Soil Moisture Sensitivity of Microbial Processing of Soil Organic Carbon

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ABSTRACT

Soil organic carbon (SOC) is the largest terrestrial C pool and understanding SOC decomposition in response to environmental factors is critical for accurate predictions of climatic change. Soil moisture is one of the most important, yet less explored, environmental factors controlling soil microbial respiration. The relationship between soil moisture and respiration also varies with soil texture. Currently, it is difficult to predict feedbacks to climatic changes from changes in soil moisture, as most earth system models lack site-specific, experimentally-derived parameters to represent soil moisture-texture-respiration relationships. The overarching goals of this dissertation are to gain a fundamental understanding of the interactive effect of soil moisture and texture on microbial processing of SOC, and to elucidate microbial response to global changes. To accomplish this, two laboratory experiments and one field study were conducted. Laboratory experiments included (i) 90-day incubation with steady-state moisture, and (ii) 140-day incubation with alternating wetting and drying (transient) and steady state moisture using three soils of different textures (sandy, loamy, and clayey). A field experiment was conducted to understand the effect of moisture to SOC dynamics under *in-situ* conditions and involved drought, rainfed, and irrigated moisture treatments. The 90-day experiment revealed that texture was the major determinant of SOC cycling despite observing different moisture optima for the highest respiration for different textures. The 140-day incubation aimed to decipher mechanism(s) fueling the Birch effect (a spike in respiration rate upon rewetting of dry soils). I found that different mechanisms caused the Birch effect in different soils. In sandy soil, metabolite accumulation and changes in bacterial community, while in loamy and clayey soils, metabolite accumulation and release of aggregate protected C contributed to the Birch effect. Results imply that response of microbial respiration to changing climate will strongly depend on

soil moisture sensitivity to textural differences. Field experiment results showed strong influence of moisture on SOC decomposition, i.e., lower CO_2 emissions from drought plots, however, higher extractable organic C, microbial biomass C and fungal hyphal abundance were observed under drought conditions. The laboratory and field experiment provide critical information for improving the moisture sensitivity of microbial parameters in ecosystem models.

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Chapter 1. General Introduction

Climate change and terrestrial carbon cycle

Rapid increases in the atmospheric CO_2 concentration due to anthropogenic perturbations have focused attention on Earth's carbon (C) stocks and cycling (Scharlemann et al., 2014). Global C cycling is a complex biogeochemical process in which elemental C is cycled in different forms across various pools including oceanic pool, pedologic or soil pool, atmospheric pool, biotic and geologic pool (Schlesinger and Andrews, 2000; Lal, 2008). Terrestrial ecosystems are a huge reserve of C in the form of plants, animals, soils, and microorganisms. The total amount of C present in the terrestrial ecosystems, including living biomass and soils, is threefold greater than the C present in the atmosphere (Falkowski et al., 2000). So, terrestrial ecosystems play a vital role in the global C cycle and it is closely linked with changing climate (Reichstein et al., 2013). The C fluxes in terrestrial ecosystems are mainly controlled by the processes of photosynthesis and soil respiration (autotrophic and heterotrophic). The difference between photosynthesis and respiration is defined as net primary production (Cao and Woodward, 1998). Though many factors influence these processes, elevated atmospheric CO_2 concentration and changing climatic factors such as temperature and precipitation are the most important as they directly or indirectly impact C fluxes (Amthor, 1995). There is still an ongoing debate about the biotic and abiotic factors driving the net C storage in terrestrial ecosystems.

Soils in terrestrial ecosystems contain a large and dynamic pool of C (2300 Pg C) which is a critical regulator of the global C budget (Johnston et al., 2004). Since soils contain 4.5 times more C than vegetation, even small changes in the processes controlling soil C cycling have the potential to enhance soil respiration and release large quantities of CO_2 to the atmosphere (Lal, 2004). In addition, climate change causes a positive feedback to soil respiration. Consequently, soil respiration accounts for the second largest flux of CO_2 between soils and atmosphere (Ryan

and Law, 2005). The global soil respiration flux is estimated to be 98 ± 12 Pg CO_2 annually (Bond-Lamberty and Thompson, 2010), which is higher than anthropogenic CO_2 emissions by an order of magnitude (Boden et al., 2010). Therefore, small perturbations in the global soil respiration flux has the potential to strongly affect the patterns of both C cycling and climate. Despite numerous past and ongoing research, there still remains a substantial amount of ambiguity of the size and stability of the soil organic C (SOC) stock (Bond-Lamberty and Thompson, 2010; Trumbore, 2006). Therefore, a better understanding of the C flows from and within soils is essential for parameterizing global C cycle models to accurately predict the climate change (Scharlemann et al., 2014). Previous studies have concluded that soil respiration is often positively correlated with temperature, thus a warmer climate will mediate progressively greater rates of terrestrial C loss in future (Bardgett et al., 2008). Major uncertainties remain, however, as to how soil moisture changes may affect soil respiration as it has received lesser attention as compared to temperature.

Major factors controlling soil microbial respiration

Microbial respiration is strongly affected by the environmental factors such as soil temperature, availability and intensity of light, and soil moisture (Kutsch et al., 2010; Moyano et al., 2013). Soil temperature directly affects respiration as it controls decomposition of organic matter by controlling the microbial activity in soil (Schuur et al., 2008). Next to temperature, soil moisture is the most important factor influencing soil respiration. Other factors such as substrate quality (Jagadamma et al., 2014) and soil C:N ratio (Silver and Miya, 2001) also play a significant role in microbial decomposition of substrates. Several studies have reported the impacts of temperature on soil respiration (Frank et al., 2015; Karhu et al., 2014; Reichstein et

al., 2013) while comparatively less has been reported about the moisture sensitivity on soil respiration.

Soil moisture

Soil moisture is essential for microbial activity (Paul, 2014), affecting the heterotrophic respiration in the soil. Reichstein et al. (2013) reported that climate extremes such as drought or heavy precipitation can affect the C cycle and lead to lagged responses. Microbial respiration forms a bell-shaped response curve based on the soil moisture status, i.e., it starts to increase with increases in soil moisture and then gradually decreases with further increases in soil moisture (Oberhauer et al., 1992). Under low moisture conditions, diffusion of substrates is hindered, and when the soil is saturated oxygen diffusion is limited (Barros et al., 1995; Prado and Airoldi, 1999). It is important to understand how microbial respiration will be affected with the changing patterns of precipitation and consequent retention of moisture in the soil. This will also be strongly affected by the intrinsic moisture retention capacity of soils.

In recent years, various earth system models have predicted less frequent rain events (Bell et al., 2009), which would lead to more frequent droughts. It is important to understand how low moisture conditions (drought) can affect microbes both in terms of immediate effects and survival strategies, as well as in a long-term view regarding microbial community structure. Borken et al. (2006) conducted a study to investigate the impacts of prolonged drought on soil respiration and results reported that prolonged droughts decrease the CO_2 efflux from soils which could cause increase in SOC storage in soils, however, they did not report if the stored C was transient or long lasting. Manzoni et al. (2012), in a meta-analysis study, reported that heterotrophic respiration decreases with decrease in soil moisture as the microbes can’t withstand water stress and this is due to substrate limitation for the microbes.

Under dry conditions, the water potential outside the cell lowers which leads to the formation of a water gradient that causes water from inside the cell to exudate out, causing the cell to dry and lose turgor. Solute concentration plays a significant role in the water gradient because microbes can increase their internal solute concentration to create osmotic water flow into the cell, reducing water gradient and retaining cellular water for other physiological activities (Boot et al., 2013). Thus, one of the methods to survive drought conditions is osmolyte production. Microbes accumulate osmolytes either by importing or by synthesizing compounds metabolically that would help them to grow and survive during times of high osmotic stress (Sagot et al., 2010).

Another way that microbes use to combat chronic drought stress is dormancy. Several studies have reported that experimental warming of soils in winter or spring increased the microbial biomass (Belay-Tedla et al., 2009) while in summers and early fall, the microbial biomass decreased (Liu et al., 2009). The ability of microorganisms to stay dormant during moisture stress is vital to the resiliency of the soil microbial communities (Sheik et al., 2011). The dormant microbes do not recover until water is available and thus reduce CO_2 efflux during the period of water stress. One more mechanism that microbes use to combat drought stress is the synthesis of extracellular polymers (EPS) which act as sponges and delay drying of microbial cells even under low water potential (Holden, 2011; Tecon and Or, 2017). Some microbes such as methanotrophs rely on gaseous or volatile substrates instead of water-soluble substrates and obtain energy by oxidizing methane gas and these organisms perform better when the soils are dry (Borken et al., 2006; Dijkstra et al., 2011; Fest et al., 2017).

Various C models have predicted altered precipitation regimes at global-scale due to climate change, leading to long term drought followed by intense precipitation (Beier et al.,

2012; Stocker et al., 2013).The sudden increases in soil moisture post prolonged dry conditions could cause a pulse in CO_2 emissions, which is termed the "Birch effect" (Birch, 1964). When water becomes available after a severe drought condition, a part of SOC will be water soluble, which provides immediate substrates to microorganisms to fuel respiration and leads to the Birch effect (Blankinship and Schimel, 2018). The existence of this water soluble SOC fraction was the reason that first order C models (such as CENTURY) were unable to predict these pulse events (Lawrence et al., 2009). Thus, it is important to understand the environmental factors that are responsible for the accumulation and dynamics of water soluble SOC. Microbial access to the water soluble SOC is critical in controlling its concurrent production and consumption, and soil moisture is the key that links microbial cells with these C substrates.

There are four main hypotheses reported in the literature to explain the Birch effect, (i) there is a sudden increase in the bacterial and fungal population abundance in response to water availability (Scheu and Parkinson, 1994), (ii) drying and rewetting of soil breaks down the aggregates which exposes the previously unavailable C for decomposition (Denef et al., 2001), (iii) due to prolonged drying, microbes die and these are rapidly decomposed by new microorganisms after rewetting (Bottner, 1985), and (iv) C pulse is due to hypo-osmotic stress response of soil microbes after sudden changes in moisture status (Fierer and Schimel, 2003; Jarvis et al., 2007).

The mechanisms that control microbial activity under a range of soil moisture conditions as well as under rapid and abrupt changes in soil moisture will affect the long-term C dynamics. Studies on single drying and re-wetting (pulse) events often reported that the C pulse is due to microbial biomass changes (Kieft et al., 1987; Fierer et al., 2003) while studies with multiple sequential pulse events attribute the C pulse to decomposition of stable C (Xiang et al., 2008).

Resolving these different perspectives is a challenge, likely because multiple mechanisms are at play to contribute to C pulse (Borken and Matzner, 2009), and the real question is the relative contribution of these mechanisms that control C dynamics.

Relationship between soil moisture and soil texture

Intrinsic soil properties, such as soil texture and total pore space, play an important role in determining the moisture status of soil. Soil texture is one of the most important soil properties that influences the moisture holding capacity of soils and thus, the SOC turnover (Oades, 1988). It can also affect soil structure by modifying conditions under which microbial decomposition occurs (Schjønning et al., 1999; Sommers et al., 1981). Numerous studies pertaining to the impacts of soil texture on soil moisture status have been conducted in context to plant available water and soil-water balance (Campbell, 1974; Clapp and Hornberger, 1978; Gupta and Larson, 1979). For example, it was found that increase in clay content leads to increased water retention by soil as water molecules become tightly bound to the clay minerals and affect the matric potential (Moyano et al., 2013). However, the role of soil texture to connect soil moisture sensitivity to soil C cycling is not well explored. It is highly likely that soil particle size distribution and associated pore space differences will have strong effects on SOC turnover because of its influence on water availability and retention. For instance, fine micropores tend to have good water holding capacity but larger macropores aid water and air to move freely in and out of the soil (Bouma and Bryla, 2000).

Some studies have also reported that even when the moisture in soil was high, the increased clay content led to a decrease in the respiration rate (Moyano et al., 2012) due to the occurrence of anoxic conditions. It is important that the soil should contain desirable moisture and oxygen for microbial activities. Clayey soils exhibit high water holding capacity and low

aeration, and vice versa for sandy soils. Moyano et al. (2012) reported that soil respiration was correlated negatively with sand content and positively with clay, silt, and SOC contents. It is quite evident that neither the saturated nor the dry conditions of soil are suited for the optimum microbial respiration. Thus, it is crucial to consider soil texture when soil respiration - moisture relationship is investigated (Moyano et al., 2013).

Research gaps

As described above, soil moisture and temperature are the two most important environmental variables that control overall microbial activities in soil. Several studies have revealed the direct effects of temperature on SOC decomposition (Carey et al., 2016; Karhu et al., 2014; Kittredge et al., 2018; Lloyd and Taylor, 1994; Wang et al., 2014) but the effects of soil moisture is still not extensively explored (Zhou et al., 2014). In addition, soil moisture retention and availability are strongly influenced by intrinsic soil properties, in particular soil texture. So, it is very important to gain fundamental understanding of the interactive influence of soil texture and moisture on microbial activities leading to CO_2 fluxes. This information is critical for improving the accuracy of future climate change predictions because most C cycling models use an approximation of moisture-respiration relationship (Moyano et al., 2013). Recently developed C cycle models such as Microbial ENzyme Decomposition (MEND) (Wang et al., 2013) and stoichiometric models developed by Sinsabaugh and Follstad Shah (2012), Sinsabaugh et al. (2013), Microbial-Mineral Carbon Stabilization (MIMICS) model (Weider et al., 2014) and microbially based soil organic carbon (SOMic) model (Woolf and Lehman, 2019) have incorporated microbial processes to a greater degree, but their applicability for predicting the response of microbial respiration to changing soil moisture is strongly restrained, primarily due to lack of quantitative relationships between model parameters and soil properties (Todd-

Brown et al., 2012; Davidson et al., 2012). Therefore, more work needs to be done for further model development to accurately simulate real world processes including unexpected and extreme soil moisture changes (Luo et al., 2016).

Results from this research will be helpful in improving the global C models as these results would provide the intricate mechanistic details about the microbial processes associated with moisture sensitivity to terrestrial C cycling and help in more realistic projections of climate change impacts on terrestrial ecosystems. As I was using distinct soil types in this research, it may help modelers to extrapolate their predictions to broader scale.

Research objectives and hypotheses:

Objective 1: Determine the sensitivity of SOC mineralization to soil moisture and texture.

Hypothesis: Different soils would exhibit different moisture optima for highest microbial respiration depending on their physico-chemical characteristics.

Objective 2: Textural control on the *"Birch effect"* under transient moisture condition with drying and re-wetting cycles in comparison to steady state moisture condition and elucidate underlying mechanisms leading to the *"Birch effect"* in different soils.

Hypotheses: (a) Cumulative SOC mineralization will be higher in transient than steady state; (b) frequent drying & rewetting breaks aggregates and release aggregate-protected C, increasing C mineralization; (c) change in microbial population with C mineralization under steady state would not be as distinct as that under transient conditions; and (d) microbial cells may undergo metabolite accumulation under dry conditions which would be released upon rewetting, thereby, adding more C substrates to soils.

Objective 3: Describe the dependence of SOC decomposition on *in-situ* soil moisture fluctuations.

Hypotheses: (a) Drought condition will reduce microbial activity; (b) the soil microbial community will adapt to continuous moisture stress; (c) legacy effect of drought will be experienced on microbial activity.

Approach

To test these hypotheses, a series of laboratory incubations and a field experiment were conducted. To test how different soil textures will respond to soil moisture manipulation, a 90-day laboratory experiment was conducted using soils of distinctly different textures, viz., sandy, loamy, and clayey. Microbial respiration was measured on a weekly basis. At each destructive harvest on days 1, 15, 60, and 90, extractable organic C, phospholipid fatty acid (PLFA) based microbial community, and extracellular enzyme activity were measured, and SOC pools and mineralization rates were estimated using double pool exponential decay model.

To elucidate the mechanisms behind the "Birch effect" in different soils, a 140-day incubation experiment was conducted in which three differently textured soils (sandy, loamy, and clayey) were subjected to transient moisture changes through five drying-rewetting cycles and at steady-state moisture condition of 55 %WHC. Microbial respiration was measured at frequent intervals under transient and steady state moisture experiments. In addition, the soils were destructively harvested at several time points to determine changes in metabolite accumulation, aggregate associated C, and soil microbial community structure.

To test moisture influence on SOC dynamics under field conditions, in-situ moisture manipulation experiment was conducted on a continuous soybean (*Glycine max* L.) field experiment at West Tennessee AgResearch and Education Center at Jackson, TN in 2018 and 2019. This experiment included drought, rainfed, and irrigated treatments in which drought was simulated using rain-out shelters throughout the growing season while irrigated treatment was

exposed to rainfall and irrigation at different reproductive growth stages of soybean. Throughout the in-field moisture manipulation in 2018 and 2019, microbial respiration was measured weekly and other parameters such as extractable organic C, microbial biomass C, hyphal length, and extracellular enzyme activity were measured at regular intervals. Results from this experiment will strengthen my understanding on how moisture stress influence carbon cycling under field conditions where several extraneous variables, in addition to soil moisture, are also at play.

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Chapter 2. Differential microbial responses to soil texture and moisture in mixed forests

Abstract

Soil microbial respiration is one of the largest sources of carbon (C) emissions to the atmosphere in terrestrial ecosystems. Soil moisture potential is strongly dependent on texture, but the combined effects of texture and moisture on microbial respiration are more complex. Therefore, this study examines the effects of soil moisture on the mineralization of soil organic C (SOC) in soils with different textures: sandy, loamy and clayey, collected from mixed forests of Georgia, Missouri, and Texas, USA, respectively. A laboratory microcosm experiment was conducted for 90 days under different moisture regimes. Soil respiration was measured weekly, and destructive harvests were conducted at 1, 15, 60, and 90 days after incubation to determine extractable organic C, phospholipid fatty acid (PLFA) based microbial community, and C-acquiring hydrolytic extracellular enzyme activities. The highest cumulative respiration in the sandy soil was observed at 50% water holding capacity (WHC), in loamy soil at 100% WHC, and in clayey soil at 175% WHC. The trends in EOC were opposite to that of cumulative microbial respiration as the treatments showing the highest microbial respiration showed the lowest EOC concentration in all soil types. Also, EEA increased with increase in soil moisture in all soils, however, microbial respiration and EEA showed a decoupled relationship in sandy and loamy soils. However, soil moisture differences did not influence microbial community composition. When my results were compared with predictions from a soil moisture-respiration model by Moyano et al. (2013), all the three soils did not follow the model predicted curve, however, the soil moisture optima observed in my study were similar to that predicted by the model for the sandy and loamy soils and not for the clayey soil. The differential respiration response of the different soil textures provides critical information for improving the moisture sensitivity of microbial parameters in ecosystem models.

Introduction

Soil organic carbon (SOC) is the largest and most dynamic terrestrial C reserve (Jobbágy and Jackson, 2000) and understanding SOC decomposition in response to environmental changes is critical for predicting CO_2 feedbacks driving future climates (Davidson and Janssens, 2006; Schmidt et al., 2011). Among terrestrial ecosystems, soils represent the largest C pool (2300 Pg) which is greater than atmospheric (760 Pg) and vegetative (560 Pg) pools combined. Globally, 60 to 80 Pg of C is emitted to the atmosphere by microbial soil respiration, which is one of the largest C fluxes between the terrestrial ecosystem and atmosphere (Raich and Potter, 1995; Raich and Schlesinger, 1992). Indeed, soil CO_2 flux is ten-fold higher than anthropogenic sources, including burning of fossil fuels, fires, and deforestation (Oertel et al., 2016). Approximately 10% of the atmospheric CO_2 cycles through soils each year and environmental changes that influence C cycling in soil impart a strong effect on atmospheric CO_2 concentrations (Rodeghiero and Cesscatti, 2005). Given the large amounts of soil C stocks and flux to the atmosphere, a major concern is that future climate change will create a positive feedback loop by increasing soil respiration (Trumbore, 1997; Davidson et al., 2000; Schlesinger and Andrews, 2000).

The existing literature shows that the soil moisture-respiration relationship is complex and site-specific, and is strongly controlled by porosity, bulk density, texture, and SOC concentration (Franzluebbers, 1999; Thomsen et al., 1999; Moyano et al., 2012; Herbst et al., 2016). However, such studies have been very few and focused mainly on determining the index of soil moisture that best predicts the microbial respiration (e.g., water-filled pore space, water potential) or elucidating the unified relationship that best describes the relationship between soil moisture and microbial respiration (e.g. linear, polynomial, etc.) (Ilstedt et al., 2000; Paul et al.,

2003). Soil C models tend to rely on empirical soil moisture-respiration functions which are developed and validated using studies from specific sites (Falloon et al., 2011; Bauer et al., 2008) and as these functions represent an average response of the moisture-respiration relationships, these functions can introduce uncertainties into predictions of the SOC budget (Sierra et al., 2015).

Soil physico-chemical properties can also impart a significant control on how moisture influences microbial mineralization of SOC such as clay content (Franzluebbers et al., 1996). While clay content does not affect the mineralization of so-called labile SOC early in an incubation, during the later stages when non-labile SOC is mineralized, high clay content slows down decomposition rates as clay protects SOC (Wang et al., 2003). On the contrary, Mutuo et al. (2006) reported poor protection of SOC against microbial respiration in sandy soil.

Moyano et al. (2012; 2013) established empirical relationships between soil moisture and respiration across 42 soils based on various soil properties such as SOC concentration, porosity, and soil texture, and reported that soil respiration was correlated negatively with sand content, and positively with clay, silt and SOC contents. However, it is unclear the extent to which the relationships between soil moisture and microbial respiration developed across soil types in Moyano et al. (2013) still hold when distinctly differently textured soils are considered separately. In effect, will soil texture change the soil moisture response enough to warrant individual models for each soil texture class. Also, the soil moisture-respiration curve provided by Moyano et al. (2013) shows close to zero relative respiration at saturated soil moisture contents for all soil types which is conceptually unreasonable as even at saturated soil moisture levels such as wetland ecosystems, there are evidences of microbial respiration fluxes due to activity of denitrifiers, manganese-, iron-, and sulfate-reducers (Ponnamperuma, 1972;

Peretyazhko and Sposito, 2005; Teh et al., 2005; Chacon et al., 2006). The relationship between soil moisture and microbial respiration are underpinned by differences in the thermodynamic energy yield of microbially catalyzed redox reactions, which suggests that even under saturated conditions, the electron acceptors such as nitrate, manganese oxides, iron oxides, sulfates are reduced in a predictable sequence (Hall et al., 2013) and allow soil microbes to respire.

Soil moisture is one of the most important drivers of microbial respiration (Davidson and Janssens, 2006; Moyano et al., 2013). Low soil moisture levels hinder microbial respiration by decreasing substrate accessibility to microbes. Under extremely dry conditions, microbes may undergo a state of low metabolic activity or dormancy (Manzoni et al., 2014; Manzoni et al., 2012). On the contrary, high soil moisture levels can also impede microbial respiration by reducing oxygen diffusion (Moyano et al., 2013). Extracellular enzyme activities, that provide information on soil microbial metabolic functioning (Stott et al., 2010) are also strongly influenced by soil moisture (Acosta-Martinez et al., 2007) and soil texture (Günal et al., 2018). Despite the critical role of soil moisture in regulating SOC mineralization, the soil moisture sensitivity of terrestrial C cycling has received less attention in comparison to temperature sensitivity (Wang et al., 2019). Therefore, this paper aims to experimentally establish soil moisture-soil respiration relationships and determine if the microbial respiration ceases under very high moisture conditions in soils with distinctly different textures against the empirical relationship provided by Moyano et al. (2012, 2013). With these objectives in mind, I conducted a laboratory-scale microcosm experiment using three soils of different textures for 90 days. I hypothesized that soil texture would interact with soil moisture regime as the dominant factor controlling the microbial respiration.

