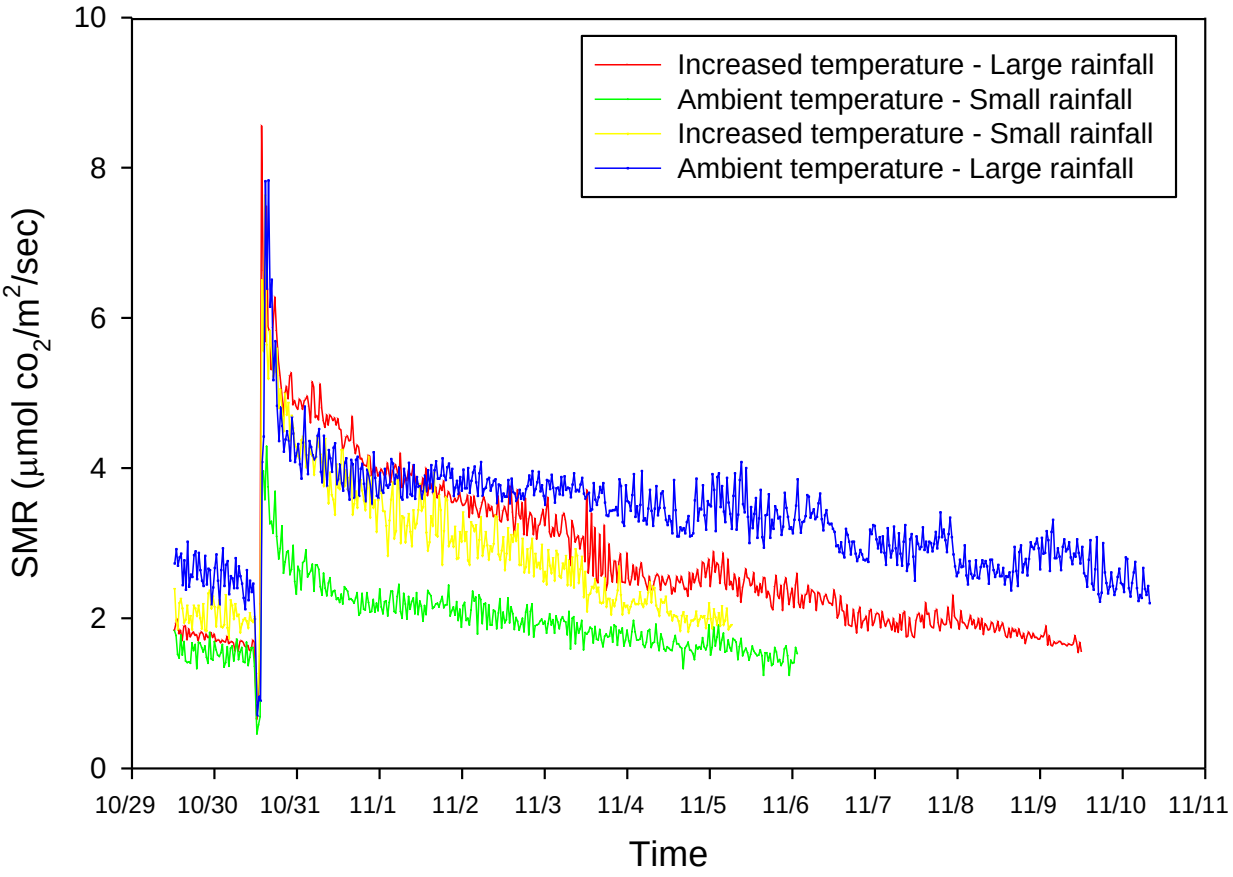

Figure 7. Time course of mean soil microbial respiration (SMR) in mixed hardwood forest soils in laboratory based incubations. Respiratory pulse is a result of small (392.5 ml) or large (785 ml) simulated rainfall event. Temperature treatments included ambient (19.6 °C ± 0.01) and warmed (24.9 °C ± 0.02). Ambient temperature was maintained at room temperature of laboratory and increased temperature was + 5°C. Measurements were taken twice hourly for duration of experiment. Individual lines terminate once 24 hour average respiration rate returns to pre pulse 24 hour avg. rate.