Materials and methods

Study sites

Three different soils - sandy, loamy, and clayey - were collected from mixed forests located in the southern US. The sandy soil belonged to the Cowart series (fine-loamy, kaolinitic, thermic Typic Kanhapludults) and was collected from Taylor County in Georgia (32.54°N, 84.22°W). The predominant tree species comprising the forest are *Quercus alba, Acer saccharum, Quercus velutina, Juniperus virginiana, Carya* spp., *Pinus clausa, Nyssa sylvatica, Magnolia grandiflora,* and *Liriodendron tulipifera*. Mean annual temperature and precipitation at this site is 20°C and 1200 mm, respectively. The loamy soil belonged to the Weller series (fine, smectitic, mesic Aquertic Chromic Hapludalfs) and was collected from the Missouri Ozark AmeriFlux (MOFLUX) site in central Missouri. This site is located at the University of Missouri Baskett Wildlife Research and Education Center (38.74°N, 92.20°W) in an upland oak-hickory forest with major tree species consisting of *Quercus alba, Carya ovata, Acer saccharum* and *Juniperus virginiana* (Gu et al., 2015; Wood et al., 2018). Mean annual temperature and precipitation at this site is 12°C and 986 mm, respectively. The clayey soil belonged to the Buxin series (very-fine, smectitic, thermic Aquic Hapluderts) and was collected from Bowie County, Texas (33.44°N, 94.48°W). At this site the predominant tree species are *Pinus taeda*, *Pinus echinata, Quercus* spp., *Carya* spp., and *Cupressus* spp. Mean annual temperature and precipitation at this site is 17°C and 900 mm, respectively. Basic properties of these three soils are reported in Table 2.1.

Soil sampling and characterization

Soil cores of 5 cm diameter were collected from several random locations at each site to a depth of 15 cm and combined to obtain a composite sample per site (Jagadamma et al., 2014;

Mavi et al., 2012). Before sampling, the uppermost layer of litter was removed. The soil samples were transported to the research laboratory at the University of Tennessee, Knoxville in coolers with dry ice under field-moist condition within two days. Three additional undisturbed cores per site were collected to determine water holding capacity (WHC) using pressure plate extractors (Klute and Dirksen, 1986). The composite samples were immediately sieved through a 2 mm sieve. One section of the sieved samples was air-dried for the initial soil characterization (n = 3), and the remainder of the composite was separated into aliquots for characterization (n = 3 per analysis) and for incubation experiments. Soil texture was determined by the Bouyoucos hydrometer method (Gee and Or, 2002). Soil pH was measured in a 1:2 soil:water suspension (Thomas, 1996) using a pH meter (Mettler Toledo, Columbus, Ohio). Total C was determined on finely ground samples by the dry combustion method using a Vario TOC cube CN analyzer in solid mode (Elementar, Hanau, Germany). As all the soils were acidic (pH < 6), total C was assumed to be equal to SOC (Al-Kaisi et al., 2005). Extractable organic C (EOC) was determined based on the method by Jones and Willett (2006). Briefly, soils were mixed with 0.5 M K_2SO_4 (1:4 soil:solution), shaken at 200 revolutions per minute for 1 hour, and centrifuged at 3500 rpm for 3 minutes. The supernatant was passed through a 0.45 μm filter paper (Whatman No. 42) and analyzed using a Vario TOC cube CN analyzer in liquid mode (Elementar, Hanau, Germany).

Soil moisture treatments and incubation experiment

This study included five soil moisture treatments: air-dried, 25% WHC, 50% WHC, 100% WHC, and 175% WHC. The WHC of the three soils was determined by placing the saturated soil cores in the pressure plate extractors at -0.33 bar and then measuring the gravimetric soil moisture content of the core (Zhou et al., 2014; Mavi et al., 2012; Bao et al.,

2016). Except for the air-dried treatments, the treatments manipulations involved adding an amount water to the composited soils based on the WHC and on the gravimetric moisture content of the composited soils.

Prior to the start of incubation, soil samples were pre-incubated for 1 week at environmental conditions similar to the experiment (i.e., at room temperature (20ºC) in the dark). Thirty grams of soil sample (on a dry weight basis) was added to plastic cups and soil moisture was manipulated according to the corresponding moisture treatment. Briefly, MilliQ water was added uniformly on top of soils using a syringe and then the soils were gently mixed using a spatula. To maintain consistency, all the soil samples were mixed the same way. The cups were then placed in 1 L glass jars and closed with lids fitted with sampling ports at the center. A total of 12 replicates per treatments were prepared to destructively harvest three replicates at day 1, day 15, day 60, and day 90 of the incubation. Soil collected from the destructive harvests were frozen at -20°C pending analysis. In order to maintain soil moisture during the course of the incubation, water loss was checked on a weekly basis by weighing each soil container and corrected by adding MilliQ water as necessary. At the most, 0.1-0.2 mL of water was added every week.

Gas sampling and CO_2 measurement

Gas samples (15 mL) from the headspace of the jars (n = 3) were collected through the sampling port on the lids on days 0, 1, 2, 5, 7, 12, 19, 26, 34, 41, 48, 55, 62, 69, 76, 83 and 90 using a syringe-needle assembly and transferred to evacuated exetainer vials (Labco, United Kingdom). The jars were sampled before opening the lids and after gas sample collection, the jars were opened, and electrical fans were used for blowing air into the jars to maintain an aerobic incubation environment. For blank correction, gas samples were collected from triplicate

empty (without soil) jars at all time points. Gas sampling was always done at the same time of day. The CO_2 concentration in the gas samples was measured using a flame ionization detector on a gas chromatograph (Shimadzu GC-2014, Japan). Microbial respiration rate was calculated using the linear response curve of CO_2 concentrations versus time (Curiel-Yuste et al., 2007). The total cumulative CO_2 emissions for each soil after 90 days was normalized with respect to the highest CO_2 emission measured in each soil to obtain the relative microbial respiration (Moyano et al., 2013). This enabled me to compare my experimentally derived relative respiration data with that predicted by Moyano et al. (2013). For data comparison, these moisture treatments were converted to volumetric soil moisture contents.

Soil extracellular enzyme assays

The activities of four C-acquiring hydrolytic enzymes, α-glucosidase (AG), β-glucosidase (BG), cellobiohydrolase (CBH), and β-xylosidase (XYL), were determined from all treatments at all the destructive sampling time points (day 1, 15, 60, and 90) in 96-well plates according to German et al. (2011b). Each column of 12 wells on each plate corresponded to one soil sample. Another plate was used to create a standard curve for each sample at 25°C. Each column in the standard curve plate contained a soil slurry with a different concentration of the 4-methylumbelliferone (MUB) standard (0, 2.5, 5, 10, 25, 50 and 100 μM) in each of the wells. The reference standards for the enzymes AG, BG, CBH, and XYL are 4-MUB-α-D-glucopyranoside, 4-MUB-β-D-glucopyranoside, 4-MUB-β-D-cellobioside, and 4-MUB-β-D-xylopyranoside, respectively. A 2.75 g of frozen soil sample from each treatment was thawed to room temperature and homogenized with 91 mL of 50 mM sodium acetate buffer (pH adjusted according to the pH of the soil types shown in Table 2.1) for 1 min in a Waring laboratory grade blender on high speed to prepare a slurry. Homogenization was followed by adding 800 μL of the soil slurry into each of the 8 wells of one column. After adding 12 samples in a plate, 200 μL

of 200 μM respective substrates were added. The plates were incubated for 3 hours at 25°C. After incubation, the plates were centrifuged at 350 x *g* for 3 minutes and 250 μL of the supernatant from each well was transferred to a 96-well black plate. A plate reader (Synergy-BioTek Instruments, Inc., Vermont, USA) was used to measure fluorescence at wavelengths 365 nm and 450 nm for excitation and emission, respectively. The standard curve plates were used to construct a linear standard curve to determine each enzymes' activity for each sample as nmol g^{-1} dry soil h^{-1}. Total activity of the C acquiring hydrolytic enzymes was defined as the sum of CBH, AG, BG, and XYL.

Phospholipid fatty acid extraction and analysis

Microbial community composition was determined using the phospholipid fatty acid (PLFA) and neutral fatty acid (NLFA) analyses (Buyer and Sasser, 2012). Briefly, 1.5-2 g of freeze-dried soil were used for Bligh-Dyer lipid extraction (Bligh and Dyer, 1959). The extracted fatty acids were dried and analyzed using a gas chromatograph (Agilent 7890A, Agilent Technologies, Wilmington, DE, USA) after trans-esterification for quantitative analysis relative to an internal standard. Fatty acid profiles were identified using MIDI PLFAD1 calibration mix and software SHERLOCK version 6.2 (MIDI, Inc., DE, USA). Bacteria, fungi, and arbuscular mycorrhizal fungi (AMF) were targeted using the fatty acid markers, where gram-positive bacteria markers were 15:0, i15:0, a15:0, i16:0, 16:0ω9, i17:0, and cy17:0, and gram-negative bacteria markers were 16:1ω7, 18:1ω7, and cy19:0 (Frostegård and Baath, 1996). The amount of PLFA 18:2ω6 was used as a marker of non-mycorrhizal fungal abundance and NLFA 16:1ω5 for AMF (Olsson et al., 1995; Olsson, 1999).

Exponential decay modeling

The CO_2 data corresponding to each moisture treatment in each soil was best fitted using a double pool exponential decay model (based on the lowest Akaike Information Criterion

values) (Farrar et al., 2012; Jagadamma et al., 2014) after testing multiple models (Das et al., 2019).

$$C_t = C_1(1 - e^{-k_1 t}) + C_2(1 - e^{-k_2 t})$$

where C_t is the total CO_2-C production per unit soil weight (μg C g^{-1} dry soil), t is the time in days, C_1 is the fast mineralizing SOC pool (active pool), C_2 is the slow mineralizing SOC pool (slow pool) within time t, and k_1 and k_2 are the mineralization rates for C_1 and C_2, respectively. For each set of data, the model was fit using Sigma plot v14 (Systat Software Inc., IL, USA). Parameter constraints while fitting the model were $k_1 > 0$ and $k_2 > 0$ and $C_1 + C_2 = 100\%$ (Das et al., 2019).

Statistical analyses

Statistical analyses were conducted using SAS software v 9.4 (SAS Institute Inc., 2002). The effect of moisture on CO_2 and EOC over the incubation period for each soil was analyzed using repeated measures ANOVA with incubation length as the repeated measure. Post hoc comparisons for determining the effect of soil moisture on respiration was performed using PROC GLIMMIX in SAS. The mean separation was done using Tukey's test. The dependence of observed microbial respiration to various soil properties including soil pore space, bulk density, SOC, pH, and sand, silt and clay contents, was determined using multiple linear regression analysis by applying stepwise model selection procedure. In all statistical tests, the mean differences were considered significant if $P \leq 0.05$. Moisture sensitivity for enzyme assays was estimated using regression analyses of measured enzyme activities for the moisture treatments, soil types, each destructive sampling time, and the total incubation length. To compare enzyme activities among soil moisture treatments on each day of destructive sampling, one-way ANOVA was used.

To understand how the microbial community composition varies across various moisture levels in all soil types, permutational multivariate analysis of variance (PERMANOVA) was used in R (R Development Core Team, 2011) on the matrix of abundances of each microbial type for all soil types and moisture treatments. The Bray-Curtis distance metric and 9999 permutations of residuals for the model were used (Kivlin et al., 2019). The Mantel test was performed to assess correlations between enzyme activity and microbial community composition in R.

Results

Microbial respiration

The CO_2 respiration rate showed significant responses to soil moisture content in all soils (Fig. 2.1) ($P < 0.0001$; $P = 0.032$ for sandy soil; $P < 0.0001$; $P = 0.004$ for loamy soil; $P < 0.0001$ for clayey soil). The lowest respiration rate was observed at the lowest moisture content (air-dried soils) in all the three soils throughout the incubation. However, the highest CO_2 respiration rate was observed at different moisture levels for the different soil types. For sandy soil, after the first 5 days of incubation, the highest respiration rate was consistently observed at 50% WHC throughout the incubation (Fig. 2.1a). For loamy soil, after a week of incubation, the highest respiration was observed at 100% WHC for the remaining incubation period (Fig. 2.1b). For clayey soil, the highest respiration rate was observed at 175% WHC consistently throughout the incubation period (Fig. 2.1c). The repeated measures ANOVA results showed significant effects of soil moisture ($P < 0.0001$), time of sampling ($P < 0.0001$), and their interaction ($P < 0.001$) on soil respiration for all soil types (Table 2.2).

SOC pool sizes and mineralization rates

Microbial respiration data was modeled by a double pool exponential decay model to determine active and slow SOC fractions mineralization at different moisture levels. Regardless

of soil texture and soil moisture differences, <1.5% of the initial SOC respired was partitioned as the active (labile) pool C1 (Table 2.4). For both loamy and clayey soils, C1 decreased with increasing moisture content. However, for sandy soil, C1 was similar for all moisture levels except the air-dried treatment. The size of the slow mineralizing pool (C2) only varied between 99.2% and 99.7% of initial SOC among all soil types and soil moisture levels, however, no significant differences were observed among soil moisture treatments.

Mineralization rate, k_1 of C_1 pool was the highest at 50% WHC (0.23 day^{-1}) for sandy soil, at 100% WHC (0.21 day^{-1}) and at 175% WHC (0.23 day^{-1}) moisture treatments for loamy soil, and at 175% WHC moisture content (0.17 day^{-1}) for clayey soil (Fig. 2.4a). Mineralization rate, k_2, of C_2 pool was one to two order of magnitude lower than k_1 and showed no significant differences across moisture levels in all the three soils (Fig. 2.4b). Comparing the soil types, sandy soil had comparatively higher k_2 followed by loamy and clayey soils.

Extractable organic carbon (EOC)

The EOC values were generally higher for the drier than wetter treatments (Fig. 2.5). For sandy soil, EOC was statistically influenced by moisture ($P < 0.001$), length of incubation ($P < 0.0001$), and their interaction ($P < 0.001$) while for loamy and clayey soils, the interaction between moisture and length of incubation was not significant ($P > 0.05$). The EOC concentration at day 1 for all moisture levels and soil types was significantly higher (60 to 115 mg kg^{-1}) than that at days 15 (33 to 103 mg kg^{-1}), 60 (24 to 94 mg kg^{-1}), and 90 (22 to 89 mg kg^{-1}). For all soil types, the driest soils contained higher EOC content throughout the length of incubation. As incubation progressed, for sandy and loamy soils, I observed no significant differences in EOC content of driest treatment with time but for other treatments EOC decreased with time. However, for clayey soil, EOC in the driest treatment also decreased with incubation

length. More importantly, beginning day 15, the lowest EOC content in each soil type was recorded from the moisture treatment which produced the highest cumulative respiration: at 50 % WHC, 100% WHC and 175% WHC for sandy, loamy and clayey soils, respectively (Figs. 2.5, S1).

Extracellular enzyme activity and microbial community composition responses

There was a consistent increase in total C acquisition enzyme activities (sum of BG, AG, CBH and XYL) with increase in soil moisture. The highest enzyme activity was observed at the highest soil moisture content for all the three soils at all time points (Fig. 2.6). Also, regardless of soil types, air-dried treatments consistently showed the lowest enzyme activity and there was a decrease in enzyme activity with the length of incubation. For sandy soil, on day 1, I observed a significant increase in enzyme activity with an increase in moisture treatments but on day 90, no statistical difference in enzyme activity was observed in the moist treatments (only air-dried treatment showed lowest activity). The regression analysis showed a significant positive correlation between soil moisture and enzyme activity (Table 2.5). Mantel test results showed that the microbial community composition derived by PLFA did not influence the enzyme activity ($P > 0.05$) (Table S1). The PERMANOVA analysis revealed that soil moisture treatments did not influence the microbial community composition in any soil type (sandy soils, $P = 0.35$; loamy soil, $P = 0.41$; and clayey soil, $P = 0.12$) (Table S2).

Experimental data versus model prediction

The relative microbial respiration for the three soils was calculated by normalizing the cumulative CO_2 respiration with respect to the highest measured CO_2 emission. The measurements were compared to predictions by Moyano et al. (2013) for sandy, loamy, and clayey soils (Fig. 2.2). For sandy and loamy soils, a bell-shaped second-order polynomial response curve was observed with the experimental data which did not follow the response

curves predicted by Moyano et al. (2013) (Figs. 2.2a and 2.2b). My data curve showed a wider curvature for both sandy and loamy soils compared to the curve generated from predicted data. Also, even at the highest soil moisture treatments in both soils, the relative respiration did not approach zero unlike predicted by Moyano et al. (2013). For the clayey soil, an increasing second-order polynomial response was observed with increasing soil moisture content, which was unlike the bell-shaped predicted response curve (Moyano et al., 2013) (Fig. 2.2c). Unlike the lab data which followed bell-shaped response curve only for sandy and loamy soils and not for clayey soil, Moyano et al. (2013) showed a bell-shaped curve across the entire range of clay content for three soils from low clay to high clay content. However, it is important to note that for the sandy soil, the highest relative respiration from the experimental data coincided well with that from the Moyano model predictions, while for the loamy soil, the peak observed relative respiration was slightly underestimated by the model. For the clayey soil, the highest relative respiration predicted by the Moyano model was at a very low moisture content (0.4 m^3 m^{-3}) compared to the peak respiration in my experimental data (0.71 m^3 m^{-3}).

The relationship between clay content and optimum soil moisture content at which the highest microbial respiration was observed was evaluated in the three soils (Fig. 2.3). The increase in clay content indeed increased the optimum soil moisture content needed for highest microbial respiration. The dependence of microbial respiration to various soil properties such as EOC concentration, moisture%, sand%, silt%, clay%, MBC concentration, and pH was determined using multiple linear regression. Results revealed that microbial respiration was significantly dependent on clay content, soil moisture, and SOC content (Table 2.3).

Discussion

In accordance with my hypothesis, soil microbial respiration was influenced by soil moisture levels and showed variable responses in different soils. In all soil types, significantly lower microbial respiration was observed in the drier treatments, likely due to limited substrate diffusion in the soil matrix that impeded microbial metabolism (Manzoni et al., 2012; Bell et al. (2009), Evans et al. (2014), Zhou et al. (2014). The moisture content at which the maximum soil respiration occurred varied across soil types. For clayey soil, CO_2 efflux consistently increased with soil moisture. The highest cumulative and relative microbial respiration positively track with soil clay content, with the clayey soil having four-fold higher soil moisture optima than the sandy soil. The clayey soil may have remnant air-filled micropores, supporting aerobic microbial metabolism despite excessive moisture conditions (Taboada, 2003). It is also important to note that the clayey soil is a Vertisol which has an exceptional shrink-swell capacity that could promote the buildup of air in micropores (Taboada, 2003). The significant increase in microbial respiration from the clayey soil with increased moisture could also be explained mechanistically by the zonal theory of organic C layering on clay minerals (Kleber et al., 2007). Based on this theory, increased moisture may facilitate easy exchange of loosely adsorbed C molecules with the soil solution, making them available for microbial acquisition (Kögel-Knabner et al., 2008). For sandy and loamy soils, the decrease in respiration beyond optimum soil moisture is probably due to the decreased diffusion of oxygen, thereby, creating anoxic conditions for microbes (Barros et al., 1995; Prado and Airoldi, 1999). The strong positive relationship between clay content and optimum soil moisture for highest cumulative microbial respiration that was observed in this study (Fig. 2.3) indicates that soil texture affects SOC decomposition by modifying the effective soil moisture available for diffusion. This observed relationship in my

study is also in accordance with that reported by Moyano et al. (2013), however, they reported the optimum soil moisture for highest respiration would be at 0.5 m^3 m^{-3}, while it was at 0.71 m^3 m^{-3} in my study. As discussed earlier, this could be due to the higher clay content in the clayey soil (71%) compared to the upper limit for clay content (57%) used in Moyano et al. (2013). The SOC content would not create a shift in the curvature of the response curve and only shift the point of maximum respiration to higher values (Moyano et al., 2012) due to higher availability of substrates for microbial uptake.

The microbial respiration data from my 90-day incubation was satisfactorily described by a double-pool exponential decay model. The air-dried soils showed the lowest k_1 and k_2, reflecting the lowest microbial activity. The moisture treatments which showed the highest cumulative microbial respiration (Fig. S1) in all soil types also showed the highest k_1 values (Fig. 2.4) indicating the tight coupling across soil texture, moisture content, and microbial activity. The mineralization rate of the slow SOC pool (k_2) was one to two orders of magnitude lower than k_1 and did not show significant differences among the moisture treatments for all the soil types despite following the order sandy > loamy > clayey. This is in line with my understanding that finer silt and clay sized particles tend to retain more SOC than coarser sandy particles (Hassink, 1997) due to enhanced physical and chemical protection. Nonetheless, the length of incubation is a key factor to determine the number of SOC pools with distinct turnover rates using kinetic modeling. Scharnagl et al. (2010) reported that long-term incubations with duration ranging from 600 to 900 days were best for determining the fast, intermediate, and slow pools of SOC unlike short-term incubations like ours which are best fit by double-pool models (Das et al., 2019; Guo et al., 2014; Guntinas et al., 2013). Moreover, my study observed that clay content was the major controller of the microbial respiration followed by soil moisture and SOC

content, and all these three variables together explained 13% of the variance in microbial respiration. In addition to these measured variables, microbial respiration could also be affected by multiple top-down and bottom-up controllers, which are not studied in this experiment. For example, phage predation, a top-down control, is known to control microbial activity by regulating the microbial biomass (Fuhrman, 1999). A decrease in phage population leads to an increase in microbial biomass and respiration (Allen et al., 2010; Lenoir et al., 2007). Also, negative species interactions may limit microbial respiration as certain microbes produce toxins that depress the activity of other species (Zhang and Zhang, 2016). Other abiotic bottom-up factors such as substrate quality and nutrient concentrations also known to control microbial respiration (Tilman, 1977).

The cumulative respiration and mineralization rate data was also supported by the change in EOC across soil moistures (Fig. 2.5). The EOC concentrations were the lowest at the moisture levels that resulted in the highest cumulative respiration, suggesting the highest rate of microbial uptake of EOC (Zsolnay, 1996). No significant decrease in EOC concentrations was observed in soils on days 60 and 90 as compared to day 15 which can plausibly be explained by the addition of C by microbial turnover (Coleman and Jenkinson, 1996; Franzluebbers et al., 1999). In addition, I observed consistently higher EOC concentrations in air-dried soils compared to higher moisture levels, similar to the trend in the size of active SOC pool, again suggesting lower microbial mineralization of SOC due to moisture limitation (Schjønning et al., 2003; Or et al., 2007).

In accordance with the general understanding that C acquiring extracellular enzymes are highly sensitive to changes in moisture (Zhou et al., 2013), I observed a strong positive effect of soil moisture on the total activity of C acquiring extracellular enzymes (BG, AG, CBH, and

XYL) (Table 2.5) (Steinweg et al., 2012). Unlike EOC and mineralization rates that followed the same trend as cumulative microbial respiration by showing sensitivity to different moisture optima for different soils, extracellular enzyme activity was decoupled from cumulative microbial respiration. These laboratory assays were short, and therefore discerned the activity of extant enzymes pool rather than newly synthesized enzymes due to substrate addition (Steinweg et al., 2012). Enzyme activities displayed a monotonic increase with increasing moisture levels, especially in sandy and loamy soils, which was probably related to enhanced enzyme diffusion under higher moisture contents (Ren et al., 2017; Burns et al., 2013). This trend was more visible during the early days of incubation but as the incubation progressed, the difference in enzyme activity among moist treatments reduced. For instance, for sandy soil, there was no significant difference in enzyme activity among all the moisture treatments except air-dried treatment on day 90. Nonetheless, the enzyme activity was the highest at 175% WHC regardless of the soil types and incubation time. The enzyme activity was the lowest in the air-dried soils for all three soils, as expected and consistent with the cumulative microbial respiration results, which could be due to substrate diffusion constraints (German et al., 2011a; German et al., 2011b) and/or adsorption of enzymes on soil particles (Kandeler, 1990; George et al., 2007). Allison and Vitousek (2005) reported that under dry conditions, enzyme production and activity are lowered as the nutrient requirement for enzyme production exceeds the nutrient availability for microbes. The enzyme activities showed a consistent decrease across the length of incubation (Fig. 2.6) probably due to substrate exhaustion (Acosta-Martinez et al., 2007).

Soil moisture did not influence microbial community composition determined by PLFA. Some past studies also reported no effect of soil moisture on microbial community composition (Griffiths et al., 2003; Buyer et al., 2010), while others reported the opposite (Williams, 2007;

Kaisermann et al., 2015). The lack of influence of moisture on microbial community composition in my study is likely due to the short length of incubation; however, changes in microbial community composition during incubation may not be an accurate reflector of in-situ microbial community activity. In the field, legacy field soil moisture content shapes the microbial community (Schimel et al., 1999; Banerjee et al., 2016). Overall, these results indicate that short-term lab scale incubations at different soil moisture levels did not change microbial community composition, however microbial activity was affected as indicated by changes in the proportion of EOC and C acquiring enzyme activities.

The experimental moisture response curves, when compared to the predictive model of Moyano et al. (2013), showed variable trends in sandy, loamy, and clayey soils (Fig. 2.2). For all the three soils, the experimental response curves did not match with the bell-shaped response curve predicted by Moyano et al. (2013). The experimental response curves generated from the incubation data did not approach zero under high soil moisture contents and there was still CO_2 efflux from saturated treatments unlike predicted by Moyano et al. (2013). My experimental results are conceptually reasonable as under saturated conditions, denitrifiers, manganese-, iron-, and sulfate-reducers are active and continue to respire. Based on my understanding of the microbially-catalyzed redox reactions, the microbes compete for electron donors, due to which, under saturated conditions, oxygen is respired and depleted first followed by nitrates, manganese and iron oxides, and sulfates (Megonigal et al., 2003). Also, as oxygen and other electron acceptors are successively depleted, the production CO_2 would also decrease over time. The experimental response curve showing CO_2 efflux even under saturated conditions indicates that other electron acceptors are also at play and help microbes to metabolize by providing more thermodynamically favorable pathways (Teh et al., 2008). The soil moisture optima for the sandy

and loamy soils obtained by experimental data were similar to that predicted by Moyano et al. (2013) but for clayey soil, the experimentally derived optimum soil moisture for highest microbial respiration was much higher than the predicted value. The predicted relationship in Moyano et al. (2012; 2013) used studies with soil clay content of $\leq$ 57%, that belonged to Inceptisols unlike my Vertisols. Perhaps the higher clay content in my clayey soil (71%) might be the reason for the deviation in moisture response from the prediction. The experimental versus model predicted data comparison provides evidence to contradict the empirical soil moisture-respiration relationship provided by Moyano et al. (2013).

Conclusions

Understanding the dependency of microbial respiration on soil moisture and soil texture is important for reducing the uncertainty in modeling SOC dynamics. Results from this study showed that the effect of soil moisture on microbial respiration was strongly controlled by soil texture, most likely due to the direct influence of texture on the moisture holding capacity of soils. The experimentally derived optimum moisture content for the highest microbial activity aligned well with the predictive results of Moyano et al. (2013) for the sandy and loamy soil, but not for the clayey soil. However, the soil moisture-respiration curve for all the three soils did not follow the one predicted by Moyano et al. (2013) as the respiration data of the incubation study did not approach zero under saturated conditions against the respiration data modeled by Moyano et al. (2013) which was zero under saturated soil moisture conditions. Different moisture optima were observed in sandy, loamy, and clayey soils for maximum microbial respiration, extractable organic C content, and SOC mineralization rates. Higher soil moisture supported higher enzyme activities and enhanced substrate availability, yet microbial respiration declined at higher soil moistures, but only for sandy and loamy soils. For clayey soils, higher

respiration and higher enzyme activities were observed for the highest soil moisture contents, consistent with increased substrate availabilities. Soil moisture and texture can become decoupled from microbial activity in more coarsely textured soils, but decoupling is much less likely in finely textured soils. Therefore, there is not a single response surface to represent soil carbon decomposition under variable moisture and texture.

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Appendix II

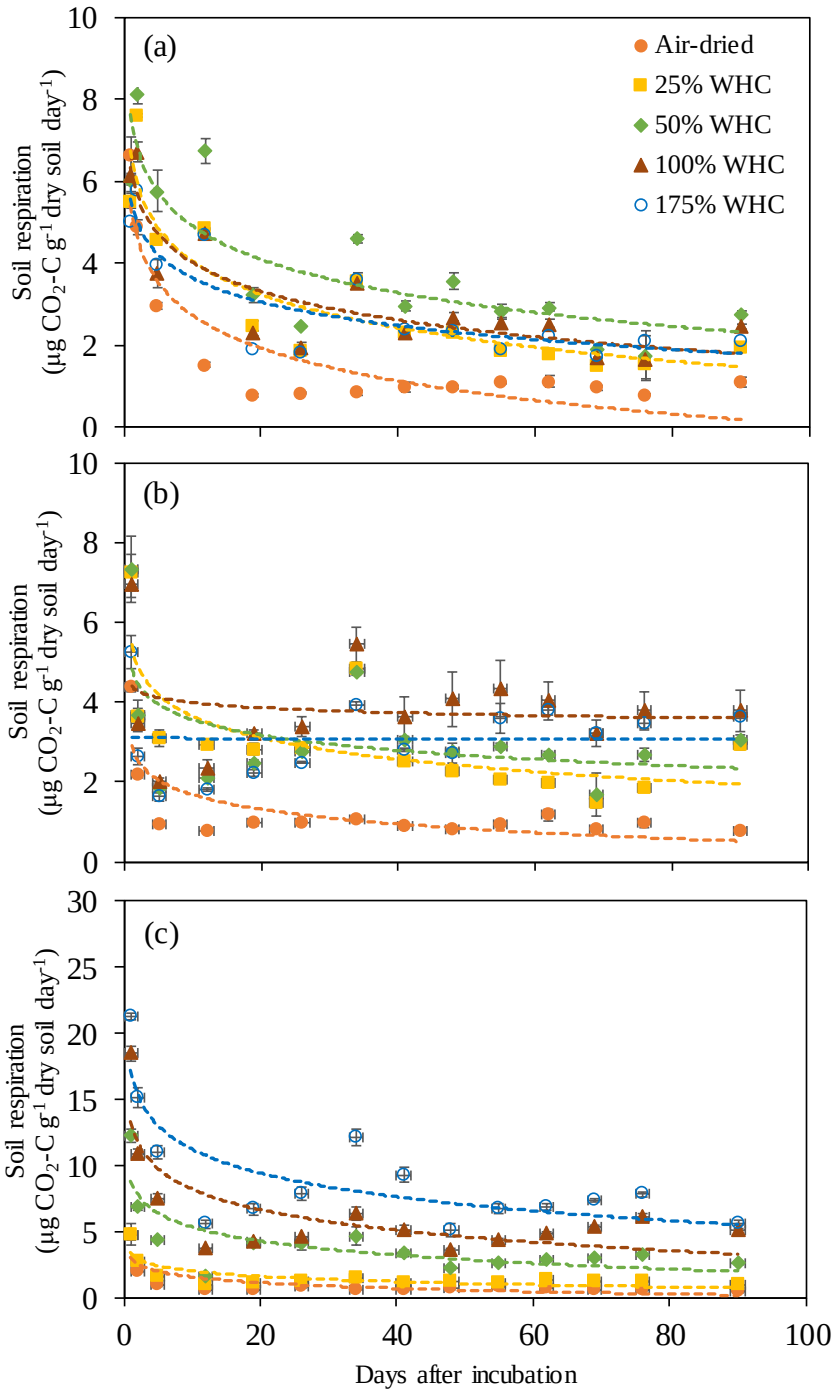


Figure 2.1. Microbial respiration rate in response to soil moisture levels in (a) sandy, (b) loamy, and (c) clayey soils.
Error bars represent standard error (n = 3). The regression functions are logarithmic for all soil moisture treatments in all soil types. Note the y axis scale is 3x larger for clay soils.

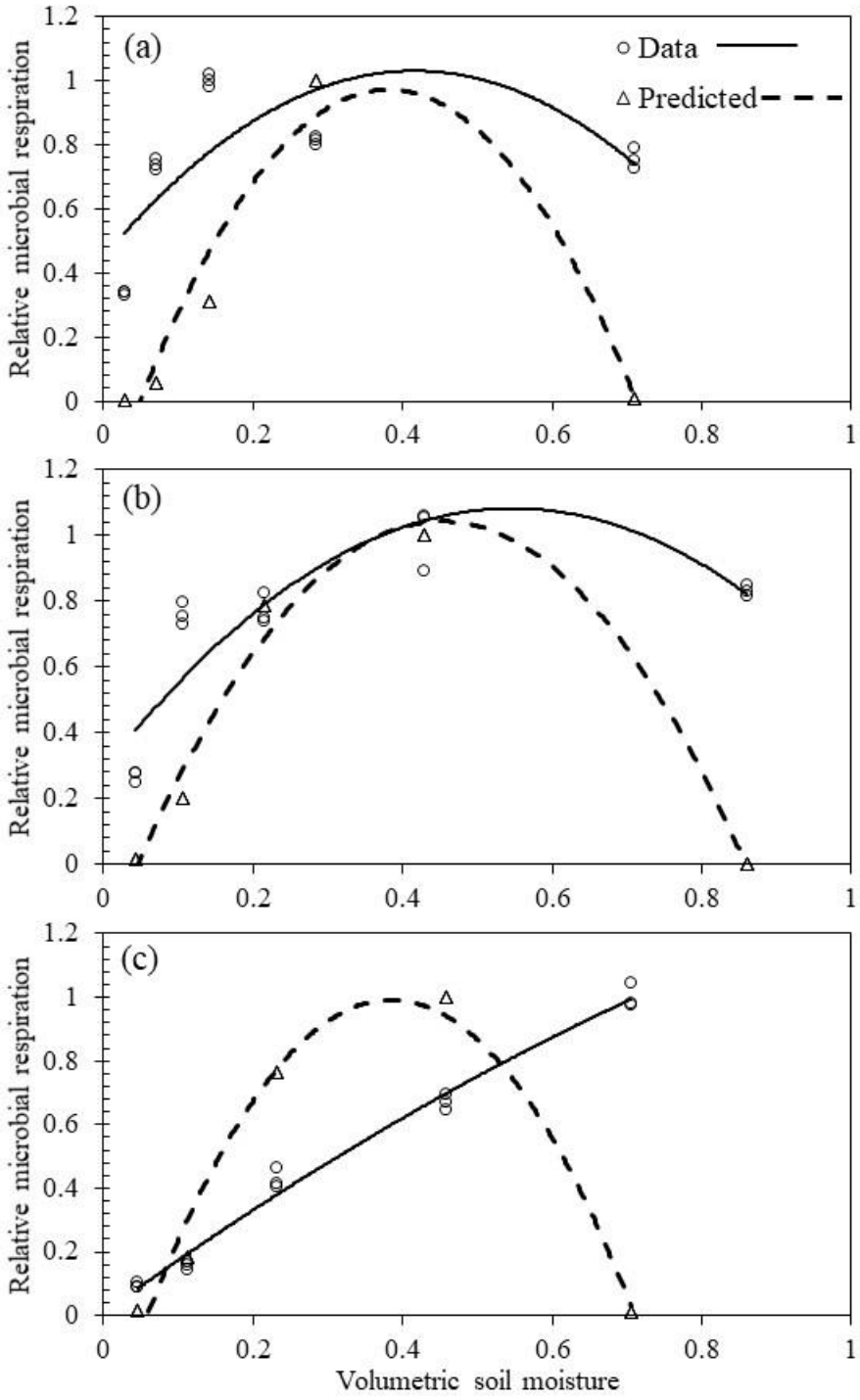


Figure 2.2. Relative microbial respiration for sandy (a), loamy (b), and clayey (c) soils in response to increasing soil moisture content.
The circles and solid lines represent the best fit response curve of relative microbial respiration to increasing moisture levels. The triangles and dashed line curves represent the data predicted by Moyano et al. (2013).

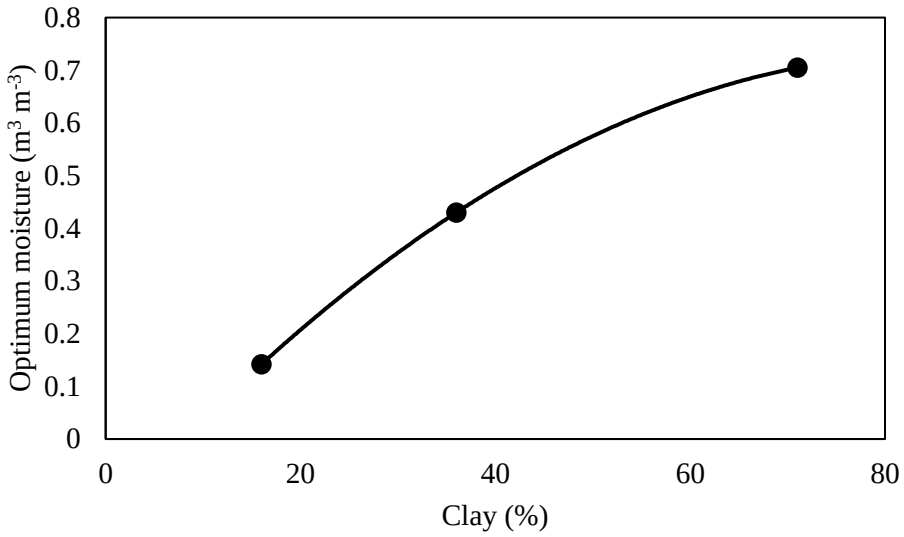


Figure 2.3. The optimal soil volumetric moisture content for highest microbial respiration as a function of clay percentage.

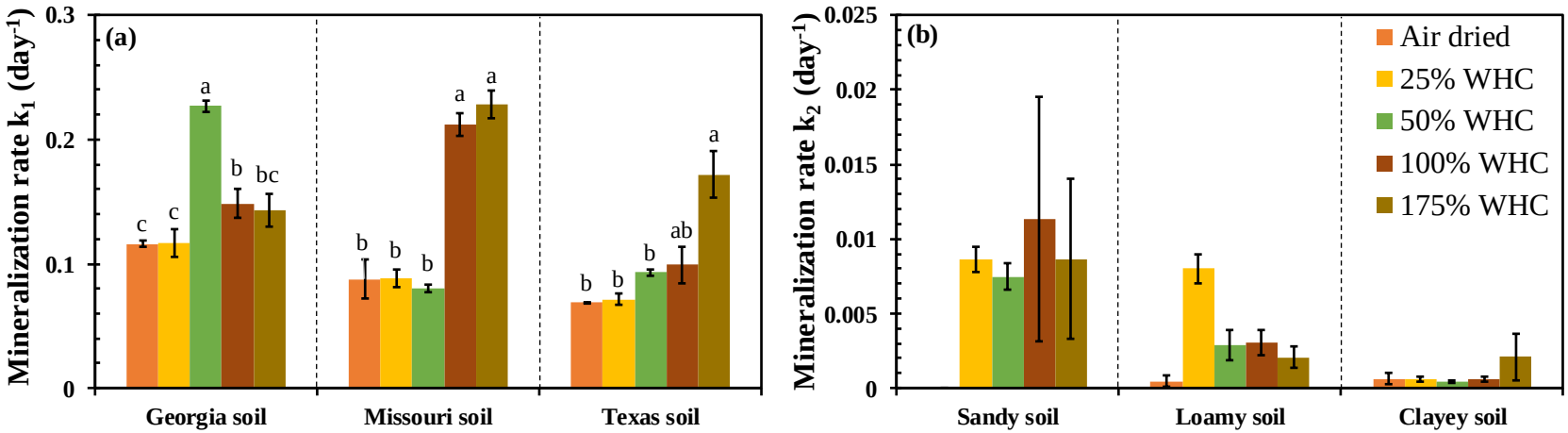


Figure 2.4. Effect of soil moisture levels on k_1, the mineralization rates of active pool (a) and k_2, the mineralization rate of slow pool (b) in sandy, loamy and clayey soils.
Different letters represent statistical significance at $P \leq 0.05$ within each soil type. No letters mean no statistical significance. Error bars represent standard error (n=3).

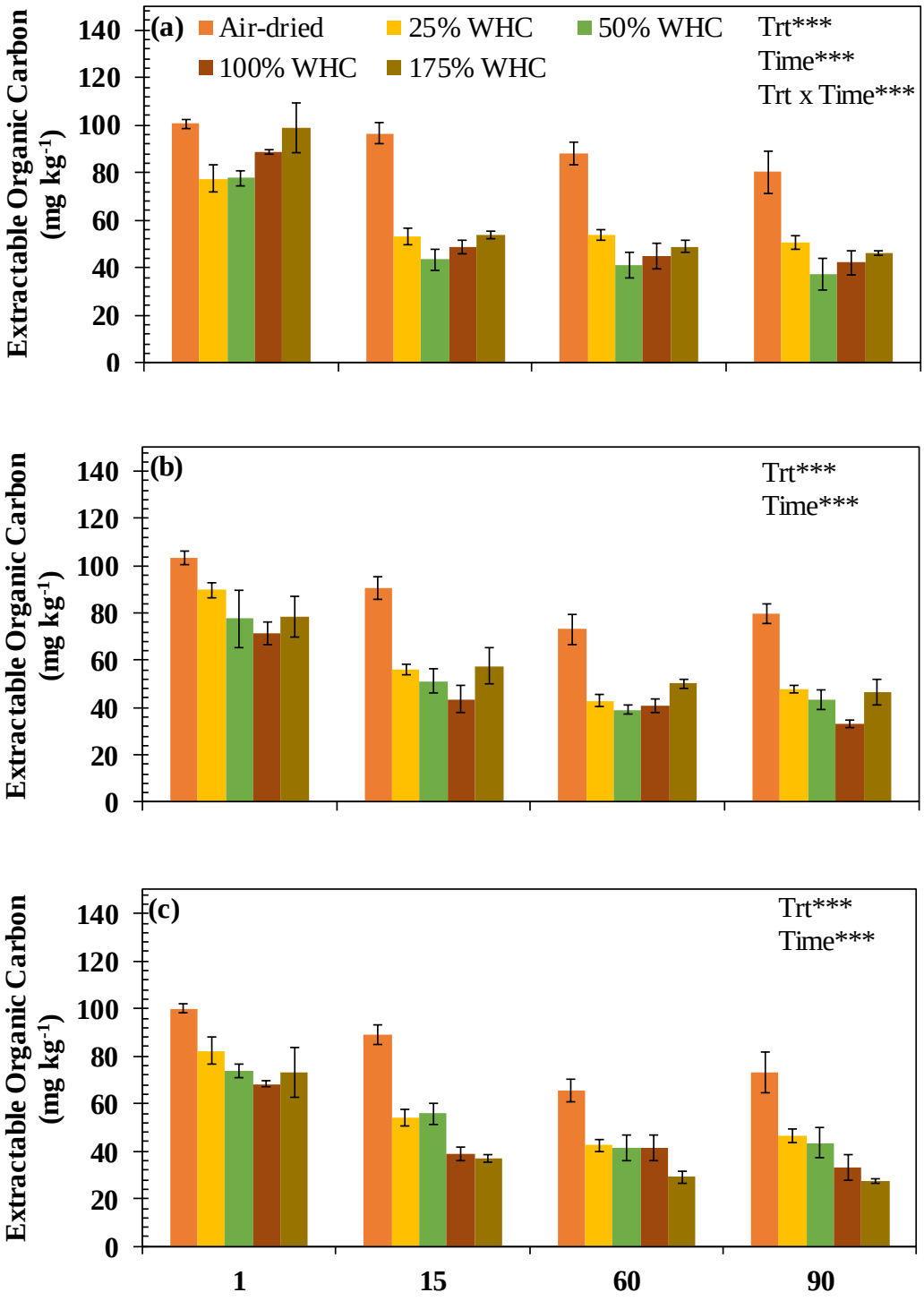


Figure 2.5. Extractable organic carbon concentrations influenced by moisture treatments in (a) sandy soil, (b) loamy soil, and (c) clayey soil at each destructive sampling time.
Error bars represent the standard errors (n=3). *** denotes P < 0.001, * denotes P < 0.05 for the effects of treatment (Trt), time, and treatment and time interaction (Trt x Time).

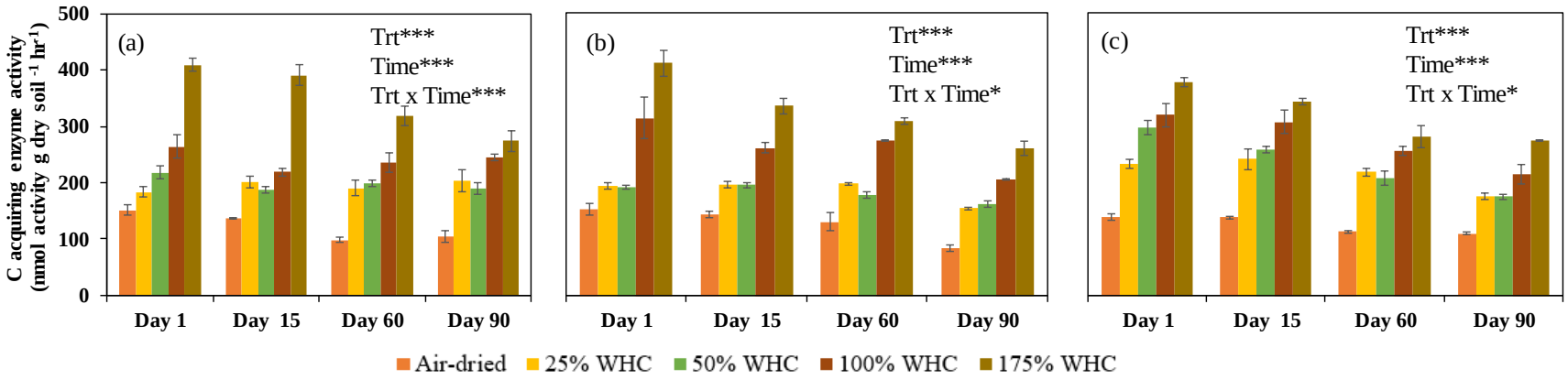


Figure 2.6. Activity of sum of four C-acquiring extracellular enzymes in sandy (a), loamy (b), and clayey (c) soils at each destructive sampling time.
Error bars represent the standard errors (n=3). *** denotes $P < 0.001$, * denotes $P < 0.05$ for the effects of treatment (Trt), time, and treatment and time interaction (Trt x Time).

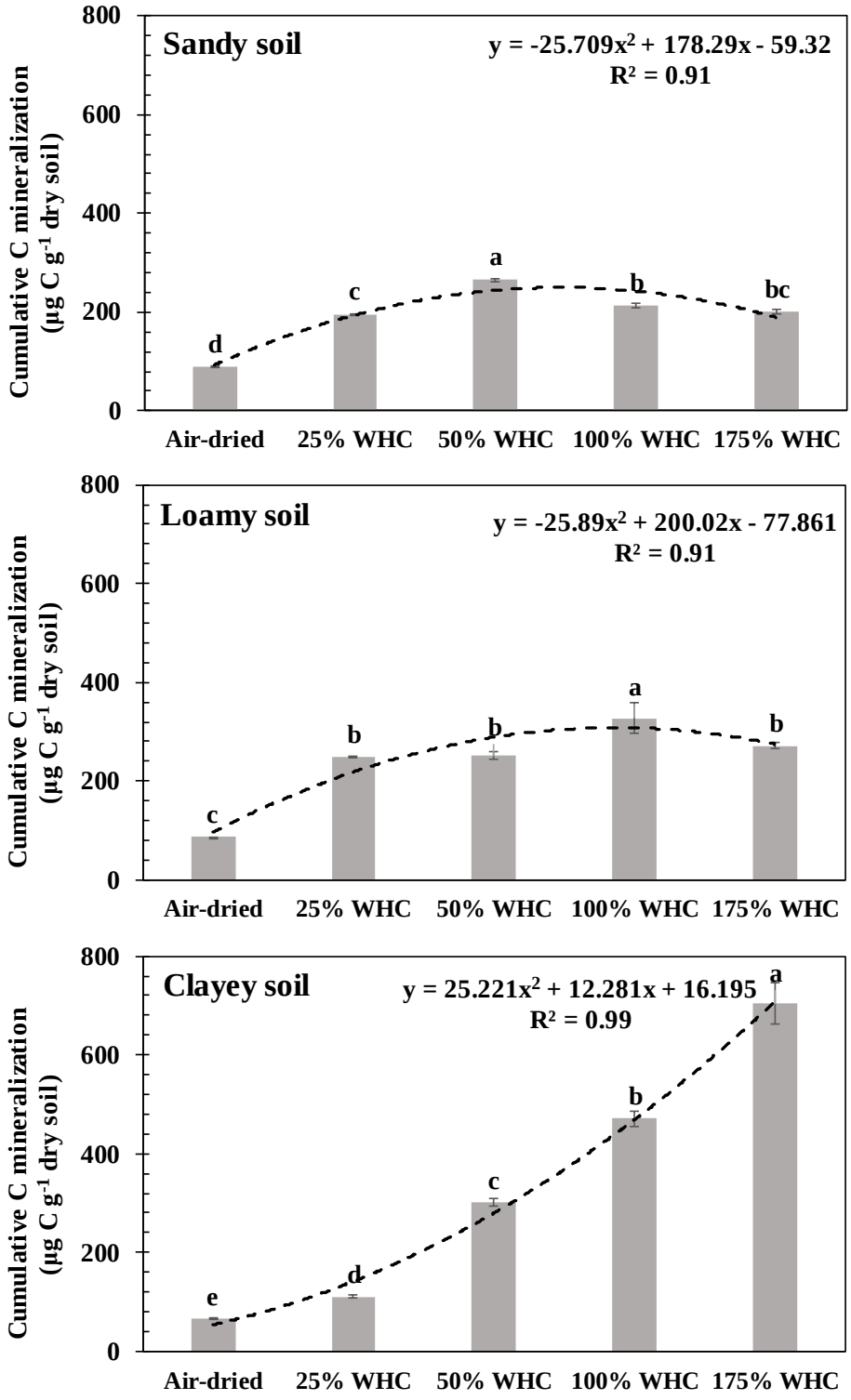


Figure S1. Cumulative C mineralization per unit dry soil after day 90 from sandy, loamy and clayey soils in response to increasing soil moisture.
Error bars represent standard errors (n = 3). Different letters represent statistical significance at $P < 0.05$.