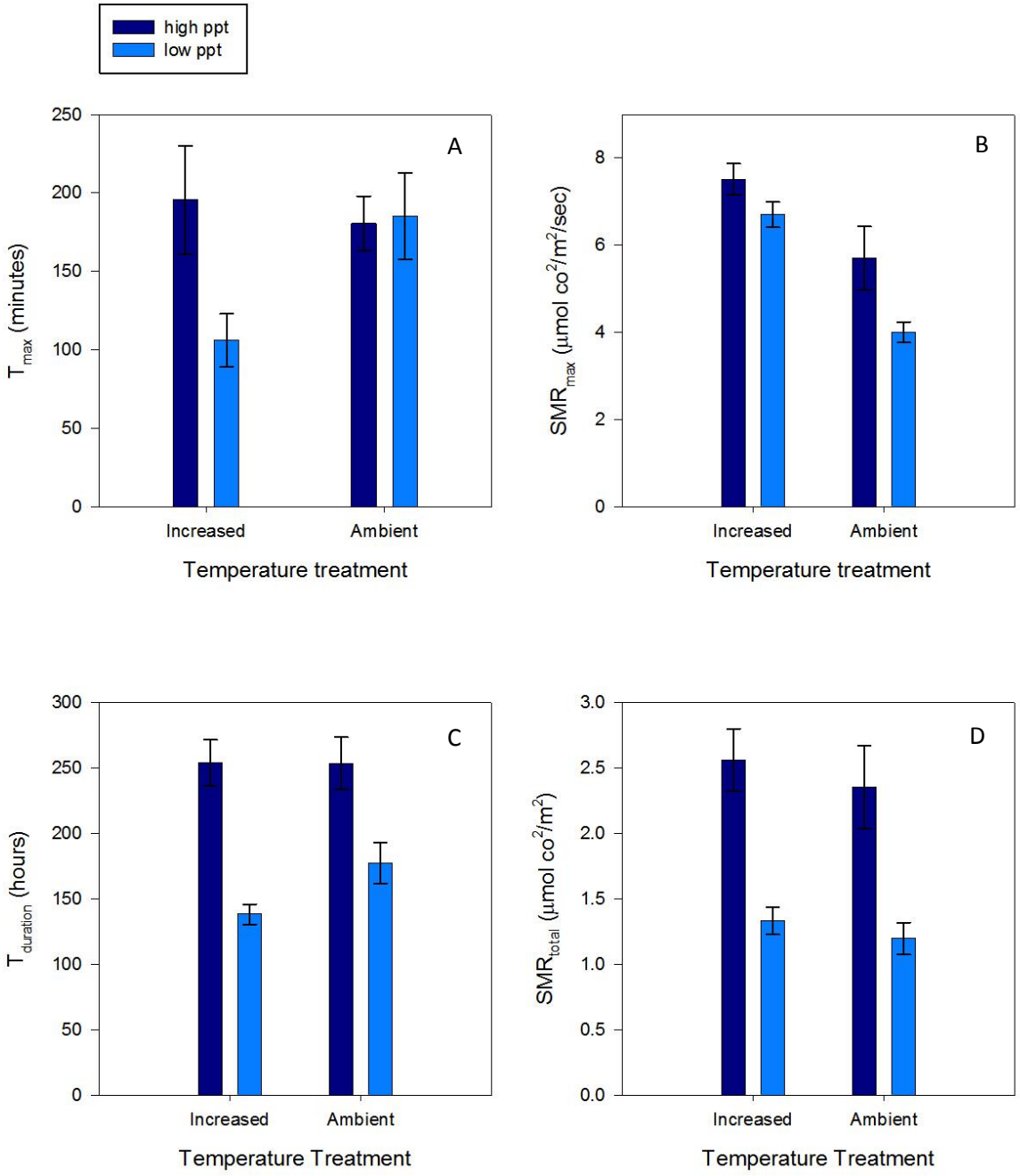

Figure 8. Relationship between heat and rainfall treatments and A. T_{max} (amount of time to reach SMR_{max}), B. SMR_{max} (maximum rate of soil microbial respiration), C. $T_{duration}$ (the amount of time each mesocosm took to return to pre-rainfall rates of SMR), D. SMR_{total} (total CO_2 production in each mesocosm) in mixed hardwood forest soil laboratory incubations.

Works Cited:

Allison, S. D. 2006. Brown ground: a soil carbon analog for the Green World Hypothesis? American Naturalist 167:619-627.

Allison, S.D., and K.K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14:2898-2909.

Allison, S.D. and J.B.H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the national academy of sciences 105:11512-11519.

Arrhenius, S.A. 1889. "Über die Dissociationswärme und den Einflusß der Temperatur auf den Dissociationsgrad der Elektrolyte". Z. Physik. Chem. 4: 96–116.

Atkin, O.K., E.J. Edwards, B.R. Loveys. 2000. Response of root respiration to changes in temperature and its relvance to global warming. New Phytologist 147: 141- 154.

Barron-Gafford, G.A., J.M. Cable, LP. Bently, R.L Scott, T.E Huxman, D.G. Jenerette, K. Ogle. 2014. Quantifying the timescales over which exogenous and endogenous conditions affect soil respiration. New Phytologist 202: 442-454.

Birch, H.F. 1958. The effect of soil drying on hummus decomposition and nitrogen, Plant and Soil 10:9-30.

Boone R.D., K.J. Nadelhoffer, J.D. Canary, J.P. Kaye. 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396: 570-572.

Borken, W., K. Savage, E.A. Davidson, S.E. Trumbore. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology 12:177-193.

Bradford, M.A, B.W. Watts, C.A. Davies. 2010. Thermal adaptation of heterotrophic soil respiration in laboratory mesocosms. Global change biology 16: 1576-1588.

Bradford, M.A., N. Fierer. 2012. The biogeography of microbial communities and ecosystem processes: Implications for soil and ecosystem models. Soil Ecology and Ecosystem Services. ed. D.H. Wall, Oxford University Press, Oxford, U.K. pgs. 189-200

Breshears, D., J.W. Nyhan, E.H. Christopher, P.W. Bradford. 1998. Effects of woody plants on microclimate in a semiarid woodland: soil temperature and evaporation in canopy and intercanopy patches. International Journal of Plant sciences 159: 1010-1017.

Carroll, G.C., D.T. Wicklow. 1992. The fungal community: Its organization and role in the ecosystem, 2nd edn. Marcel Dekker, New York

Coe, K.K, J. Belnap, J.P. Sparks. 2012. Precipitation-driven carbon balance controls survivorship of desert bio-crust mosses. Ecology 20: 1626-1636

Coleman, D.C., D.A. Crossley, and P.F. Hendrix. 2004. Fundamentals of soil ecology. 2nd edition. Elsevier.

Conrad, R. 1996, Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, NO). Microbial Review 60:609

Craine, J.W., Wedin, D.A., Chapin, F.S., 1999. Predominance of ecophysiological controls on soil CO2 flux in a Minnesota grassland. Plant Soil 207, 77–86.

Davidson, E.A., E. Belk, R.D. Boone. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global change biology 4:217-227.

Davidson E.A., I.A. Janssens, 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165-173.

De Boer, W., L.B. Folman, R.C. Summerbell, L. Boddy. 2005. Living in a fugal world: impact of fungi on soil bacterial niche development. FEMS Microbiological review 29: 795-811.

Degens, B.P., L.A. Schipper, G.P. Sparling, L.C. Duncan. 2001. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biology and Biochemistry 33:1143-1153

Delarue, F., A. Buttler, L. Bragazza, L. Grasset, V.E.J. Jassey, S. Gogo, F. Laggoun-Defarge. 2014. Experimental warming differentially affects microbial structure and activity in two contrasted moisture sites in a Sphagnum-dominated peatland. Science of the Total Environment 511: 576-583.