Table 2.1. Properties of the soils used in the study.

Properties	**Sandy Soil**	**Loamy Soil**	**Clayey Soil**
Location	Taylor County, GA	Boone County, MO	Bowie County, TX
Soil series	Cowart	Weller	Buxin
Soil order	Ultisols	Alfisols	Vertisols
Sand (%)	77 ± 0.3	13 ± 0.2	15 ± 0.1
Silt (%)	7 ± 0.2	51 ± 0.6	14 ± 0.5
Clay (%)	16 ± 0.4	36 ± 0.6	71 ± 0.2
Texture	Sandy	Silt loam	Clayey
Porosity (%)	73.1 ± 0.003	67.5 ± 0.004	73.4 ± 0.001
Bulk density (Mg m^{-3})	0.71 ± 0.01	0.86 ± 0.01	0.70 ± 0.01
pH	4.4 ± 0.11	5.0 ± 0.04	5.9 ± 0.11
Water holding capacity (%)	40 ± 0.7	50 ± 1.2	65 ± 1.6
Soil organic C (g kg^{-1})	13 ± 0.10	9.3 ± 0.08	16 ± 0.12
Extractable organic C (mg kg^{-1})	88 ± 2.7	86 ± 0.9	80 ± 1.8
Microbial biomass C (μg g^{-1} dry soil)	156 ± 7.8	206 ± 8.5	378 ± 27

Values are mean ± standard error (n = 3).

Table 2.2. Repeated measures ANOVA statistics (P value) for soil moisture and time effects on microbial respiration.

Soil type	Moisture	Time	moisture x Time
Sandy	<0.0001	<0.0001	<0.001
Loamy	<0.0001	<0.0001	<0.001
Clayey	<0.0001	<0.0001	<0.001

Table 2.3. Multiple linear regression metrics for microbial respiration.

Parameters	Partial R^2	slope
Clay content	0.08	2.3
Soil moisture content	0.04	1.2
Soil organic carbon	0.01	3.7

Table 2.4. Effects of soil moisture on soil organic carbon pool sizes as a percentage of initial soil organic carbon.

Soils	Moisture Treatments	Active SOC Pool (C_1)	Slow SOC pool (C_2)
		----------(%)----------	
Sandy	Air-dried	0.61± 0.07 a†	99.39 ± 0.07 b
	25% WHC	0.31 ± 0.03 b	99.69 ± 0.03 a
	50% WHC	0.36 ± 0.03 b	99.64 ± 0.03 a
	100% WHC	0.42 ± 0.05 b	99.58 ± 0.06 a
	175% WHC	0.34 ± 0.04 b	99.66 ± 0.04 a
Loamy	Air-dried	0.78 ± 0.06 a	99.22 ± 0.06 c
	25% WHC	0.48 ± 0.09 b	99.52 ± 0.09 b
	50% WHC	0.35 ± 0.02 bc	99.65 ± 0.02 ab
	100% WHC	0.31 ± 0.02 bc	99.69 ± 0.02 ab
	175% WHC	0.30 ± 0.05 c	99.70 ± 0.06 a
Clayey	Air-dried	1.43 ± 0.14 a	98.57 ± 0.14 c
	25% WHC	0.67 ± 0.03 b	99.33 ± 0.03 b
	50% WHC	0.65 ± 0.03 b	99.35 ± 0.03 b
	100% WHC	0.49 ± 0.02 bc	99.51 ± 0.02 ab
	175% WHC	0.29 ± 0.06 c	99.71 ± 0.06 a

Values are mean ± standard error (n = 3).
†Letters represent statistical significance at $P \leq 0.05$ within a column and soil type.

Table 2.5. Linear regression statistics of moisture sensitivity of C acquiring extracellular enzyme assays.

Days	Soils	Slope	Intercept	R^2	*P* value
1	Sandy	2.6	158	0.99	0.0002
	Loamy	2.7	149	0.96	0.0032
	Clayey	2.1	179	0.83	0.0303
15	Sandy	2.4	144	0.95	0.0043
	Loamy	1.9	155	0.96	0.0038
	Clayey	1.8	178	0.82	0.0342
60	Sandy	1.9	144	0.85	0.0271
	Loamy	1.7	152	0.84	0.0278
	Clayey	1.4	153	0.73	0.0630
90	Sandy	1.3	157	0.66	0.0923
	Loamy	1.6	112	0.88	0.0182
	Clayey	1.5	125	0.91	0.0116

Slopes and intercepts are not statistically significant.

Table S1. Mantel test statistics for extracellular enzyme activity and microbial community composition in the sandy, loamy and clayey soils.

Soil types	R^2	*P* value
Sandy soil	0.067	0.13
Loamy soil	0.008	0.39
Clayey soil	0.013	0.35

Table S2. The PERMANOVA results for microbial community structure in the sandy, loamy, and clayey soils.

Soil types	R^2	*P* value
Sandy soil	0.042	0.35
Loamy soil	0.024	0.41
Clayey soil	0.110	0.12

Chapter 3. Soil organic carbon mineralization under transient and steady state moisture conditions in different textured soils

Abstract

Global climate change is predicted to intensify temperature and precipitation extremes, exposing soils to more frequent and/or severe alternating wetting and drying regimes. Rewetting a dry soil causes a burst of respiration called the “Birch effect”. This spike in respiration can be attributed to: (i) release of cellular solutes (metabolites) accumulated during drought due to rapid increase in water potential upon re-wetting; (ii) sudden death of certain microbes serving as carbon (C) sources for surviving microbes, leading to microbial community shifts; and (iii) release of physically protected C for microbial mineralization due to aggregate breakdown upon repeated drying and wetting. The relative importance of these mechanisms may change in different soils and the extent to which these mechanisms influence the Birch effect in different soil textures is poorly understood. In this study, I evaluated the effects of repeated drying and wetting cycles (transient state moisture conditions) on Birch effect and elucidate the underlying mechanisms that contribute to the same in different textured soils in comparison to a steady state moisture condition at 55% water holding capacity (WHC). Soil samples of three distinct textures (sandy, loamy and clayey) were incubated for a period of 140 days under five alternate cycles of drying (10% WHC) and wetting (100% WHC) conditions. Soil respiration was measured 52 times in 140 days. Destructive harvests were conducted on days 1, 29, 33, 56, 112, 116, and 140 in transient incubation while on days 1, 33, 116, and 140 in steady state incubation. Microbial biomass C (MBC), extractable organic C (EOC), metabolites, microbial community structure, and changes in aggregate associated C were determined at each destructive harvest time point. Results showed that soil texture strongly influenced respiration rates throughout the incubation as sandy soil showed the lowest respiration rate followed by loamy and clayey soils. Also, cumulative SOC loss was higher under transient moisture state compared to steady state. My

results showed that different mechanisms dominated the Birch effect in different soils. In sandy soils, metabolite accumulation and changes in microbial community structure contributed to the Birch effect while in loamy and clayey soils, metabolite accumulation and release of aggregate protected C contributed to the Birch effect. Deciphering the mechanisms underpinning increased SOC turnover from different soils upon wetting and drying is of interest to improve the understanding of terrestrial C cycling in response to extreme events.

Introduction

Earth system models predict frequent extreme weather events with more intense drought and more variable precipitation intensities at regional and global scales (Jentsch et al., 2007; IPCC, 2013), resulting in repeated drying and wetting of soils (Planton et al., 2008). These drying and rewetting cycles strongly influence the microbial transformations of terrestrial soil organic carbon (SOC) (Knapp et al., 2008; Wu et al., 2010). As soil respiration is the second largest source of terrestrial carbon (C) flux (Bond-Lamberty and Thomson, 2010), it is critical to elucidate how microbial respiration responds to repeated drying and wetting. Water limited conditions decrease microbial respiration (Muhr et al., 2010) by inhibiting substrate diffusion and accessibility for microbes (Voroney and Heck, 2015), as well as by causing microbial dormancy (Pulleman and Tietema, 1999). On the contrary, wetting of these dry soils will lead to a large increase in respiration, called the "Birch effect" (Birch, 1958; Fierer and Schimel, 2003; Kim et al., 2011; Xu et al., 2004). Therefore, transient soil moisture conditions created by the repeated drying and wetting cycles is considered as one of the crucial factors influencing soil C fluxes. Currently, the specific mechanisms underlying the Birch effect are unclear.

Kim et al. (2011) attributed the Birch effect as a contributor to substantial emissions of C from terrestrial ecosystems to atmosphere. Several mechanisms could potentially increase substrate availability after wetting, leading to the Birch effect. These mechanisms include, (i) cell lysis due to desiccation under drought releases cellular materials which become available upon re-wetting (Griffiths and Birch, 1961; Jager and Bruins, 1975; Orchard and Cook, 1983; Scheu and Parkinson, 1994), (ii) microbial osmolyte accumulation during drought and release with the rewetting flush (Fierer and Schimel, 2002, 2003), (iii) disruption of macroaggregates by repeated drying-wetting cycles, which expose aggregate-protected C for microbial uptake (Denef et al.,

2001), and (iv) sudden change in the bacterial and fungal population abundance in response to water availability (Scheu and Parkinson, 1994). To date, there is very limited information that supports the concept of microbial cell lysis and release of microbial necromass due to wetting (Schimel, 2018). Some studies reported the accumulation of compatible solutes (osmolytes) inside microbial cells under water limitation that help in decreasing the solute potential and maintain turgor. These osmolytes include small sugars, amino acids, quaternary ammonium compounds and pyrimidine derivatives (Csonka, 1989; Hasegawa et al., 2000; Wood et al., 2001) and previous studies have supported the theory of osmolyte accumulation (Slessarev et al., 2020; Slessarev and Schimel, 2020). These solutes are potentially released to soil upon re-wetting a dry soil leading to greater availability of C for microbial respiration. However, this mechanism has been questioned by other studies (Williams and Xia, 2009; Boot et al., 2013; Kakumanu et al., 2013; Warren, 2014). Also, upon rewetting of dry soils, connectivity of water films increases (Smith et al., 2017), increasing microbial access to substrates, potentially generating a pulse of respiration. Additionally, disruption of macroaggregates upon repeated drying and wetting tends to release the occluded C in soil for microbial uptake (Denef et al., 2001; Consentino et al., 2006). In reality, multiple mechanisms could simultaneously contribute to the Birch effect and the relative contribution of these mechanisms may change based on soil biogeochemical properties including texture, structure, SOC content, and microbiota (Sanaullah et al., 2011; Xiang et al., 2008). Though several studies reported the occurrence of the Birch effect, the specific mechanism(s) leading to SOC turnover under dry and wet phases have not been investigated in great detail.

Soil texture exerts a major control on SOC turnover during drying-wetting events due to its differential sensitivity to soil moisture changes (Barnard et al., 2020; Thomsen et al., 1999).

Cable et al. (2008) reported that magnitude of respiration pulse was higher in fine-textured soils than coarse-textured soils. This was mainly because of the differences in water holding capacity, pore size distribution, and pore connectivity of fine-textured soils that prolonged the moisture availability to microbes (Franzluebbers et al., 2000; Jobbágy and Jackson, 2000; Taylor et al., 2002). Additionally, different textures may be differently susceptible to aggregate breakdown and release of C substrates upon extreme moisture changes. The activities of enzymes in different pore size classes can also influence the depolymerization and transfer of substrate to microbes. Soil microbial community structure could also be altered differently in different soils by repeated drying and rewetting, thereby altering the magnitude of C respiration pulse (Meisner et al., 2015). Despite the key role in SOC decomposition, the extent of textural control and the associated mechanisms contributing to Birch effect are poorly understood (Harrison-Kirk et al., 2013; Thomsen et al., 1999). The relative importance of these multiple mechanisms and their interactions are still a source of uncertainty for projecting the magnitude of C emissions in response to soil moisture extremes (Falloon et al., 2011; Suseela et al., 2012).

This study aims to quantify the Birch effect in differently textured soils and examine the mechanism(s) contributing to it under transient moisture conditions in comparison to steady state moisture condition. The specific objectives of this study are: (i) to understand the sensitivity of soil organic C mineralization to transient state moisture conditions compared to steady state moisture condition in differently-textured soils, (ii) to understand the changes in microbial community structure and microbial physiology (metabolite accumulation) over time in response to transient and steady state moisture contents in differently textured soils, and (iii) to evaluate if aggregate protected C contributes to difference in C mineralization under transient and steady state moisture conditions in different soils.

I hypothesized that (i) cumulative C mineralization will be higher in transient than steady state moisture regimes, (ii) metabolite accumulation will be greater under dry-phase of the transient moisture condition, leading to elevated Birch effects upon rewetting, (iii) microbial community structure will change more under transient state moisture than under steady state moisture conditions and these changes will drive the Birch effect, and (iv) frequent drying and rewetting breaks aggregates and this will contribute to Birch effect by exposing physically protected C to decomposition. By testing these different hypotheses in different textured soils, I aimed to identify the relative dominance of mechanism(s) for Birch effect in different soils under extreme moisture changes.

Materials and methods

Study sites

Three soils with different textures - sandy, loamy, and clayey - were collected from mixed forests located in the southern US. The sandy soil was collected from Taylor County in Georgia (32.54°N, 84.22°W) and belonged to the Cowart series (fine-loamy, kaolinitic, thermic Typic Kanhapludults). The predominant tree species comprising the forest are *Quercus alba, Acer saccharum, Quercus velutina, Juniperus virginiana, Carya* spp., *Pinus clausa, Nyssa sylvatica, Magnolia grandiflora,* and *Liriodendron tulipifera*. Mean annual temperature and precipitation at this site is 20°C and 1200 mm, respectively. The loamy soil belonged to the Weller series (fine, smectitic, mesic Aquertic Chromic Hapludalfs) and was collected from the Missouri Ozark AmeriFlux (MOFLUX) site in central Missouri (38.74°N, 92.20°W) in an upland oak-hickory forest with major tree species consisting of *Quercus alba, Carya ovata, Acer saccharum* and *Juniperus virginiana* (Gu et al., 2015; Wood et al., 2018). Mean annual temperature and precipitation at this site is 12°C and 986 mm, respectively. The clayey soil

belonged to the Buxin series (very-fine, smectitic, thermic Aquic Hapluderts) and was collected from Bowie County, Texas (33.44°N, 94.48°W). At this site the predominant tree species are *Pinus taeda*, *Pinus echinata*, *Quercus* spp., *Carya* spp., and *Cupressus* spp. Mean annual temperature and precipitation at this site is 17°C and 900 mm, respectively. Basic properties of these three soils are reported in Table 3.1.

Soil sampling and characterization

Soil cores of 5 cm diameter were collected during summer 2019 from several random locations at each site from 0-15 cm depth and mixed to obtain a composite sample per site (Jagadamma et al., 2014; Mavi et al., 2012). Prior to soil sampling, the uppermost litter layer was removed. The soil samples were transported to the research laboratory at the University of Tennessee, Knoxville in coolers with dry ice within a day or two. Three additional cores were sampled from each site and the water holding capacity (WHC) for each soil was determined using pressure plate extractors (Klute and Dirksen, 1986). One section of the samples was air-dried for the initial soil characterization (n = 3). Soil texture was determined by the Bouyoucos hydrometer method (Gee and Or, 2002) and soil pH was measured in a 1:2 soil:water suspension (Thomas, 1996) using a pH meter (Mettler Toledo, Columbus, Ohio). Total C was determined on pulverized soil samples by the dry combustion method using a TOC Variomax analyzer (Elementar, Hanau, Germany). Extractable organic C (EOC) was determined based on the method by Jones and Willett (2006). Briefly, soils were mixed with 0.5 M K_2SO_4 (1:4 soil:solution), shaken at 200 revolutions per minute for 1 hour, and centrifuged at 3500 rpm for 3 minutes. The supernatant was passed through a 0.45 μm filter paper (Whatman No. 42) and analyzed using a Vario TOC cube CN analyzer in liquid mode (Elementar, Hanau, Germany). Microbial biomass C (MBC) was determined using the chloroform fumigation extraction method

(Vance et al., 1987) as the difference in C concentration in non-fumigated (EOC) and fumigated soil samples (extracted using 0.5 M K_2SO_4) and dividing that with kEC (extraction efficiency) value of 0.45 (Beck et al., 1997).

Incubation experiment setup

Two sets of incubation experiments were conducted for this study, viz., transient state and steady state. The transient state incubation comprised of five cycles of soil drying (~10% WHC) and wetting (100% WHC) while a constant soil moisture (55% WHC) level was maintained for the steady state experiment (Fig. 3.1). After sieving the soils, samples were air-dried till they reached a moisture level of ~10% WHC. Soil samples were then incubated at 25°C under either transient or steady-state moisture status. For the transient-state experiment, air-dried soils (60 g dry weight equivalent) were incubated in glass mason jars and soil respiration was measured every day for 3 days (gas sampling and measurement details are provided in section 2.4). Following this, MilliQ water was added to the soil to reach WHC of each soil and incubated for 3 additional days. Headspace gas samples were taken every 3 hours (for 12 hours) after rewetting and then every day for 3 days and analyzed for CO_2. To repeat 5 drying and rewetting cycles, soil samples were allowed to dry following the 3 days of rewetting and until the moisture content reached 10% WHC. A total of 21 replicates per soil type were prepared for transient state incubation experiment to destructively harvest three replicates at day 1, day 29, day 33, day 56, day 112, day 116, and day 140 of the incubation. Out of these seven destructive harvest times, day 33 and day 116 were during the wet phases while others were dry phases of the transient state experiment. Simultaneously, another set of jars were set up at steady state moisture level of 55% WHC and headspace samples were collected on a weekly basis. To maintain soil moisture at 55% WHC during the incubation, water loss was monitored on a weekly basis by weighing the

jars and corrected by adding MilliQ water as necessary. A maximum of 0.1-0.2 mL of water was added every week. For steady state incubation experiment, a total of 12 replicates were prepared to destructively harvest three replicates at day 1, day 33, day 116, and day 140 of the incubation. Soils collected from destructive harvests from both experiments were frozen at -20 °C pending analysis. These soil samples were later analyzed for EOC, MBC, metabolites, aggregate associated C changes, and microbial (bacterial and fungal) community composition.

Gas sampling and CO_2 measurements

Gas samples were collected by inserting a syringe needle (15 mL) into the septum on the jar lid and transferred to evacuated exetainer vials (12 mL) (Labco, United Kingdom). The headspace was mixed before withdrawing gas samples by pumping the syringe a few times. After gas sample collection, the jar lids were opened and aerated using a hand-held fan for one minute such that the gas concentration returned to ambient CO_2 levels, before being sealed. For blank correction, gas samples were collected from triplicate empty (no soil containing) jars at all time points of sample collection. The CO_2 concentration in the gas samples were measured using a flame ionization detector on a gas chromatograph (Shimadzu GC-2014, Japan). Microbial respiration rate was calculated using linear response curve of change in CO_2 concentrations over time (Curiel-Yuste et al., 2007).

Metabolite extraction and analysis

Soil samples that were destructively harvested from the transient and steady state experiments were analyzed for metabolites. The analysis was conducted at the University of Tennessee Chemistry Core (Knoxville, TN, USA). Soil samples, stored at -20 °C, were ground using liquid nitrogen for rapid quenching and immediately extracted at 4 °C in a cold room with pre-chilled solvent (1300 µL of 40:40:20 HPLC grade acetonitrile: methanol: water with 0.1 M

formic acid). After extraction, the supernatants were dried under a stream of nitrogen and resuspended in 300 µL of MilliQ water before analysis (Lu et al., 2008). Samples were immediately analyzed for metabolite concentrations using a mass spectrometer. The mass spectrometer was run in full scan mode following Lu et al. (2008). The chromatographic eluent was ionized through electrospray ionization source under negative mode and coupled to an Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, United States) at University of Tennessee Chemistry Core (Knoxville, TN, USA). Metabolites were identified and integrated using known masses and retention times. The numbers were reported as relative abundances to the highest abundant ion for that metabolite.

Microbial community composition

I extracted DNA from 0.25 g of soil using the DNeasy PowerSoil Pro Kit (Qiagen, Valencia, California, USA) following the manufacturer's suggested protocol with slight modifications. DNA was quantified using a Qubit Fluorometer and was standardized to approximately 10 ng μL^{-1} for each sample. An approximately 450 base region of 16S bacteria DNA was amplified with bacteria specific primers S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Klindworth et al., 2013) modified with adapters on the 5' end for the Illumina MiSeq platform (Illumina Corporation, San Diego, CA, USA). Each polymerase chain reaction (PCR) contained: 21.5 µl of Platinum PCR Supermix (Invitrogen, Carlsbad, CA, USA), 1.25 µl of each primer (10 µM), 0.5 µl of BSA (20 mg ml^{-1}), and 2 µl of DNA. The reactions ran with a hot start at 95°C for 5 min, 25 cycles of 95°C for 60 s, 55°C for 2 min, 72°C for 60 s and a final extension step of 72°C for 7 min. Fungal community composition was determined by targeting ITS region and the primers used were 5.8S-Fun (5'- AACTTTYRRCAAYGGATCWCT-3') and ITS4-Fun (5'- AGCCTCCGCTTATTGATATGCTTAART-3') (Taylor et al., 2016). The reactions ran with

a hot start at 96°C for 2 min, 35 cycles of 94°C for 30 s, 58°C for 40 s, 72°C for 2 min and a final extension step of 72°C for 10 min. For each sample, PCR was run in duplicate. Results from PCR were confirmed by running each sample on a 1% agarose gel. Initial PCR products with the Illumina adapters were cleaned with AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). The samples were then pooled and sequenced as 2 x 300 bp reads on one lane of an Illumina MiSeq v3 sequencer at the University of Tennessee Genomics Core (Knoxville, TN, USA).

Divisive Amplicon Denoising Algorithm 2 (DADA2), a parametric model, was used to infer true biological sequences from reads (Prodan et al., 2020) and was run as an R script (in R v.3.6) (R Development Core Team, 2011) using its R package dada2 v.1.7. For 16S sequences, the sequences were quality filtered using the "filterAndTrim" function. Error rates were determined from a set of subsampled reads and also estimated separately for each sequencing run. Reads were then deduplicated, chimeras were removed using "removeBimeraDenovo" and Amplicon Sequence Variants (ASVs) were inferred. I then used the Ribosomal Database Project (RDP) classifier against the RDP 16S bacteria to assign taxonomy to each ASV. Samples were then normalized as relative abundances for statistical analyses. Similar pipeline was used for ITS (https://benjjneb.github.io/dada2/ITS_workflow.html). Due to high variability of the ITS region, all possible orientations of forward and reverse primers were removed from forward and reverse sequences using "cutadapt" function. The UNITE database (Kõljalg et al., 2013) was used to assign taxonomy to each ASV. Sequences were deposited in the NCBI Sequence Read Archive (SRA).

Aggregate size distribution analyses

Aggregate size separation was conducted by the wet sieving method adapted from Elliott (1986). Briefly, 25 g of soil sample (<8 mm) was sieved through a series of three sieves to obtain

four aggregate size fractions: >2000 μm (large macroaggregates), 250-2000 μm (small macroaggregates), 53-250 μm (microaggregates) and <53 μm (silt and clay sized fractions). The soil sample was submerged into water at room temperature on top of the 2000 μm sieve for ~5 minutes before sieving. After that, the sieve was moved manually up and down 50 times during a period of 2 min. Water along with the soil that passed through the sieve was poured onto the next smaller size sieve and same procedure was repeated (Denef et al., 2001). The aggregate fractions retained on the sieves were oven dried at 50°C for 24 h, weighed and stored in glass vials. Mean weight diameter (MWD) was calculated using Equation 1 (Youker and McGuinness, 1957).

$$\text{MWD} = \sum_{i=1}^{n} x_i w_i \quad (1)$$

where, x_i is the mean diameter of aggregates size fraction on each sieve size (mm), w_i is the weight of aggregates retained on top of each sieve size and n is the number of aggregate size fractions. Aggregate associated SOC concentration in each aggregate size was determined using the Elementar CN analyzer (Elementar vario Max cube, Hanau, Germany).

Statistical analyses

Statistical analyses were conducted using SAS software v 9.4 (SAS Institute Inc., 2002). The effect of moisture (dry, wet, and steady) on CO_2 respiration rate over the incubation period for each soil was analyzed using repeated measures ANOVA with length of incubation as the repeated measure. To compare EOC, MBC, and aggregate associated SOC among soil moisture treatments on each day of destructive sampling, one-way ANOVA was used. Post hoc comparisons for determining the effect of soil moisture on soil respiration was performed using PROC GLIMMIX in SAS. The mean separation was done using Tukey's test. In all statistical tests, the mean differences were considered significant if $p \leq 0.05$.

To understand how the metabolite concentration and microbial community composition varied across various moisture levels in all soil types, permutational multivariate analysis of variance (PERMANOVA) was used in R (R Development Core Team, 2011) on the matrix of abundances of each microbial type and metabolites for all soil types and moisture treatments. The Bray-Curtis distance metric and 9999 permutations of residuals for the model were used with R vegan function adonis. Pairwise analysis was performed separately for dry wet, wet steady and dry steady moisture treatments. To identify the metabolites and microbial classes that were significantly enriched in either dry, wet or steady state moisture, I conducted an indicator species analysis in R (R Development Core Team, 2011). The indval score for each metabolite or microbial class is the product of the relative frequency and relative abundance with each soil moisture treatment. Only the metabolites or microbial classes with a *p* value less than 0.05 were considered significant. The diversity indices including Richness, Evenness, Inverse Simpson and Shannon's index were also determined using functions from the vegan package in R (R Development Core Team, 2011).

Results

Microbial respiration rate and cumulative CO_2 emissions

The microbial respiration rate peaked after every wetting cycle (Fig. 3.2a) in all the three soils and decreased gradually as the moisture depleted while drying. The peak respiration rate showed a gradual decrease from cycle 1 to 5 for all soils with mean values decreased from 20 μg C g^{-1} SOC day^{-1} to 4 μg C g^{-1} SOC day^{-1} for sandy soil, 37 μg C g^{-1} SOC day^{-1} to 14 μg C g^{-1} SOC day^{-1} for loamy soil, and 32 μg C g^{-1} SOC day^{-1} to 15 μg C g^{-1} SOC day^{-1} for clayey soils. I observed lowest respiration rate in sandy soil while no differences between loamy and clayey soils across the cycles. During the entire incubation period, the dry phases of dry-wet cycles

showed the lowest respiration rates in all three soils. In steady state incubation, I observed no differences in respiration rates during the first week of incubation, however following that, the respiration rate was highest in loamy soil followed by clayey soil and the lowest in sandy soil (Fig. 3.2b). As the incubation progressed, the respiration rates decreased from 10 μg C g^{-1} SOC day^{-1} to 0.8 μg C g^{-1} SOC $day^{-1}$for sandy soil, 16 μg C g^{-1} SOC day^{-1} to 5 μg C g^{-1} SOC day^{-1} for loamy soil, and 15 μg C g^{-1} SOC day^{-1} to 3 μg C g^{-1} SOC day^{-1} for clayey soil.

Over the incubation period of 140 days, transient state incubation averaged 127%, 35%, and 69% higher cumulative CO_2 emissions compared to steady state incubation in sandy, loamy and clayey soils, respectively (Fig. 3.2c). Also, across the three soils, the cumulative CO_2 emissions were the lowest for sandy soil compared to loamy and clayey soils under both moisture conditions.

Soil microbial biomass C and extractable organic C

Microbial biomass C (MBC) varied differently under transient and steady state moisture conditions (Figs. 3.3a and b). In transient state conditions, MBC was higher under the dry phases (days 1, 29, 56, 112 and 140) compared to the wet phases (days 33 and 116) in all soil types (Fig. 3.3a). The change in MBC under steady state condition showed a decreasing trend with time (Fig. 3.3b). Specifically, the sandy soil showed a decreasing trend from day 1 to day 116. For loamy soil, MBC on days 1 and 33 was similar and was significantly higher than that on days 116 and 140. For clayey soil, each consecutive destructive sampling showed a significant reduction in MBC. It is to note that sandy soil showed the lowest MBC compared to the loamy and clayey soils under both moisture conditions. The trends in EOC were the same as that of MBC under both moisture conditions (Fig. 3.3c and d). The dry phases under the transient incubation showed higher accumulation of EOC compared to the wet phases (Fig. 3.3c). In

steady state incubation, I observed a consistent and significant decrease in EOC with time (Fig. 3.3d). Also, clayey soils showed the lowest EOC compared to the sandy and loamy soils.

Aggregate structural change and aggregate associated C turnover

The breakdown of large aggregates into smaller aggregates was higher in transient state incubation with repeated drying and rewetting phases as compared to the steady state incubation (Fig. 3.4a, b, c). In general, sandy soils were less aggregated compared to loamy and clayey soils, indicated by 3% large macroaggregates compared to 74% in loamy and 73% in clayey soils. Consequently, sandy soils were dominated by small macroaggregates, which were broken down to <0.25 mm fractions over time (Fig. 3.4a). This disruption of smaller aggregates was slightly more evident under transient moisture condition than steady condition. In loamy and clayey soils, larger aggregates broke down at a much higher and faster rate into smaller fractions (Figs. 3.4b and c). Similar to sandy soils, aggregate disruption under steady state moisture condition was not as drastic as under transient condition in loamy and clayey soils. Consequently, the mean weight diameter decreased drastically as the incubation progressed under transient than steady state experiment, and the decrease was more prominent in loamy (Fig. 3.4e) and clayey soils (Fig. 3.4f) as compared to sandy soil (Fig. 3.4d). At the end of experiment (day 140), the mean weight diameter in sandy, loamy, and clayey soils was reduced by 21%, 82% and 81%, respectively, compared to the start of the incubation (day 1) under transient condition. However, under steady state condition, only clayey soil showed a decrease (16%) (Fig. 3.4f).

The change in aggregate associated C across the steady and transient conditions was different in the three soils (Fig. 3.4g, h, and i). For sandy soil, the change in aggregate associated C did not vary between the steady and transient incubations (Fig. 3.4g). However, at the end of

the incubation, there was a 29% and 28% decrease in the aggregate associated C of loamy and clayey soils, respectively, under transient than steady state incubation (Fig. 3.4h, i).

Metabolites

The NMDS ordination plots showed that the three soil textures (Fig. 3.5a) (R^2 = 0.032, p = 0.01) and three soil moisture states (steady state, and wet and dry phases of transient state) (Fig. 3.5b) (R^2 = 0.04, p = 0.006) significantly influenced the metabolite concentrations. Post hoc pairwise comparison analysis showed no differences in metabolite concentrations between dry and steady soil moisture (p = 0.08) but significant differences between steady and wet soil (p = 0.02). I found that 22, 19, and 12 metabolites were significantly affected by soil moisture in sandy, loamy, and clayey soils, respectively (Fig. 3.5d, e, and f). A general trend of higher concentration of most metabolites was observed under dry phase of transient condition (days 1, 29, 56, 112 and 14) compared to the subsequent wet phases (days 33, 116). Little to no change in metabolite concentrations was observed throughout the experiment under the steady state condition. Osmolytes such as disaccharides, myo-inositol, and biogenic amino acids were all enriched under dry treatments. Other metabolites such as homovanillic acid, hydroxyisovalerate, inosine, aconitic acid, and methyladenosine, which are markers of stressed metabolism, were also higher under dry treatment except adenosine that showed decrease under dry than wet conditions. In accordance with these results, the indicator species analysis results showed the highest number of indicator species under dry phase (13) compared to wet phase (6) of the transient experiment as well as the steady state experiment (1).

Changes in microbial community structure

Bacterial community composition

Bacterial community composition was influenced by soil texture ($p = 0.0001$) (Fig. 3.6a) and soil moisture ($p = 0.0039$) (Fig. 3.6b). Post hoc pairwise comparisons also showed that bacterial community composition of each texture and moisture level significantly differed from one another (Table 3.2a). I also observed more bacterial community changes in transient incubation compared to steady state incubation (Fig. 3.6c). Various diversity indices for the bacterial community composition were analyzed for sandy, loamy and clayey soils (Figs. 3.7, 3.8, and 3.9, respectively).

For sandy soil, I observed significantly higher richness, evenness, inverse Simpson index, and Shannon index in transient incubation on days 1 and 116 (Fig. 3.7). A total of 90 bacterial indicator ASVs were detected for sandy soil across dry, wet, and steady moisture content treatments (Fig. 3.7e). Major indicator ASVs for each moisture state (with corresponding phylum in parenthesis) include Acetobacteraceae (Proteobacteria) and Hyphomicrobiaceae (Firmicutes) under dry phases of transient experiment, Sphingomonadaceae (Acidobacteria) and Rhizomicrobium Proteobacteria) under wet phases of transient incubation experiment, and Actinomycetales (Actinobacteria), Alicyclobacillaceae (Acidobacteria), Bdellovibrionaceae (Firmicutes), Bradyrhizobiaceae (Acidobacteria), Caulobacteraceae (Proteobacteria), Oxalobacteraceae (Proteobacteria), and Rhizomicrobium (Proteobacteria) under steady state experiment.

For loamy soil, the richness of bacterial community showed no difference between transient and steady state incubation (Fig. 3.8a) while evenness was higher in steady state incubation compared to transient incubation (Fig. 3.8b) except on day 1. Both inverse Simpson

(Fig. 3.8c) and Shannon (Fig. 3.8d) indices were also higher under steady state than transient state, but only on days 116 and 140. For this soil, a total of 70 indicator species were identified across wet, dry, and steady moisture contents (Fig. 3.8e). Major indicator ASVs for each moisture state (with corresponding phylum in parenthesis) include Armatimonadaceae (Proteobacteria), Bdellovibrionaceae (Proteobacteria), Methylobacteriaceae (Armatimonadetes), and Chthonomonadaceae (Chloroflexi) under dry treatments, while, Actinomycetales (Proteobacteria), Rhizomicrobium (Actinobacteria), and Ktedonobacteraceae (Chloroflexi) under wet treatments, and Acidimicrobiales (Proteobacteria), Actinomycetales (Actinobacteria), Bradyrhizobiaceae (Acidobacteria), Caulobacteraceae (Proteobacteria), and Hyphomicrobiaceae (Acidobacteria) under steady state condition.

For clayey soil, no significant differences were observed between transient and steady state incubations for any of the indices (Figs. 3.9 a-d). A total of 28 bacterial indicator species were observed in clayey soil across dry, wet and steady state moisture conditions (Fig. 3.9e). Major indicator species for each moisture level (with corresponding phylum in parenthesis) belonged to Actinomycetales (Actinobacteria), Anaerolineaceae (Chloroflexi), Actinobacteridae (Acidobacteria), and Sorangiineae (Proteobacteria) for dry phase of transient experiment and Acidimicrobiales (Proteobacteria), Actinomycetales (Actinobacteria), Hyphomicrobiaceae (Acidobacteria), Rhodobiaceae (Gemmatimonadetes), Rhodospirillaceae (Chloroflexi), and Sphingomonadaceae (Acidobacteria) under steady state experiment. No prominent indicator species was observed under wet phase of the transient experiment.

Fungal community composition

Fungal community composition was also influenced by texture ($p = 0.0001$) (Fig. 3.10a) and soil moisture ($p = 0.0171$) (Fig. 3.10b). Post hoc pairwise comparisons also showed similar

results (Table 3.3) in which all textures and moisture levels were significantly different from each other. Temporal changes in fungal community composition showed that the fungal community at the end of the experiment (day 140) from the transient incubation was different than that in the earlier days in transient incubation as well as all days in steady state incubation (Fig. 3.10c).

For sandy and loamy soils, I found no significant differences in any of the diversity indices throughout the experiment (Fig. 3.11 a-d, Fig. 3.12 a-d). In sandy soil, 33 indicator species were identified. Major indicator species for each moisture level (with corresponding phylum in parenthesis) belonged to Russulaceae (Ascomycota), Sordariales (Ascomycota), and unidentified fungi (Unidentified fungi) under dry phase of the transient state incubation while only Rhizophlyctidaceae (Chytridiomycota) under wet treatment (Fig. 3.11e), and Agaricaceae (Basidiomycota), Thermoascaceae (Ascomycota), Trichocomaceae (Ascomycota), Umbelopsidaceae (Zygomycota), and some unidentified Chytridiomycota under steady state moisture condition. In loamy soil, a total of 50 indicator species were identified out of which no indicator species were observed under wet treatment (Fig. 3.12e). Major indicator species (with corresponding phylum in parenthesis) belonged to Gloniaceae (Ascomycota), Russulaceae (Basidiomycota), and Thelephoraceae (Basidiomycota) under the dry phase while unidentified Ascomycota, Clavicipitaceae (Ascomycota), Herpotrichiellaceae (Ascomycota), Hypocreaceae (Ascomycota), Umbelopsidaceae (Zygomycota), and Thelephoraceae (Basidiomycota) under steady state moisture condition.

For clayey soil, significant differences in evenness (Fig. 3.13b) and inverse Simpson index (Fig. 3.13c) were found between transient and steady state conditions. On day 1, transient incubation showed significantly higher evenness while on days 116 and 140, steady state

incubation showed significantly higher evenness. Similar trends were observed for inverse Simpson index except on day 33 when steady state incubation showed significantly higher inverse Simpson index. A total of 40 indicator species were observed under dry and steady state moisture conditions (Fig. 3.13e). Major indicator species (with corresponding phylum in parenthesis) belonged to Boliniaceae (Ascomycota), Glomeraceae (Glomeromycota), Herpotrichiellaceae (Ascomycota), Magnaporthaceae (Ascomycota), Meripilaceae (Basidiomycota), Microascaceae (Ascomycota), Nectriaceae (Ascomycota), and Paraglomeraceae (Glomeromycota) under dry phase of the transient incubation and Bionectriaceae (Ascomycota), Clavicipitaceae (Ascomycota), Cryphonectriaceae (Ascomycota), and unidentified Ascomycota, Chytridiomycota, and Basidiomycota under steady state moisture condition. No prominent indicator species was observed under wet phase of the transient experiment.

Discussion

Transient and steady moisture controls on microbial respiration and active C fractions

The results showed that microbial respiration rate under transient incubation peaked after every wetting event (Birch effect) and started to decrease immediately and continuously as the soils began to dry, showing evidence for the strong control of soil moisture on microbial activity (Blazewicz et al., 2014; Brockett et al., 2012; Cook and Orchard, 2008; Pulleman and Tietema, 1999). This finding is consistent with previous studies that tested the effect of wetting and drying on soil respiration (Consentino et al., 2006; Schindlbacher et al., 2012). I observed similar peak respiration rates upon wetting in cycles 2, 3, and 4, which indicate that the microbes are being exposed to additional C for metabolism when dried soils are re-wetted (Fig. 3.2a), while that was not the case with constant moisture in the steady state experiment (Fig. 3.2b) probably due to

depletion of substrates (Fang et al., 2005; Kirschbaum, 2006). Regardless, the cumulative CO_2 loss from transient incubation was higher than steady state incubation for all the soils (Fig. 3.2c) which is also reported in previous studies (Fierer and Schimel, 2002, 2003; Priemé and Christensen, 2001; Xiang et al., 2008) and is in accordance to my hypothesis. This implies that the decreased respiration rate during dry phase of the transient state experiment was greatly compensated by the Birch effect, so the cumulative respiration was higher compared to steady state moisture conditions.

My finding that MBC was higher under dry- than wet-phase of transient state experiment (Figs. 3.3a and b) and lower respiration rates add to the increasing body of evidence that microbes may undergo dormancy under dry conditions (Lennon and Jones, 2011; Alvarez et al., 1998; Placella et al., 2012; Aanderud et al., 2015; Salazar et al., 2018), and then quickly respond to re-wetting, leading to the Birch effect (Stenström et al., 2001; Blagodatskaya and Kuzyakov, 2013; Barnard et al., 2015; Salazar-Villegas et al., 2016). Also, microbes may die upon wetting of soils, leading to addition of substrates for the surviving microbes. These microbial metabolic changes have strong implications to C cycling (Bottner, 1985; Alvarez et al., 1998; Placella et al., 2012; Aanderud et al., 2015; Barnard et al., 2015; Salazar-Villegas et al., 2016). The increased concentration of EOC under water deficit conditions (Figs. 3.3c and d) imply reduced microbial processing of EOC, as reported by several studies (Blankinship et al., 2014; Manzoni et al., 2016; Schaeffer et al., 2017; Xiang et al., 2008; Homyak et al., 2018). Increase in EOC under dry conditions could also be due to the formation of extracellular polysaccharides (EPS) (Schimel, 2018; Lebre et al., 2017; Flemming et al., 2016) that prevent desiccation of microbial cells. I further investigated the possible mechanisms behind changes in the dynamics of C with drying-rewetting events leading to the Birch effect and are discussed in the following sections.

Possible mechanisms to explain Birch effect

Changes in aggregate associated C

In accordance with my hypothesis, repeated drying and wetting in the transient state experiment significantly reduced mean aggregate size, as indicated by the reduction in mean weight diameter (Fig. 3.4d-f), compared to the steady state condition. Macroaggregates that typically have more macropores are more susceptible to disruption than microaggregates due to faster water infiltration and greater number of planes of weakness (Dexter, 1988; Kay, 1990; Denef et al., 2001). Consequently, repeated drying and wetting events destabilize C protected within the macroaggregate structure for microbial respiration or redistribute the C to smaller-sized aggregates for stronger stabilization (Denef et al., 2001; Cosentino et al., 2006; Borken and Matzner, 2009; Navarro-García et al., 2012).

This breakdown of macroaggregates under repeated drying and wetting exposes more C and could be a potential mechanism for the Birch effect. Based on my results, this holds true only for loamy and clayey soils. For sandy soil, I did not observe any differences in the total aggregate associated C between steady and transient state incubation experiments (Fig. 3.4g) indicating that Birch effect in sandy soil, albeit the lowest (Fig. 3.2a) among the three soils, was not contributed by C release from aggregate breakdown. However, for loamy and clayey soils, I found evidence for decreased aggregate associated C from transient state experiment as compared to the steady state experiment towards the end of the experiment, indicating that C released by aggregate breakdown might have contributed to the Birch effect during the later cycles of re-wetting.

Accumulation of metabolites

In accordance to my hypothesis, greater accumulation of compatible solutes (metabolites) was observed in the dry phase of the transient state experiment in all the three soil types (Figs.

3.5d, e, and f). The accumulation of metabolites under water stress conditions was also reported by previous studies (Kieft et al., 1987; Halverson et al., 2000; Fierer and Schimel, 2003; Schimel et al., 2007). This demonstrates physiological response of microbial cells to drought conditions as a tradeoff against growth (by approximately 90%) by diverting their energy investment towards synthesis of compatible metabolites (Schimel et al., 2007; Killham and Firestone, 1984). When the soils are rewet, the microbial cells may undergo plasmoptysis, releasing the metabolites into the soils (Kempf and Bremer, 1998; Halverson et al., 2000; Welsh, 2000; Warren, 2019 and 2020) which can be assimilated rapidly by other microorganisms, contributing to Birch effect. Death of microbial cells upon rewetting was also reported by previous studies (Chowdhury et al., 2019; Malik et al., 2019; Slessarev et al., 2020; Warren, 2020). However, older studies such as Boot et al. (2013) and Kakumanu et al. (2013) found no evidence of osmolyte accumulation under water deficit conditions. I observed increased concentration of all metabolites under drier conditions except adenosine. Malik et al. (2019) also reported that adenosine, one of the growth metabolites, was lower under dry conditions. In addition to common osmolytes such as amino acids, simple sugars, alcohols, methylamines, and pyrimidine derivates, I also observed some metabolites (e.g., homovanillic acid, hydroxyisovalerate, inosine, aconitic acid, and methyladenosine), which are indicators of stressed metabolism in bacterial cells, as they undergo water limitation.

The metabolites results corroborated the trends observed in MBC and EOC. For example, higher concentration of metabolites in microbial cells under dry soils may be helpful in explaining the higher MBC I observed under dry soils (Fig. 3.3a). Conversely, lower MBC was observed after re-wetting, which can be attributed to microbial death as cells undergo uncontrolled influx of water to counteract the reduced solute potential, and eventually cells burst

and release more C substrates to soils (Halverson et al., 2000; Welsh, 2000; Warren, 2019) which consequently can be assimilated by microbes. Although metabolite accumulation under water deficit condition was consistent in all soil types, I found only 12 metabolites in clayey soil compared to 22 and 19 in sandy and loamy soils. This implies the possibility of moisture availability in the micropores of clayey soils even under drought conditions, helping microbes to not undergo complete stress as in other soils.

Change in microbial community structure

The results in general showed an increase in certain bacterial phyla under dry and wet phases of transient state moisture which was common in the three soils. Fierer et al. (2007) proposed that oligotrophs might be more competitive than copiotrophs under stress. This is because oligotrophs are able to survive under stressed conditions because of their resource efficient and slow growing life strategy compared to copiotrophs which tend to reduce under stressed condition. In addition, oligotrophs also invest more energy for the production of EPS (Eichorst et al., 2007). This reduction/death of certain microbial classes could lead to increased C addition in the soils that will be available for microbes to utilize when soils are rewet. On the other hand, the stress resistant microbes survive under stress albeit at low abundance as they are just able to maintain enough activity to survive stress conditions and invest more energy for EPS production. My observation of Acidobacteria under wet conditions is in agreement with Barnard et al., (2015). On the contrary, relative abundance of Actinobacteria and Firmicutes, thick-walled, Gram-positive, spore-forming, and EPS-producing bacterial groups, increased under dry conditions which was also reported by Zvyagintsev et al. (2007). Under dry conditions, the relative abundance of thick-walled, Gram-positive, spore-forming, and EPS-producing bacterial groups increase which are Actinobacteria and Firmicutes. Under dry conditions, I also observed

an increase in some Proteobacteria orders and decrease in thin-walled, Gram-negative groups such as Acidobacteria and Chloroflexi (Placella et al., 2012; Angel and Conrad, 2013; Zhou et al., 2016). My findings of more fluctuations in bacterial compared to fungal abundances with moisture changes is in accord with the general understanding that bacteria are more sensitive to changes in soil moisture than fungi (Manzoni et al., 2012). The increased abundance in Ascomycota found in my study under both dry and wet conditions which was due to more resistance to stress (Liu et al., 2019). My study also shows that oligotrophic life strategy enables the specific microbial phyla to sustain and survive under stressed conditions. These results suggest that change in fungal population may not contribute to Birch effect, however, change in bacterial population fueled the Birch effect in sandy soil and not in loamy and clayey soils.

My results demonstrated changes in bacterial and fungal community structure differently in different soils, therefore, indicating that texture influenced the microbial community under transient and steady state moisture conditions. In sandy soil, I observed higher bacterial diversity indices under transient incubation, therefore, my hypothesis that, microbial community structure will show considerable changes under transient state moisture than under steady state moisture conditions and these changes will drive the Birch effect, holds true for only bacterial community composition in sandy soils. The bacterial indicator species analysis revealed the indicator taxa under dry conditions belonged to Firmicutes phyla while that under wet conditions belonged to Acidobacteria (Fig. 3.7e). This trend of observing Firmicutes under dry conditions and Acidobacteria under wet conditions are also supported by Barnard et al. (2015), Liu et al. (2019) and Schimel (2020). The fungal indicator species under dry conditions belonged to Ascomycota phyla (Fig. 3.11e) which is reported to survive drought conditions more successfully (Schimel, 2020).