Fay, P.A., D.M. Kaufman, J.B. Nippert, J.D. Carlisle, C.W. Harper. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. Global Change Biology 14:1600-1608.

Fay, P.A., J.M. Blair, M.D., Smith, J.B. Nippert, J.D. Carlisle, and A.K. Knapp. 2011. Relative effects of precipitation variability and warming on tallgrass prairie ecosystem function. Biogeosciences 8:3053-3068.

Fierer, N., and J.P. Schimel. 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry, 34: 777–787.

Fierer, N., J.P. Schimel, P.A. Holden. 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology 1:63-71.

Fierer, N., J.A. Jackson, R. Vilgalys, R. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71: 4117-4120.

Frostegard, A., E. Baath. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Ganguly, A.R., K. Steinhaeuser, D.L. Erickson. 2009. Higher trends but larger uncertainty and geographic variability in 21st century temperature and heat waves Proceedings of the National Academy of Sciences of the United States of America 106: 15555-15559.

Guggenberger, G., S.D. Frey, J. Six, K. Paustian, E.T. Elliot. 1999. Bacterial and fungal cell-wall resides in conventional and no-tillage agroecosystems. Soil Science of America Journal 63: 1188-1198.

Guntiñas, M.E., F. Gil-Sotres, M. C. Leirós, C. Trasar-Cepeda 2013. Sensitivity of soil respiration to moisture and temperature. Journal of Soil Science and Plant Nutrition 13:445-461

Hanson, P.J., N.T. Edwards, C.T. Garten, J.A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48: 115-146.

Heimann, M., M. Reichstein. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451: 289-292.

Hogberg, P., D.J. Read. 2006 Towards a more plant physiological perspective on soil ecology. Trends in Ecology and Evolution 21:548-554.

Holland, E.A., D.C. Coleman. 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosytem. Ecology 68:425-433

Houghton, R. A. 2003. Revised estimates of the annual net flux of carbon to the atmosphere from changes in land use and land management. Tellus 55: 378-390.

Huntington T.G. 2006. Evidence for intensification of the global water cycle: review and synthesis. Journal of Hydrology 319: 83–95.

Huxman, T.E., K. Snyder, D. Tissue, A.J. Leffler, W. Pockman, K. Ogle, D. Sandquist, D.L. Potts, S. Schwinning. 2004. Precipitation pulses and carbon balance in semi-arid and arid eco-systems. Oecologia 141:254-268

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate

Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, U., Morgenstern, K., Clement, R., Oltchev, S., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.-D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulmans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology 7: 269-278

Jenerette, G.D., A. Chatterrjee. 2012. Soil metabolic pulses: water, substrate, and biological regulation. Ecology 93: 959-966

Kalbitz, K., S. Solinger, J.H. Park, B. Michalzik, E. Matzner. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Science 165:277-304.

Kirschbaum, M.U.F. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol. Biochem. 27, 753–760.

Kirschbaum, M.U.F. 2006. The temperature dependence of organic-matter decomposition – still a topic of debate. Soil Biology and Biochemistry 38: 2510-2518.

Lavigne, M.B., R.J. Foster, G. Goodine. 2004. Seasonal and annual changes in soil respiration in relation to soil temperature, water potential and trenching. Tree Physiol 24: 415–424.

Lee, X., H. Wu, J. Sigler, C. Oishi, T. Siccama. 2004. Rapid and transient response of soil respiration to rain. Global Change Biology 10: 1017-1026.

Linderholm, H.W. 2006. Growing season changes in the last century. Agricultural and forest meteorology 137:1-14.

Liski, J., H. Ilvesniemi, A. Makela, C.J. Westman. 1999. CO2 emissions from soil in response to climatic warming are overestimated- the decomposition of old soil organic matter is tolerant of temperature. Ambio 28, 171–174.