In loamy soil, I found higher evenness, inverse Simpson index, and Shannon index for bacterial community under steady state than transient state conditions, while no difference in fungal community diversity indices. My hypothesis that change in microbial community under transient state would be more pronounced than under steady state to fuel the Birch effect does not hold true for loamy soil. Most of the bacterial indicator species found under dry treatment (Fig. 3.8e) have been previously reported to increase under dry conditions (Barnard et al., 2015; Starke et al., 2016; Liu et al., 2019). Under wet conditions, Actinomycetales, Rhizomicrobium and Ktedonobacteraceae taxa increased and all belonged to Acidobacteria phylum. Fungal indicator taxa that increased under dry conditions belonged to Ascomycota and Basidiomycota (Fig. 3.12e), which previously have been reported to survive dry conditions (Andreo-Jimenez et al. 2019).

In clayey soil, my hypothesis that microbial community change will show significant changes under transient state compared to steady state incubation was rejected in clayey soil as in the case of loamy soil. The bacterial indicators in clayey soil belonged to Actinobacteria (Fig. 3.9e) under dry conditions while no indicators under wet conditions. Similar to loamy soils, fungal indicators under dry conditions belonged to Ascomycota and Basidiomycota (Fig. 3.13e). No changes in bacterial and fungal community composition with moisture changes in clayey soil could be attributed to presence of water film associated with micropores even under drought conditions. Micropores are less in loamy and sandy soils compared to clayey soils.

This study looked at various mechanisms contributing to Birch effect in sandy, loamy, and clayey soils. Sandy soil tends to dry faster than the loamy and clayey soils, due to which the soils are exposed to longer periods of drought as compared to loamy and clayey soils. For sandy soil, change in bacterial population and metabolite accumulation may have contributed to the

Birch effect. The loamy soil, in addition to metabolite accumulation and subsequent C release from their release to soil, release of C from the breakdown of macroaggregates may also have contributed to the Birch effect. In this soil, there was no change in bacterial and fungal populations in transient state experiment. Similar to loamy soil, clayey soil also showed metabolite accumulation and aggregate breakdown exposing more C as major mechanisms controlling the Birch effect. The higher clay content explains the possibility of these soils not undergoing extreme dry conditions (due to water in micropores) and extreme wet conditions (due to swelling of clay on adding water and creating aerobic conditions), which might have regulated microbial population change.

Conclusions

The large CO_2 pulse (Birch effect) that is produced upon rewetting of dry soils could be due to multiple mechanisms. This study investigated several mechanisms leading to Birch effect in different textured soils. Continued spike in respiration rates upon multiple re-wetting cycles revealed the availability of variable C sources contributing to the Birch effect. The results also indicated decline in EOC and MBC when respiration rates peaked after wetting of dry soils. I also evaluated three potential mechanisms driving Birch effect viz., (i) release of cellular metabolites upon rewetting, adding more C substrates to soils, (ii) physical breakdown of aggregates upon repeated drying and wetting, exposing unavailable C for microbial assimilation, and (iii) drastic changes in bacterial and fungal population under repeated drying and wetting, enhancing microbial activity. Results showed that for sandy soil, metabolite accumulation and bacterial population change fueled Birch effect while for loamy and clayey soils, aggregate breakdown and metabolite accumulation mechanisms contributed to Birch effect. The metabolites mechanism, being common in the three soils, indicates the energy costs of microbial

physiological adaptations to dry conditions against their growth. The results revealed that soil texture was a major controller of microbial activity under extreme moisture changes, which ultimately influenced the mechanisms driving the Birch effect.

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Appendix III

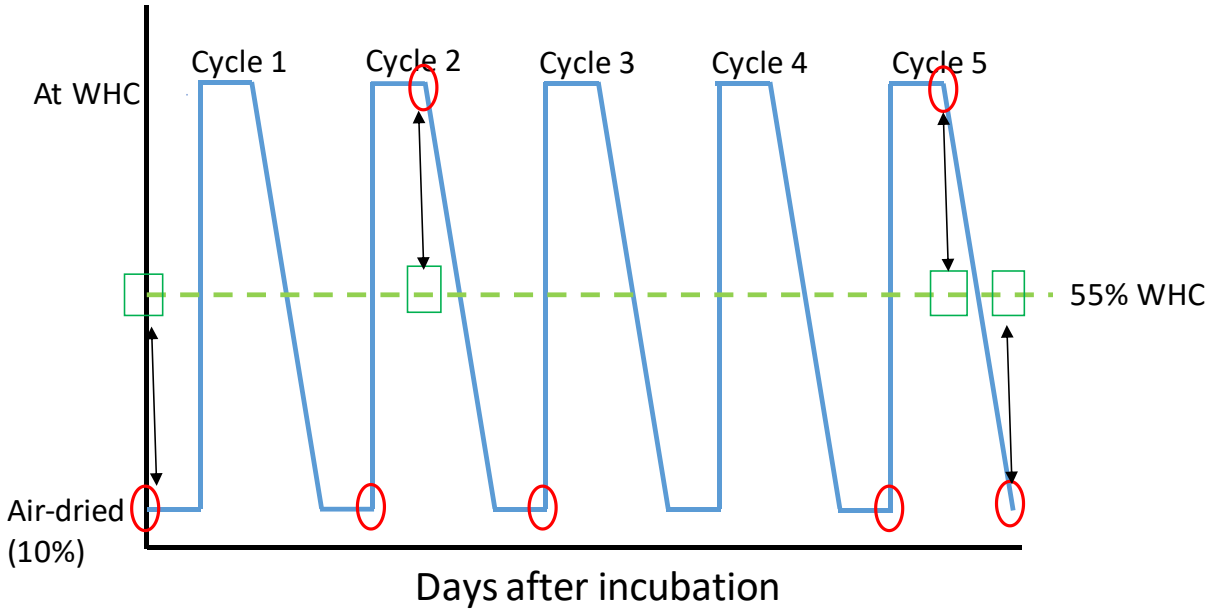


Figure 3.1. A schematic diagram of the experimental design showing the incubation at transient state (in blue) and steady state (in green dashed line) soil moisture conditions.
Red circles and green squares represent the destructive harvest time points in transient and steady state incubation experiments, respectively. Red circles represent days 1, 29, 33, 56, 112, 116 and 140 while green squares represent days 1, 33, 116 and 140. Black arrows highlight the concurrent destructive harvests in both the experiments.

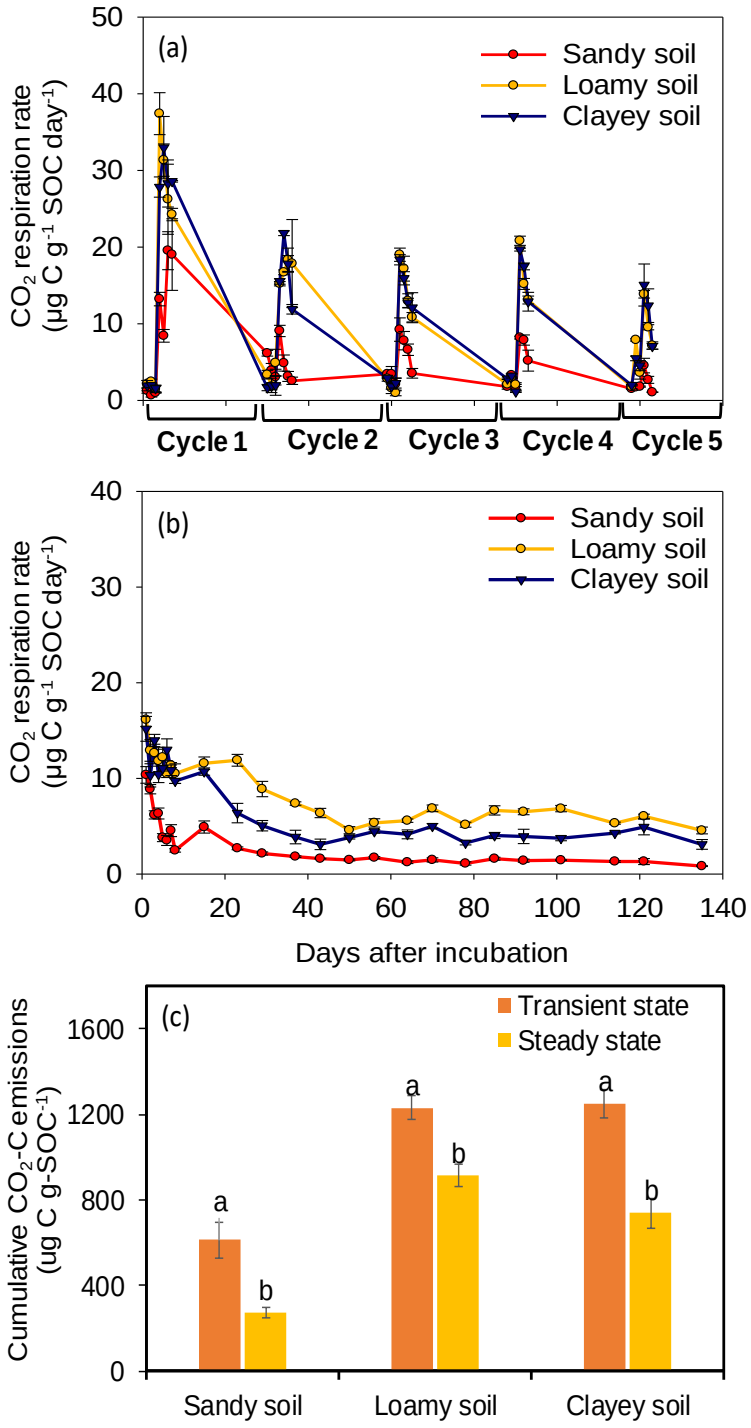


Figure 3.2. Microbial respiration rates in (a) transient state and (b) steady state incubation experiments. Cumulative CO_2-C emissions per unit SOC basis (c) after day 140 from the sandy, loamy, and clayey soils.
Error bars represent standard errors (n = 3).

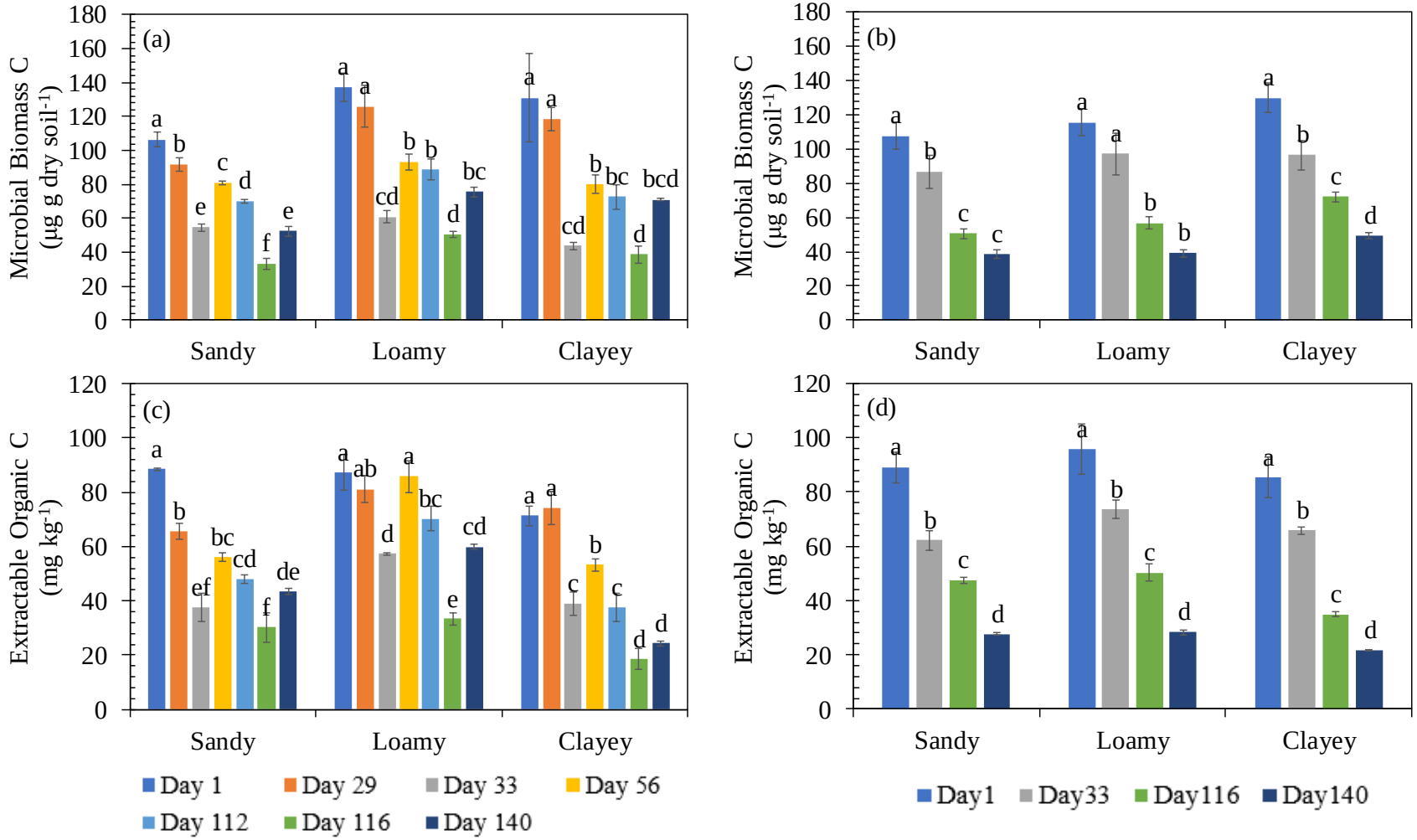


Figure 3.3. Microbial biomass C and extractable organic C from experiments under transient state (a, c) and steady state (b, d) moisture conditions.
Error bars represent standard errors (n = 3). Different letters represent statistical significance at $p \leq 0.05$ with each soil type. No letters mean no statistical significance.

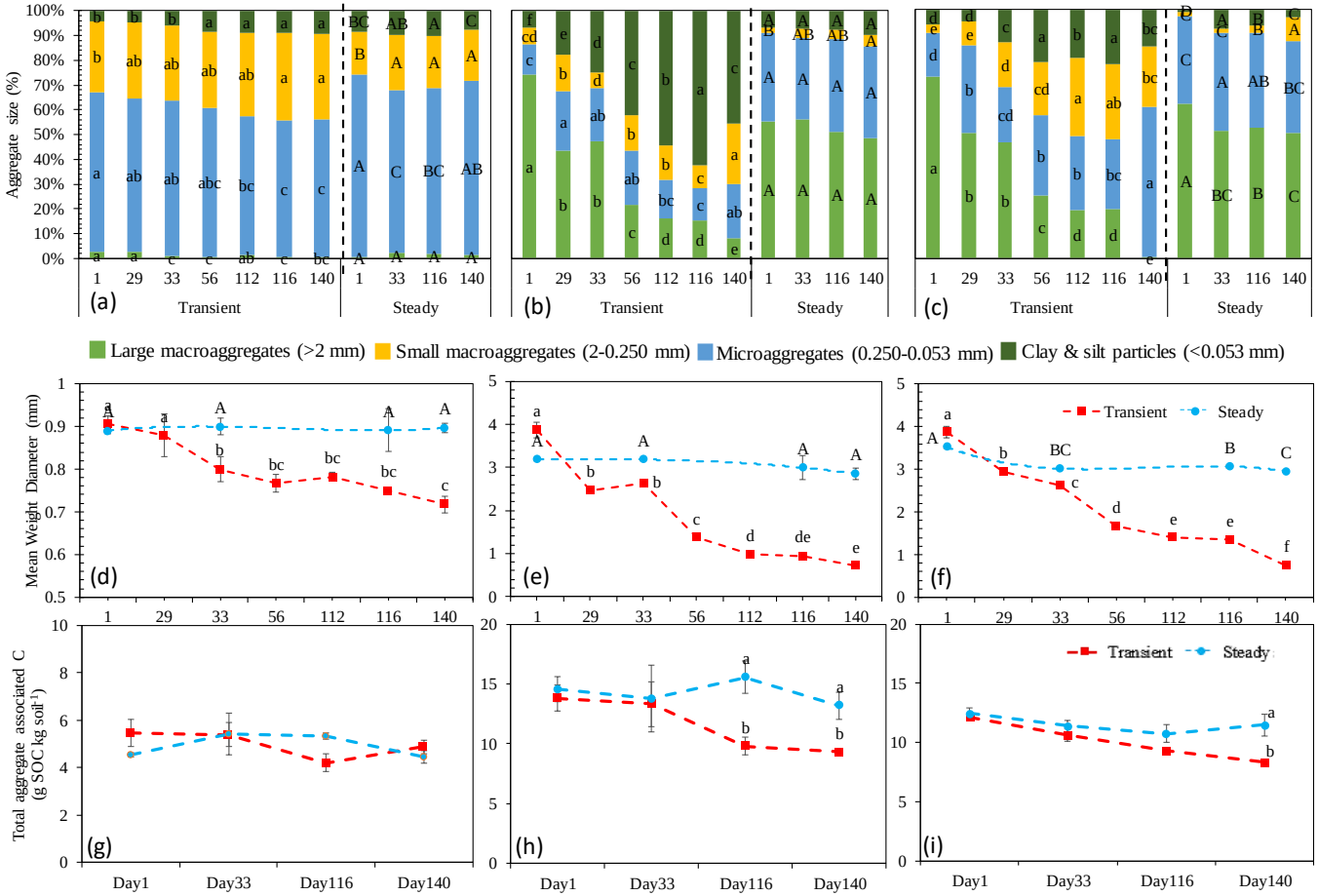


Figure 3.4. Aggregate size distribution mean weight diameter, and aggregate associated C in sandy (a, d, g), loamy (b, e, h), clayey (c, f, i) soils.

Error bars represent the standard errors (n=3). In mean weight diameter figures (d, e, f), uppercase and lowercase letters represent temporal changes in mean weight diameter in steady and transient state experiments, respectively. In total aggregate associated C figures (g, h, i), different letters represent statistical difference between steady and transient state experiments at each destructive timepoint within each soil type. No letters mean no statistical significance.

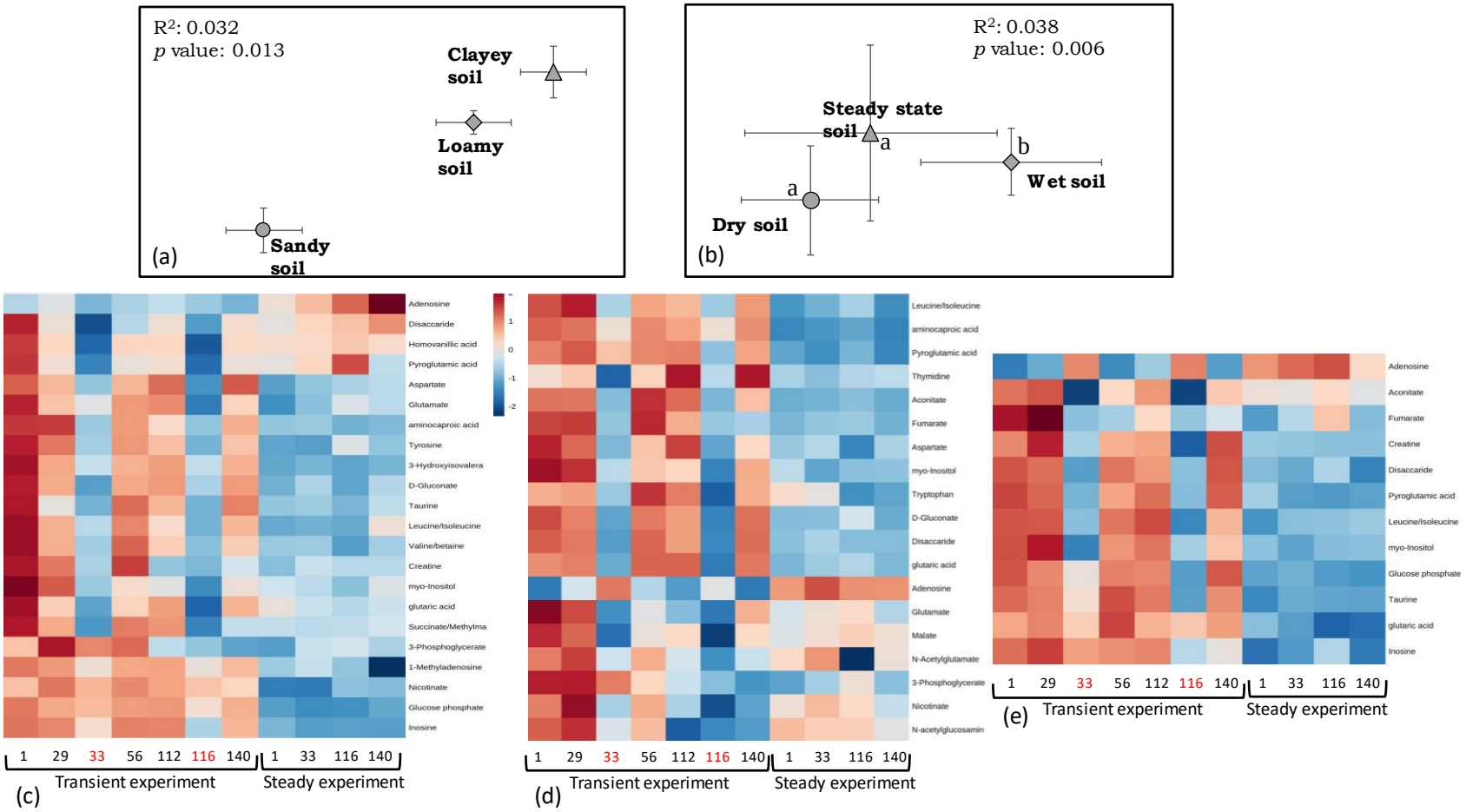


Figure 3.5. Two dimensional NMDS ordination of (a) different textures and (b) different moisture levels for metabolites in steady and transient state experiments. Heatmaps showing the metabolite concentrations in (c) sandy, (d) loamy, and (e) clayey soils at different destructive time points from transient and steady state incubation experiments. Days in red represent the wet phases of the transient state experiment.

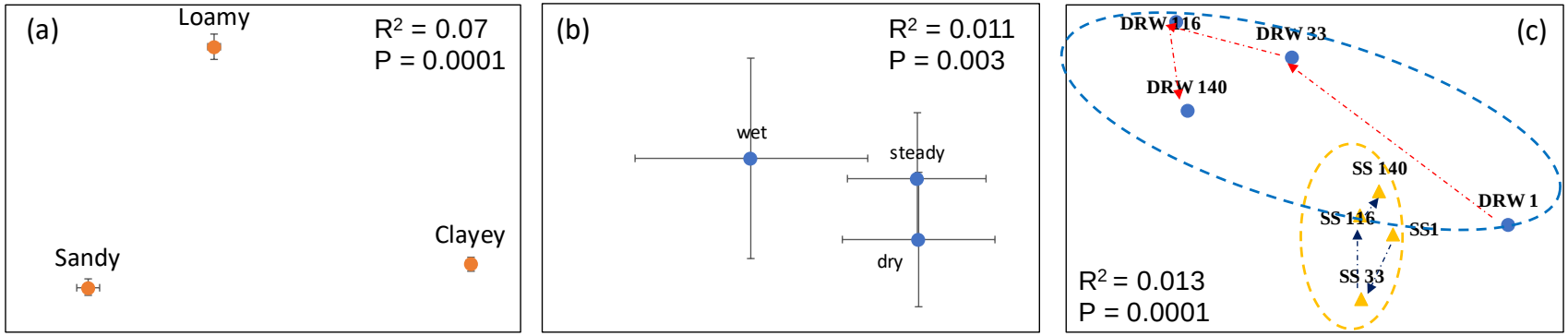


Figure 3.6. Two dimensional NMDS ordination for (a) different textures, (b) different moisture, and (c) different destructive harvest timepoints for bacterial community composition.
In figure (c), SS represents steady state experiment and DRW represents transient state experiment.

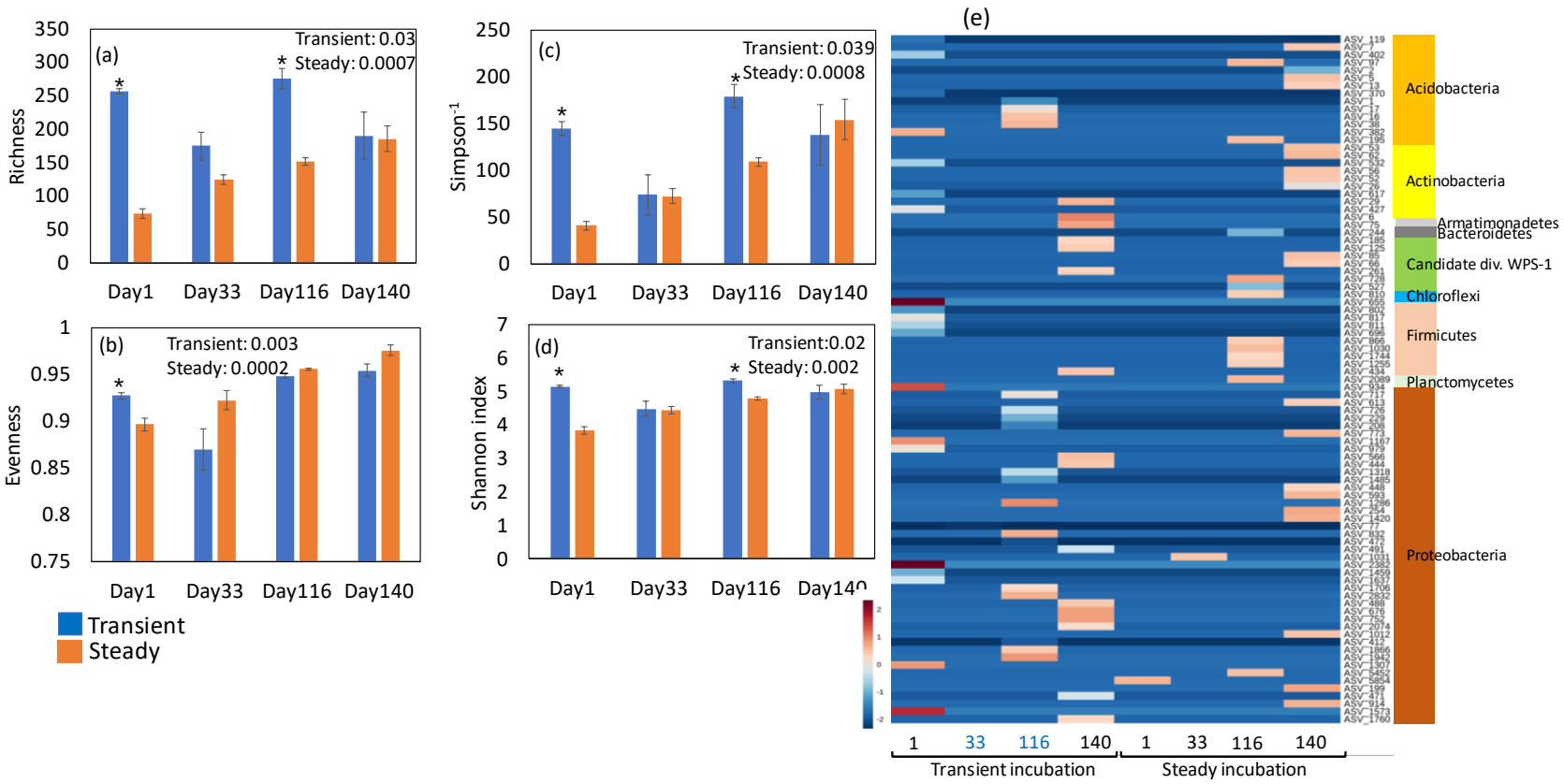


Figure 3.7. Diversity indices for bacterial community composition including (a) richness, (b) evenness, (c) Inverse Simpson index, and (d) Shannon index for sandy soil. Indicator species for bacterial community composition for sandy soil (e). Transient and steady state p values represent temporal changes in the diversity indices.
* represents statistical significance between transient and steady state experiments at each time point.

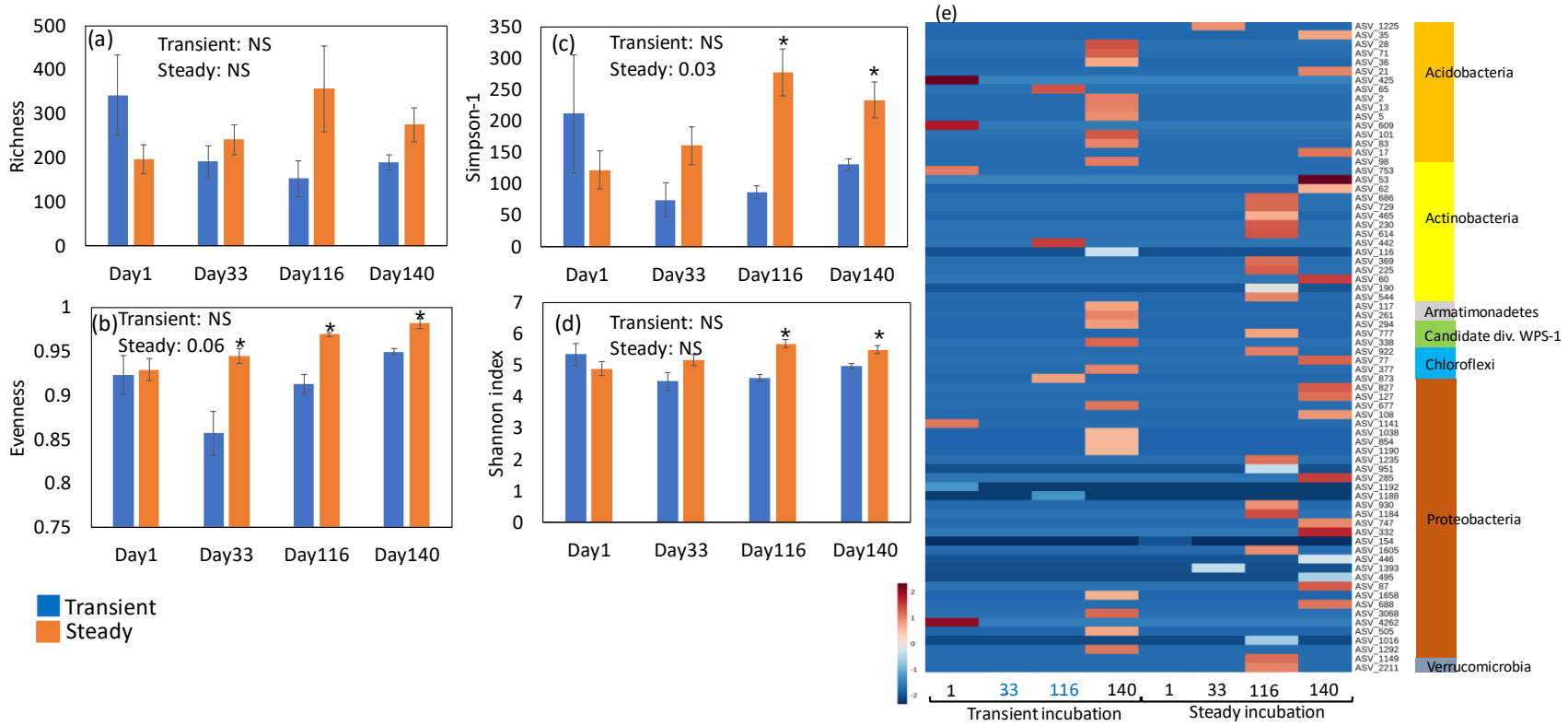


Figure 3.8. Diversity indices for bacterial community composition including (a) richness, (b) evenness, (c) Inverse Simpson index, and (d) Shannon index for loamy soil. Indicator species for bacterial community composition for loamy soil (e). Transient and steady state p values represent temporal changes in the diversity indices.
NS = non-significant; * represents statistical significance between transient and steady state experiments at each time point.

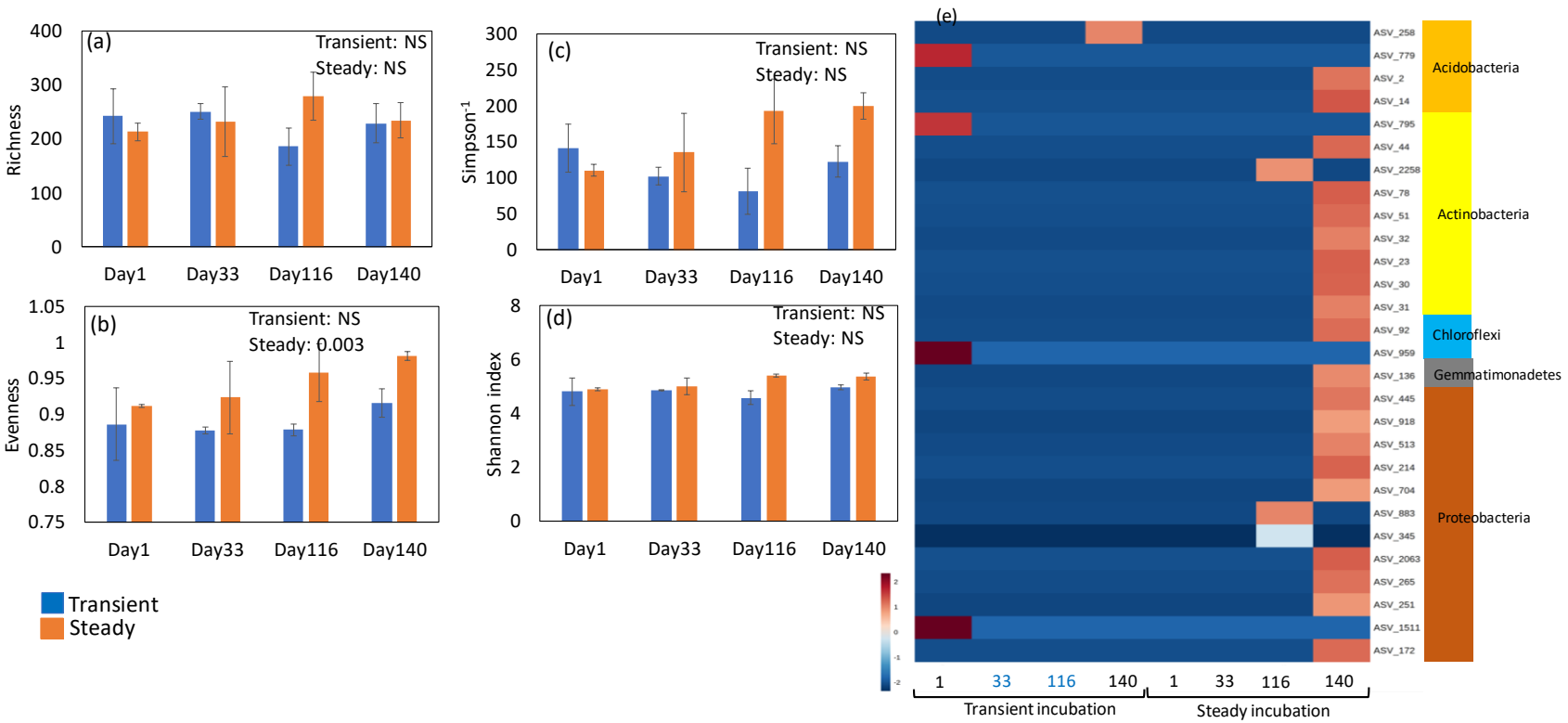


Figure 3.9. Diversity indices for bacterial community composition including (a) richness, (b) evenness, (c) Inverse Simpson index, and (d) Shannon index for clayey soil. Indicator species for bacterial community composition for clayey soil (e). Transient and steady state p values represent temporal changes in the diversity indices.
NS = non-significant; * represents statistical significance between transient and steady state experiments at each time point.

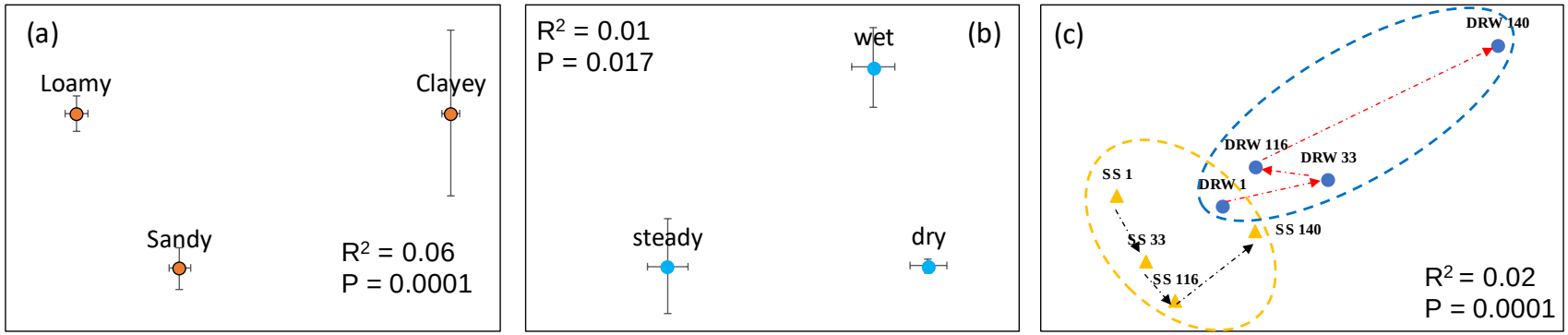


Figure 3.10. Two dimensional NMDS ordination for (a) different textures, (b) different moisture, and (c) different destructive harvest timepoints for fungal community composition. In figure (c), SS represents steady state experiment and DRW represents transient state experiment.

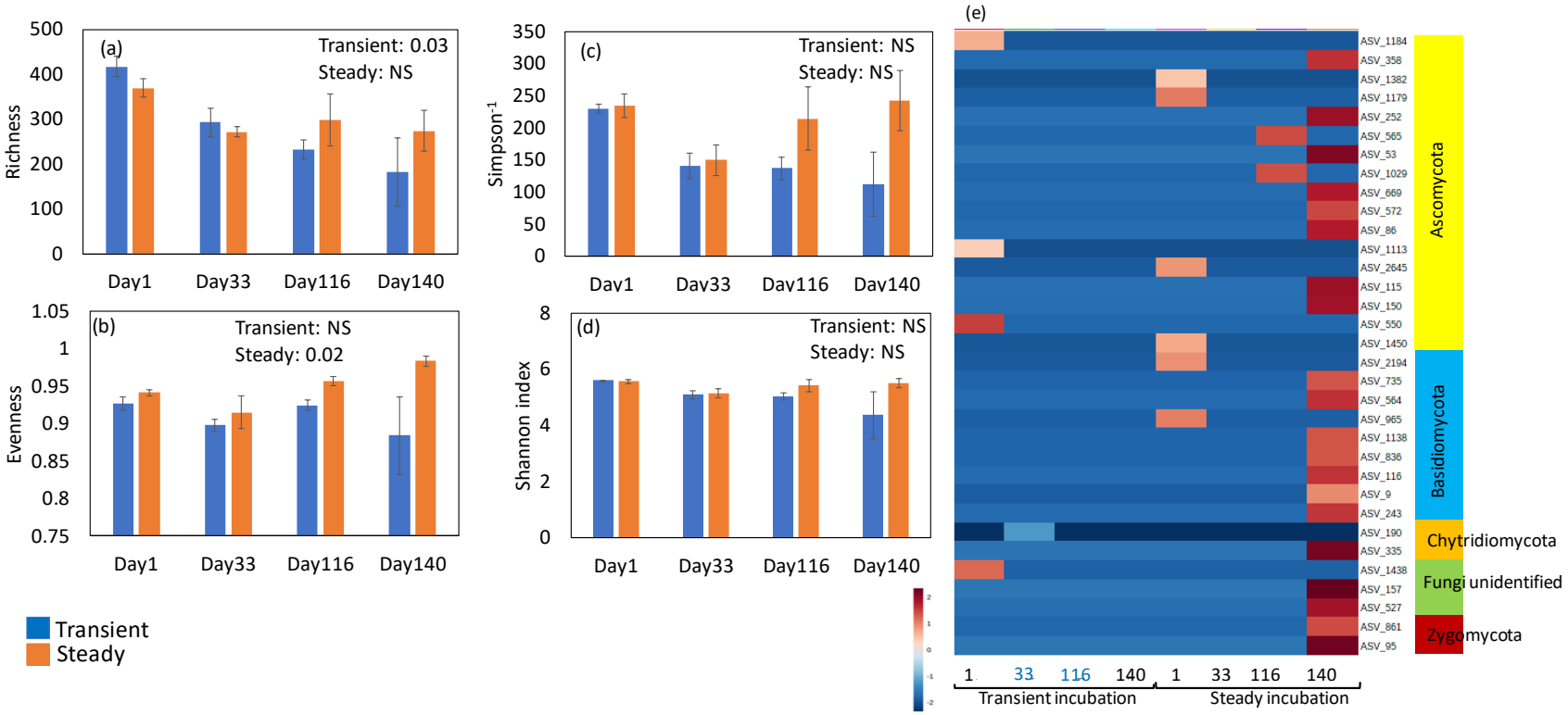


Figure 3.11. Diversity indices for fungal community composition including (a) richness, (b) evenness, (c) Inverse Simpson index, and (d) Shannon index for sandy soil. Indicator species for fungal community composition for sandy soil (e). Transient and steady state p values represent temporal changes in the diversity indices.
NS = non-significant; * represents statistical significance between transient and steady state experiments at each time point.

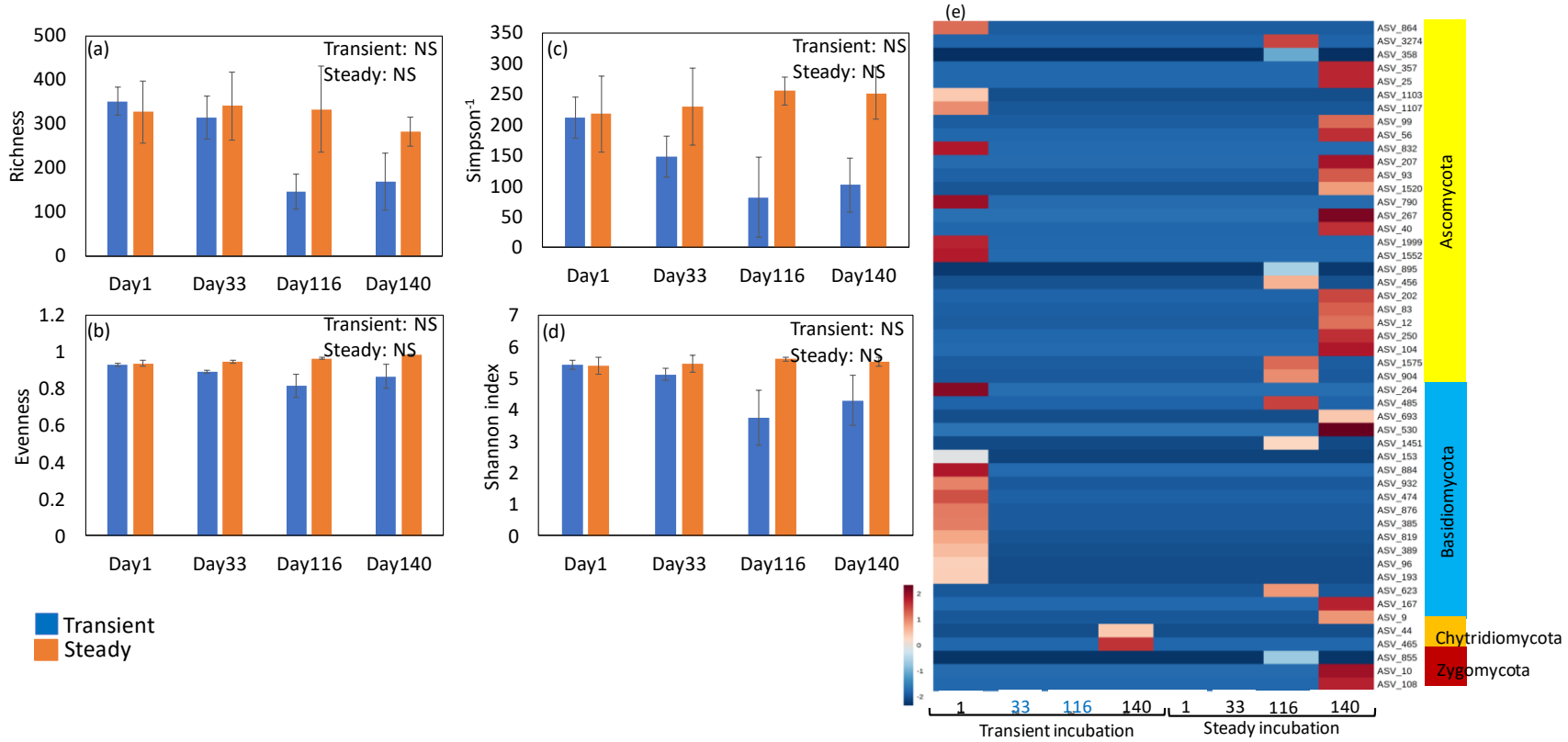


Figure 3.12. Diversity indices for fungal community composition including (a) richness, (b) evenness, (c) Inverse Simpson index, and (d) Shannon index for loamy soil. Indicator species for fungal community composition for loamy soil (e). Transient and steady state p values represent temporal changes in the diversity indices.
NS = non-significant; * represents statistical significance between transient and steady state experiments at each time point.

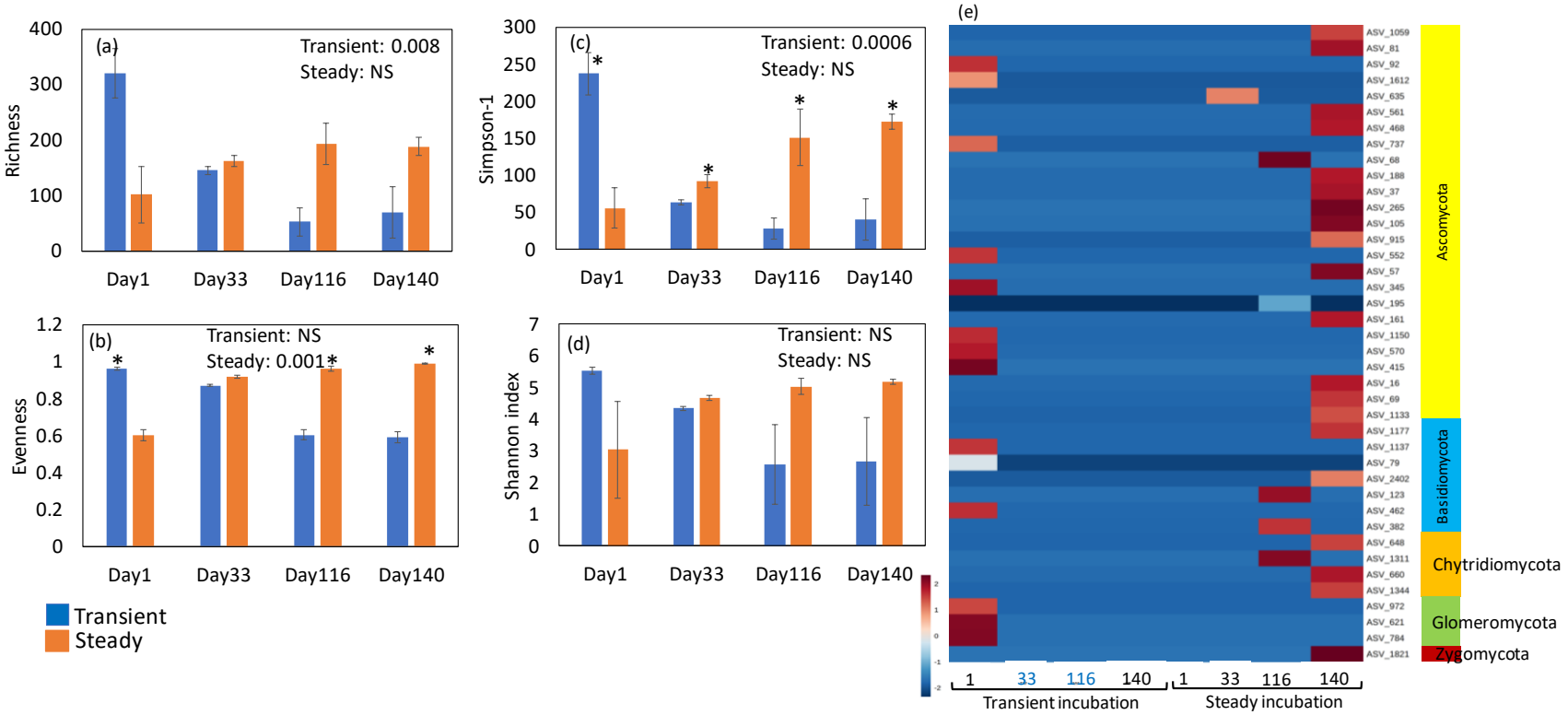


Figure 3.13. Diversity indices for fungal community composition including (a) richness, (b) evenness, (c) Inverse Simpson index, and (d) Shannon index for clayey soil. Indicator species for fungal community composition for clayey soil (e). Transient and steady state p values represent temporal changes in the diversity indices.
NS = non-significant; * represents statistical significance between transient and steady state experiments at each time point.