Liu, W., Z. Zhang, S. Wan. 2008. Predominate role of water in regulating soil and microbial respiration and their response to climate change in a semi-arid grassland. Global Change Biology 15:184-195.

Loik, M.E., D.D. Breshears, W.K. Lauenroth, J. Belnap. 2004. A multi-scale perspective of water pulses in dry land ecosystems: climatology and Ecohydrology of the western USA. Oecologia 141: 269-281.

McLauchlan, K.K., S.E. Hobbie. 2004. Comparison of labile soil organic matter fractionation techniques. Soil Science Society of America Journal 68: 1616–1625

Meehl, G.A., C. Tebaldi. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305: 994-997.

Melillo, J. M., P.A. Steudler, J.D. Aber, K. Newkirk, H. Lux, F.P. Bowles, C. Catricala, A. Magill, T. Ahrens, S. Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298: 2173-2175.

Moyano, F.E., S. Manzoni C. Chenu. 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biology and Biochemistry 59: 72-85

Odum EP (1985) Trends expected in stressed ecosystems. Bioscience 35:419-422

Nelson, D.W., L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 2, 2nd ed., A.L. Page et al., Ed. Agronomy.9:961-1010. Am. Soc. of Agron., Inc. Madison, WI.

Opel, et al. 2010. A Study of PCR Inhibition Mechanisms Using Real Time PCR. J. Forensic Sci. 55: 1-9.

Or, D., B.F. Smets, J.M. Wraith, A. Dechesne, S.P. Friedman. 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous mediae a review. Advances in Water Resources 30: 1505-1527.

Pan, Y., R.A. Birdsey, J.Y. Fang, R. Houghton, P.E. Kauppi, W.A. Kurz, O.L. Phillips, A. Shvidenko, S.L. Lewis, J.G. Canadell, P. Ciais, R.B. Jackson, S.W. Pacala, A.D. McGuire, S.L. Piao, A. Rautiainen, S. Sitch, D. Hayes. 2011. A large and persistent carbon sink in the world's forests. Science 333: 988–993.

Pastor, J., W.M. Post. 1986. Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. Biogeochemistry 2: 3–27.

Paul, E.A., S.J. Morris S. Bohm. 2001. The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers. Assessment methods of soil carbon 19: 193–205.

Pietikainen, J., M. Pettersson, E. Baath. 2005. Comparison of temperature effects on soil respiration and bacterial; and fungal growth rates. FEMS Microbial Ecology 52:49-58.

Placella, S.A., E.L. Brodie, M.K. Firestone. 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. PNAS 109: 10931-10936.

Potts, D.L., G.A. Barron-Gafford, D.G. Jenerette. 2014. Metabolic acceleration quantifies biological systems' ability to up-regulate metabolism in response to episodic resource availability. Journal of Arid Environments 104:9-16.

Potts, D.L., T.E. Huxman, B.J. Enquist, J.F. Weltzin, D.G. Williams. 2006. Resilience and resistance of ecosystem functional response to a precipitation pulse in a semi-arid grassland. Journal of Ecology 94: 23-30.

Potts, D.L., T.E. Huxman, J.M. Cable, N.B. Englishm D.D. Ignace, J.A. Eilts, M.J. Mason, J.F. Waltzin, D.G. Williams. 2006. Antecedent moisture and seasonal precipitation influence the response of canopy-scale carbon and water exchange to rainfall pulses in a semi-arid grassland. New Phytologist 170: 849-860.

Raeymaekers, L. 2000. Basic principles of quantitative PCR. Molecular Biotechnology 15:115–122.

Raich, J.W., and W.H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44: 81-99.

Rustad LE, J.L. Campbell, G.M. Marion. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental warming. Oecologia 126: 543–562.

Ryan, M.G., B.E. Law. 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73: 3-27.

Schjonning, P., I. Thomsen, P. Moldrup, B. Christensen. 2003. Linking soil microbial activity to water- and air-phase contents and diffusivities. Soil Science Society of America Journal 67:156-165.

Schimel, J.P., J.M. Gulledge, J.S. Clein-Curley, J.E. Lindstrom, J.F. Braddock. 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology and Biochemistry 31: 831-838.

Schimel, J., T.C. Balser, M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Schimel, D. S., B.H. Braswell, E.A. Holland, R. McKeown, D.S. Ojima, T.H. Painter, W.I. Parton, A.R. Townsend. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. Glob. Biogeochem. Cycles 8: 279–294.

Seneviratne, S. I., D. Luthi, M. Litschi, and C. Schar. 2006. Land-atmosphere coupling and climate change in Europe. Nature 443:205–209

Six, J., C. Feller, K. Denef, S.M. Ogle, J.D.C. Sa, A. Albrecht. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils - Effects of no-tillage. Agronomie 22: 755-775.

Six, J., S.D. Frey, R.K. Thiet, K.M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70: 555–569.

Sorensen, L.H. 1972. Stabilization of newly formed amino acid metabolites in soil by clay minerals. Soil Science 114: 5-11.

Tisdall, J.M., J.M. Oades. 1982. Organic matter and water stable aggregates in soils. Journal of Soil Science 2:141-163.

Trumbore, S. 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecology Applications 10:399-411.

Trumbore, S.E., G. Bonani, W. Wolfli. 1990. The rates of carbon cycling in several soils from AMS 14C measurements of fractionated soil organic matter. In: Bouwman, A.F. (Ed.), Soils and the Greenhouse Effect. John Wiley, New York, NY, pp. 405–414

Vu Tien, Phuong-Chi. "A Vegetational Analysis of Selected Sites at Beaver Meadow Wildlife Refuge, Wyoming Count, New York." Buffalo State College (1977): 1-82. Print.

Wallenstein, M.D., A.M. Hess, M.R. Lewis, H. Steltzerae, E. Ayres. 2010. Decomposition of aspen leaf litter results in unique metabolomes when decomposed under different tree species. Soil Biology and Biochemistry 42:484-490.

Wardle, D.A. 1998. Controls of temporal variability of the soil microbial biomass. Soil Biology and Biochemistry 30: 1627-1637.

Weltzin, J.F., Loik, M.E., Schwinning, S., Williams, D.G., Fay, P.A., Haddad, B.M., Harte, J., Huxman, T.E., Knapp, A.K., Lin, G.H., Pockman, W.T., Shaw, M.R., Small, E.ESmith, M.D., Smith, S.D., Tissue, D.T., Zak, J.C. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. Bioscience 53:941-952

Yang, L. H., K. F. Edwards, J. E. Byrnes, J. L. Bastow, A. N. Wright, and K. O. Spence. 2010. A meta-analysis of resource pulse–consumer interactions. Ecological Monographs 80:125–151.

Yeming, Y., W. Juan, H. Xueman, T. Zuoxin, L. Shirong, J.S. Osbert. 2014. Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. Ecology and Evolution 4: 633–647.

Yuste, J.C., Janssens, I.A., Carrara, A., Meiresonne, L., Ceulemans, R. 2003. Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest. Tree Physiology 23: 1263-1270.

Appendix A.

Community Structure

Predictions:

The potential responses of soil community structure to the treatments can be described in one of four (equally likely) ways, so I present multiple alternative hypotheses (Allison and Martiney 2008; Figure 2):

1. The community will be resistant to changes in temperature and precipitation regime, the treatments will have no significant effect on community structure. Resulting in the same fungal to bacterial ratio before, during, and after the experiment.
2. The community will be resilient to changes in temperature and precipitation regime, with a change in bacteria to fungal ratio for the duration of the disturbance, but returning to pre-treatment conditions after drying occurs.
3. The community will be functionally redundant such that, the community composition will be altered with the new community persisting post-disturbance, but will perform functionally, as indicated by respiration rates, the same as the pre-disturbance community.
4. The community will be altered and will exhibit a different functional response to the treatments.

Methods:

To quantify how temperature and rainfall interact to dynamically influence community composition, I periodically sampled soils from each mesocosm before, during and after the experiment. To sample soil, I collected soils at four locations within the mesocosm to the entire depth. This sample was homogenized and 1 gram of the homogenized sample was stored in 1-ml Eppendorf tube, and kept at -80°C.

To quantify soil microbial community structure, I extracted soil microbial DNA from the frozen samples using a commercially available kit (Axygen AxyPrep MAG Soil, Stool and Water DNA Kit, Thermo Fisher Scientific, Waltham, MA USA). Extracted DNA was analyzed using qPCR assays (BIO-RAD CFX96 Touch™ Real-Time PCR Detection System, BIO-RAD, Hercules, California, USA). Forward primers each 25 µl reaction contained the following 12.5 µl SYBR Green Real-Time PCR Master Mix (Life Technologies, Thermo Fisher Scientific, Waltham, MA USA), 5 µl purified water, 1.25 µl (1 µM) of forward and reverse primers, and 5 µl template DNA (1-3 ng/µl). PCR conditions were 15 minutes 95°, followed by 40 cycles at 94 ° for 30 seconds, 30 seconds at 53 ° annealing temperature, and 72° for 30 seconds. Primers (Table 1) and annealing temperatures (Table 2) were adapted from Fierer et al. (2005) and modified for optimization of equipment. Primers were chosen that target all bacterial groups (forward - EUB338, reverse – EUB518), and all fungal group (forward – 5.8s, reverse ITS1F).

Results:

Despite my best efforts, I was unable to describe microbial community structure using DNA extraction and analyses. After numerous attempts to adjust PCR conditions, including using a gradient to help determine appropriate annealing temperatures and adjusting reaction quantities, I spiked the soil DNA with *E.coli* DNA. Using the all bacteria primers, I was able to determine that metal ions in the soil water solution may have inhibited PCR (Opel et al. 2010). An ethanol precipitation was employed to attempt to further purify DNA, but this was also unsuccessful. We established that a DNA purification kit may have accomplished purification but due to time and financial constraints we were unable to attempt this option.

Discussion:

The role of soil temperature

I was ultimately unsuccessful in my efforts to examine the resistance, resilience, or redundancy of the soil microbial community (Allison and Martiney 2008) by quantifying changes in soil microbial community structure in response to simulated rainfall and increased temperature using qPCR. To the best of my knowledge, this was a unique experimental design and therefore I was unable to find studies that supported or refuted my specific hypotheses. However, I can speculate as to how rainfall event magnitude and soil warming may have influenced dynamic shifts in the soil microbial community.

Direct effects of increased temperatures could have promoted increases in the bacterial community, who have a rapid turnover associated with an accelerated generation time, and whose numbers have been shown to increase in warmer temperatures compared to slower growth rate of fungi (Pietikainen et al. 2005). This idea is supported by a recent study which found that warming treatments in forest soils were positively correlated with an increase in bacteria and negatively correlated with fungi (Wei et al. 2014). However, Schindlebacher et al. (2011) found that warming over time had no impact on microbial community structure in forest soils. Temperature could also have indirectly influenced community structure by accelerating soil drying time and limiting the duration of water availability (Placella et al. 2012).

The role of soil moisture

The absence of studies addressing my hypothesis and lack of results from qPCR data do not allow me to address my research objectives directly however, I can speculate on potential soil microbial community structure changes. Physiological stress associated with soil drying could result in a reduction of microbial diversity by favoring the groups that are best adapted to dealing with water stress (Schimel et al. 1999). This could potentially favor fungal communities, which are more tolerant to drought, as a result of their ability to access and transfer moisture from micropores with their hyphae (de Boer et al. 2005). This idea is supported by the findings of Bell et al. (2009) who reported an increase in the fungal component of the microbial community in response to drying in grassland soils with no change in overall bacterial community size, despite shifts in functional groups.