Table 3.1. Basic properties of the soils used in the study.

Properties	**Sandy Soil**	**Loamy Soil**	**Clayey Soil**
Location	Taylor County, GA	Boone County, MO	Bowie County, TX
Soil series	Cowart	Weller	Buxin
Soil order	Ultisols	Alfisols	Vertisols
Sand (g kg^{-1})	770 ± 26	130 ± 15	150 ± 13
Silt (g kg^{-1})	70 ± 20	510 ± 55	140 ± 50
Clay (g kg^{-1})	160 ± 35	360 ± 58	710 ± 17
Texture	Sandy	Silt loam	Clayey
pH	4.8 ± 0.16	4.9 ± 0.25	5.3 ± 0.21
Water holding capacity (%)	40 ± 0.7	50 ± 1.2	65 ± 1.6
Soil organic C (g kg^{-1})	7.0 ± 0.60	13.0 ± 1.1	12 ± 0.07
Extractable organic C (mg kg^{-1})	46 ± 2.7	57 ± 9.4	62 ± 5.5
Microbial biomass C (μg g^{-1} dry soil)	122 ± 9.8	200 ± 9.9	288 ± 19

Values are mean ± standard error (n = 3).

Table 3.2. Pairwise comparison statistics for bacterial community composition in different textures and moisture contents.

Texture	*p* value	R^2	Moisture	*p* value	R^2
Sandy-Loamy	0.0001	0.04	Dry-Wet	0.009	0.02
Loamy-Clayey	0.0001	0.06	Dry-Steady	0.0001	0.02
Sandy-Clayey	0.0001	0.07	Wet-Steady	0.0001	0.03

Table 3.3. Pairwise comparison statistics for fungal community composition in different textures and moisture contents.

Texture	*p* value	R^2	Moisture	*p* value	R^2
Sandy-Loamy	0.001	0.02	Dry-Wet	0.03	0.02
Loamy-Clayey	0.0002	0.02	Dry-Steady	0.0001	0.02
Sandy-Clayey	0.0007	0.02	Wet-Steady	0.0004	0.02

Chapter 4. The influence of in-situ soil moisture variation on microbial mineralization of soil organic carbon

Abstract

Current climate models reveal that increase in concentrations of atmospheric CO_2 and other greenhouse gases will consequently increase global temperatures and alter precipitation regimes. The combination of extended dry periods with high intensity rainfall will lead to greater variability in soil water content, imposing a greater uncertainty to the microbial processes pertinent to soil C decomposition. Field-scale studies to understand the effect of extreme changes in soil moisture to C cycling are limited, particularly in the southeastern U.S., a region that experiences rapidly changing precipitation patterns. The specific objective of this study was to determine how prolonged drought in an agroecosystem affects C mineralization processes during and after the drought period. A field experiment was conducted in 2018 and 2019 on a cropland planted with soybean (*Glycine max* L.) at the West Tennessee Research and Education Center in Jackson, TN with three soil moisture treatments: drought, rainfed, and irrigated. Drought was simulated in the field using rainout shelters from June to October of each year, the rainfed treatment received the natural rainfall, and the irrigated treatment received irrigation at critical growth stages of soybean in addition to the rainfall. Soil respiration was measured weekly using static chamber approach from May to November. Soil samples were collected in May, July, September and November 2018 and 2019 from 0-5, 5-15 and 15-30 cm depths to determine microbial biomass C (MBC), extractable organic C (EOC), extracellular hydrolytic enzyme activities, and fungal hyphal length in response to moisture changes. Compared to rainfed and irrigated treatments, cumulative CO_2 emissions under drought treatment were lower by 32% and 33%, respectively, in 2018 and 38% and 45%, respectively, in 2019. Significantly higher MBC and EOC concentrations were observed under drought treatment even after the shelters were removed. Fungal hyphal abundance was also higher under the drought treatment which was

consistent with the MBC results. Specific enzyme activity data showed less metabolically active microbes under drought conditions as compared to rainfed and irrigated treatments. The effect of moisture manipulation on microbial activity and active C fractions was only evident in the top 15 cm soil. My results show that under field conditions, the microbes were sensitive to changes in soil moisture and even though I observed higher MBC under drought treatments, the microbes were metabolically less active. These results strengthen my understanding on how moisture limitation influences microbial processing of soil C under field conditions.

Introduction

Elevated atmospheric concentrations of CO_2 and other greenhouse gases (GHGs) are expected to increase earth's surface temperatures and alter precipitation regimes (Trenberth, 1999; IPCC, 2012). Coupled Model Intercomparison Project Phase 5 (CMIP5) revealed that future water regimes will be characterized by altered global water cycle leading to changes in amount, frequency, timing, variability, and intensity of precipitation patterns, and prolonged drought (Chadwick et al., 2016; Duffy et al., 2015; Maloney et al., 2014). Historical climate data suggest that rainfall patterns have already shifted over the past centuries, and there has been a 10% increase in total precipitation in the United States (Karl and Knight, 1998) with approximately half of the increase being due to very high intensity rainfall events of > 40-50 mm day^{-1}. The combination of increased drought periods interspersed with high intensity precipitation events will lead to greater variability in soil moisture regimes (Jackson et al., 2001) with potential influence on sources and sinks of GHGs in terrestrial ecosystems (Harper et al., 2005). Although terrestrial ecosystems are highly sensitive to changes in precipitation patterns (Feng et al., 2013), the extent to which the predicted altered rainfall regimes influence soil C cycling and storage in terrestrial ecosystems is currently not clear.

Soil moisture content acts as a key variable influencing microbial processing of soil organic C (SOC) (Borken and Matzner, 2009; Schimel and Schaeffer, 2012). Extremes of moisture conditions (e.g., drought or flooding) tend to adversely affect the rate of microbial respiration. Drought limits the enzyme and substrate diffusion by breaking the water films and flooded condition promotes anaerobic decomposition through limiting oxygen diffusion (Davidson and Janssens, 2006). The response of microbial respiration to changing precipitation patterns has been a subject of numerous studies (Clein and Schimel, 1994; Lee et al., 2002; Muhr

and Borken, 2009; Borken et al., 2003). However, the impacts of droughts, whose intensity and frequency are predicted to increase under changing climate (IPCC, 2013), on microbial respiration remain uncertain. The limited available data based on in-situ moisture/precipitation manipulation experiments revealed that the effects of reduced precipitation on microbial respiration are variable (Borken et al., 2006; Cleveland et al., 2010; Doughty et al., 2015). In addition, the influence of drought on microbial respiration may also differ during the growing and non-growing seasons (Zhang et al., 2015).

Predicting microbial functional responses to increased variability in soil water content is challenging. Water deficits or drought conditions during inter-rainfall periods may result in reduced microbial respiration as a result of microbial stress. Under such conditions, microbes tend to adapt through several mechanisms such as by accumulating osmolytes to grow and survive to cope with moisture stress (Sagot et al., 2010), undergoing dormancy by investing energy in creating structures around microbial cells such as endospores that support long-term survival (Jones and Lennon, 2010), producing extracellular polymer in their microenvironment that acts as sponges for microbial cells and delay drying (Or et al., 2007), or changing community composition by the selection of drought tolerant taxa (Schimel, 2018; Martiny et al., 2017). Conversely, precipitation events may increase microbial activity as substrate diffusion is enhanced, leading to higher CO_2 fluxes, although this response may not be consistent (Fierer and Schimel, 2002). Intense or longer precipitation events may temporarily reduce microbial respiration due to anoxic conditions leading to reduced microbial activity (Bouma and Bryla, 2000). Also, sudden increase in soil moisture could lead to microbial cell lysis leading to a decrease in microbial population, which could supply substrates to the surviving microbes. As a result of these complex responses of microbial respiration and the tight coupling of soil moisture

and microbial functioning, it is difficult to anticipate *a priori* the responses of microbial respiration to changes in precipitation patterns. Most past studies were conducted by manipulating soil moisture in microcosms under controlled environmental settings where soils are disturbed, and C inputs are prevented. These laboratory-scale experiments are valuable, but they lack the opportunity to study soil biogeochemical changes from in-field moisture variability that happen in tandem with other biotic and abiotic changes. Field-based experiments better represent native ecological conditions compared to laboratory experiments and may follow or contradict my understanding of microbial responses to moisture changes derived from controlled experiments.

The present study was conducted with the objectives to: (1) assess the dependence of soil organic C dynamics to *in situ* soil moisture changes, and (2) determine if prolonged drought has legacy effects on soil microbial activity. The hypotheses were: (1) drought conditions would reduce microbial activity, resulting in reduced microbial respiration fluxes as compared to rainfed and irrigated conditions; (2) soil microbes would adapt to continuous moisture stress under field conditions; and (3) legacy effect on microbial activity would be observed after cessation of drought.

Materials and methods

Study description and experimental design

The study site was located at the University of Tennessee's West Tennessee AgResearch and Education Center in Jackson, TN (35º37'22" N, 88º50'47" W; elevation 125 m). The study site experiences a mean annual temperature and precipitation of 15.6 ℃ and 1375 mm, respectively (based on 30-year average). Rainfall and temperature data were obtained from the National Oceanic and Atmospheric Administration Global Historical Climatology Network

Weather Station (GHCND: USC00404561) located at the immediately adjacent the experimental field each year. Soils of the study site are classified as well-drained Lexington series (fine-silty, mixed, thermic, Ultic Hapludalfs) with 0 to 2 percent slope. The moisture manipulation experiment was conducted on a soybean cropping system (*Glycine max* L.). There were three moisture treatments, viz., (i) drought, (ii) rainfed, and (iii) irrigated. The experimental plots for each treatment were 3.4 m × 3 m size with 1 m alley between the plots. The soybeans were planted on 2nd May 2018 and 6th May 2019 with four rows per plot. The genotype considered for the current study in both years was Ellis. The soybeans were fertilized with 33.6 kg ha^{-1} P, 112 kg ha^{-1} K, and 17 kg ha^{-1} S in 2018 while 33.6 kg ha^{-1} P, 90 kg ha^{-1} K, and 13.5 kg ha^{-1} S in 2019 based on soil test results. All plots were treated with 3.36 kg ha^{-1} alachlor (2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide) and 0.42 kg ha^{-1} metribuzin (4-Amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) for pre-emergence weed suppression and 0.13 kg ha^{-1} clethodim (2-[1-[[(E)-3-chloroprop-2-enoxy] amino] propylidene]-5-(2-ethylsulfanylpropyl) cyclohexane-1,3-dione) for post-emergence weed control. The treatments were arranged in a completely randomized design (CRD) with three replicates. Drought treatment was imposed on the experimental plots by covering the plots using rainout shelters. The shelters were established in the plots from second week of June to first week of October. The crops under shelter plots received 16 cm of water as rainfall in May (2018 and 2019) for seedling emergence before the shelters were established. The rainfed plots received rainfall throughout the entire growing season in both years (Table 4.1). These plots received 103 cm and 83 cm of cumulative rainfall in 2018 and 2019, respectively. In addition to the rainfall amount the irrigated treatment received 11 cm of water in 2018 and 8 cm in 2019 through irrigation at critical growth stages from flowering to seed fill.

Soil sampling and analyses

Soil samples were collected from 0-5, 5-15, and 15-30 cm depths in May, July, September, and November of 2018 and 2019. Multiple soil cores were collected from inter-row area in each plot using a probe of 2.5 cm diameter and combined to obtain a composite sample per plot. Soil sampling in May was conducted to determine the baseline soil properties (before moisture manipulation). In November, the soil sampling was conducted after the shelters were removed to determine the legacy effect of moisture manipulations on microbial activity. The samples were kept in airtight plastic bags and transferred to the laboratory in coolers with ice packs. Gravimetric soil moisture content was determined immediately. The composite samples were sieved through 2 mm sieve to remove plant materials and rocks.

Soil microbial biomass C (MBC) was determined using the chloroform-fumigation and extraction method (Vance et al., 1987). Briefly, 10 g of soil sample was fumigated in the dark for 48 hours after which C content of the fumigated and unfumigated samples were extracted using 0.5 M K_2SO_4 (1:4 soil:0.5 M K_2SO_4), shaken at 200 revolutions per minute for 1 hour, and then centrifuged at 3500 rpm for 3 minutes. The supernatant was passed through a 0.45 μm filter paper and filtrate was analyzed for C using a CN analyzer (Vario TOC cube in liquid mode, Elementar, Hanau, Germany) (Jones and Willett, 2006). MBC was determined as the difference in C concentration in non-fumigated and fumigated soil samples and dividing that with k_{EC} (extraction efficiency) value of 0.45 (Beck et al., 1997). The C concentration of non-fumigated soil is considered as extractable organic C (EOC).

Soil fungal abundance was measured after extracting the hyphae in 3% sodium hexametaphosphate (Kivlin and Hawkes, 2016). Briefly, this method involves disaggregation of soil to extract fungal hyphae by constant stirring of soil in hexametaphosphate solution followed

by filtering a 5 mL aliquot through 0.45 μm filter paper to separate the hyphae from liquid. The hyphae are then stained using 0.01% acid fuchsin dye and mounted on a slide. This is followed by adding polyvinyl alcohol-lactic acid-glycerol (PVLG) mounting media on the slides and carefully placing a cover slip. Both septate and aseptate hyphae were quantified using compound microscope under 20X magnification with 50 fields of view using the grid-line intercept method (McGonigle et al., 1990). Hyphal lengths were reported as sum of both septate and aseptate hyphae.

The activity of six extracellular hydrolytic enzymes, viz., α-glucosidase (AG), β-glucosidase (BG), cellobiohydrolase (CBH), β-xylosidase (XYL), N-acetyl glucosaminidase (NAG), and phosphatase (AP) were determined using 96-well plates method outlined by German et al., (2011). The reference substrates for AG, BG, CBH, XYL, NAG, and PHOS were 4-methylumbelliferone (MUB)-α-D-glucopyranoside, 4-MUB-β-D-glucopyranoside, 4-MUB- β -D-cellobioside, 4-MUB-B-D- xylopyranoside, 4-MUB-N-acetyl- β -D-glucosaminide, and 4-MUB phosphate, respectively. Briefly, 2.75 g of soil sample was homogenized in a Waring blender with 91-mL of 50 mM sodium acetate buffer on high setting for one minute. Following that, 800 μL of the soil slurry was transferred into each of the 8 wells of one column. Another plate was prepared to create a standard curve for each sample at 25 °C. Each column in the standard curve plate contained a soil slurry with a different concentration of the 4-MUB standard (0, 2.5, 5, 10, 25, 50, and 100 μM) in each of the wells. After adding 12 samples in a plate, 200 μL of 200 μM respective substrates were added. The plates were incubated at 25 °C for 3 hours. Post incubation, the plates were centrifuged at 350 x *g* for 3 minutes and 250 μL of the supernatant from each well was transferred to a 96-well black plate. A plate reader (Synergy-BioTek Instruments, Inc., Vermont, USA) was used to measure fluorescence at wavelengths 365

nm and 450 nm for excitation and emission, respectively. The standard curve plates were used to construct a linear standard curve to determine each enzymes' activity for each sample as nmol g^{-1} dry soil h^{-1}. Specific enzyme activity was calculated by dividing the total enzyme activity by MBC values (Waldrop et al., 2000).

Soil surface CO_2 measurement

Soil surface CO_2 emissions were measured from June through November in 2018 and 2019. Gas sampling was done on weekly interval depending on weather conditions. After the shelters were removed, gas sampling was conducted on biweekly intervals. Vented polyvinyl chloride static chambers were installed in between soybean rows in late May (Lehman and Osborne, 2013). The collars were only removed while harvesting soybeans and then re-established. Gas sampling was typically done between 9:00 AM and 12:00 PM to minimize the effect of temperature on CO_2 fluxes. Chamber temperature, soil temperature, and soil moisture were measured during each sampling time at each plot. Gas samples were collected at 0, 20, and 40 min, after securely placing a lid on the chambers, using a 15 mL syringe and then transferred to 12 mL evacuated exetainer vials (Labco, United Kingdom). Gas samples were analyzed for CO_2 concentrations using a flame ionization detector on a gas chromatograph (Shimadzu GC-2014, Japan) using N_2 as a carrier gas. Soil CO_2 fluxes were calculated as the change in the headspace CO_2 concentration over time within the enclosed chamber volume (Wegner et al., 2018; Abagandura et al., 2020). Cumulative fluxes for each treatment from June through November were calculated using linear interpolation method (Wegner et al., 2018).

Statistical analyses

Statistical analyses were conducted using the GLIMMIX procedure in SAS v9.4 (SAS, 2012). Analysis of variance (ANOVA) was conducted for each soil depth and each sampling

time point separately to compare the impacts of *in-situ* moisture manipulations on CO_2 fluxes, MBC, EOC, extracellular enzyme activity, and specific enzyme activity with moisture treatments as fixed effects. The effect of time and moisture treatments and their potential interactions on the above-mentioned parameters were determined using a repeated measures ANOVA with sampling time as the repeated measure variable. Mean differences were considered significant at $p \leq 0.05$. The mean separation was done using Tukey's test. The results from the repeated measures ANOVA for all the parameters are included in Table 4.2.

Results

Microclimate conditions and soil water content

Daily maximum and minimum air temperature and precipitation for 2018 and 2019 are shown in Fig. 4.1. The area received 103 and 83 cm of rainfall from May through first week of October in 2018 and 2019, respectively. Therefore, the total amount of water received by the rainfed treatment in 2018 and 2019 was 103 and 83 cm, respectively. The irrigated treatment received a total of 114 and 91 cm of water in 2018 and 2019, respectively. Mean air temperature during 2018 and 2019 were similar during the growing season. Soil temperature and moisture for 2018 and 2019 are shown in Fig 4.2a and b, respectively. In 2018, soil temperature was the same for all three treatments till early July, after that drought treatments showed 0.5-0.7 °C higher temperature than the rainfed and irrigated treatments. In late September, soil temperature under drought was 2 °C higher than rainfed and irrigated treatments. There was a consistent decrease in soil temperature post-harvest of soybeans, and it was reduced to 7 °C for all the three treatments by the end of November. In 2019, soil temperatures were similar in the three treatments until July last week, after that drought treatment showed 0.5-5 °C higher temperature than rainfed and irrigated treatments. Similar to 2018, soil temperature was reduced to 8 °C by the end of

November. Soil moisture was similar in the three treatments till mid-July in 2018 and after that, soil moisture was consistently lowest in drought treatments. After removal of shelters, soil moisture was increased in the three treatments for both years and no differences were observed among the three treatments.

Soil CO_2 efflux in response to soil moisture

The repeated measures ANOVA showed that treatment (2018: $p < 0.0001$; 2019: $p = 0.0002$), time of sampling (2018: $p < 0.0001$; 2019: $p < 0.0001$), and their interaction (2018: $p = 0.0004$; 2019: $p = 0.0002$) influenced the CO_2 emissions (Table 4.2). Soil CO_2 efflux consistently followed the soil moisture trends with the lowest CO_2 efflux observed from the drought treatment compared to rainfed and irrigated treatments in both years (Fig. 4.2c and d). In 2018, the first baseline sample collected on June 14 did not show differences in CO_2 emissions across the moisture treatments (Fig. 4.2c). From July 19, CO_2 emissions from drought treatment were the lowest compared to rainfed and irrigated treatments until the first week of October (Fig. 4.2c). After the shelters were removed in first week of October, three more gas samplings were conducted and no significant differences in CO_2 emissions were observed among the three treatments. The CO_2 emissions were significantly reduced during the last three sampling events for the treatments compared to the previous sampling events. Cumulative CO_2 emissions from June through November under drought treatment averaged 32% and 33% lower than rainfed and irrigated treatments, respectively (see inset in Fig. 4.2c). In 2019 also, the baseline data showed no significant differences among the three treatments and after that the drought treatment exhibited the lowest CO_2 emissions as compared to rainfed and irrigated treatments (Fig. 4.2d) until October 2. Similar to 2018, the last three gas sampling events in 2019 after shelter removal showed reduced emissions with no significant differences among the three treatments.

Cumulative CO_2 emissions from June through November under drought treatment averaged 38% and 45% lower than rainfed and irrigated treatments, respectively (see inset in Fig. 4.2d).

Active soil C pools in response to soil moisture

In general, EOC showed significant differences at 0-5 (Figs. 4.3a and b) and 5-15 cm (Figs. 4.3c and d) depths while no differences were observed at 15-30 cm (Figs. 4.3e and f) depth. Results showed no significant differences among the three treatments in May 2018 and 2019, before the shelter establishment, for both 0-5 and 5-15 cm depths. However, in both the years, drought treatment showed higher EOC compared to rainfed and irrigated treatments in July and September at 0-5 (Figs. 4.3a and b) and 5-15 cm depths (Figs. 4.3c and d). In November, six weeks after the shelters were removed, drought treatment still showed higher EOC than rainfed and irrigated treatments at both 0-5 and 5-15 cm depths. More specifically, at 0-5 cm depth, EOC under drought averaged 55% and 90% higher in November 2018 (Fig. 4.3a) and 82% and 69% higher in November 2019 (Fig. 4.3b) than rainfed and irrigated treatments, respectively. I observed a significant decrease in EOC over time of sampling. The EOC showed a consistent decrease with depth and over time within each depth for all the three treatments.

Similar to EOC, MBC also showed significant differences among the moisture treatments at 0-5 cm (Figs. 4.4a and b) and 5-15 cm (Figs. 4.4c and d) depths while no differences were observed at 15-30 cm depth (Figs. 4.4e and f). In 2018 and 2019, no differences were observed in May and July at 0-5 and 5-15 cm depths, while in September and November, drought treatment showed the highest MBC compared to rainfed and irrigated treatments. I observed a general decrease in MBC over time at each sampling depth except at 5-15 cm depth in 2018. Also, there was a significant decrease in MBC with soil depth. Fungal hyphal lengths, measured at 0-5 and 5-15 cm depths, showed similar trends with MBC (Fig. 4.4). At both depths, fungal hyphal lengths did not show any differences among moisture treatments in May and July, while

higher hyphal lengths under drought treatment was observed in September and November compared to rainfed and irrigated treatments in both years (Fig. 4.4a to d). The increased fungal abundance under drought was more evident in 2019 than 2018.

Extracellular hydrolytic enzyme activity and specific enzyme activity

In general, extracellular hydrolytic enzyme activity increased with an increase in soil moisture (Fig. 4.5). For all depths, drought treatment showed significantly lowest activity compared to rainfed and irrigated treatments when treatment differences were significant. In both 2018 and 2019, no significant differences in enzyme activity was observed in May at all depths (Figs. 4.5a to f). The lowest enzyme activity under drought as compared to the rainfed and irrigated treatments was also observed in November, six weeks after the shelter removal (Figs. 4.5a to d). Also, there was a significant decrease in enzyme activity with soil depth (Fig. 4.5). Specific enzyme activity was also similar among the moisture treatments in May 2018 and 2019 (Fig. 4.6). At other time points, drought treatment showed the lowest specific enzyme activity at 0-5 and 5-15 cm depths in 2018 (Figs. 4.6a and c) and at all the depths in 2019 (Figs. 4.6b, d, and f).

Discussion

Soil moisture changes and microbial activity

In accordance with my hypothesis, I observed the lowest CO_2 efflux under drought treatment compared to rainfed and irrigated treatments (Figs. 4.2c and d). The reduction in microbial respiration of C under drought conditions is most probably due to reduced physical accession of C substrates under moisture deficit conditions (Suseela et al., 2012; Wang et al., 2014; Blankinship and Schimel, 2018). Several past studies also reported decreased microbial respiration under water limitation (e.g., Xu et al., 2004; Zhou et al., 2007; Allison and Treseder,

2008; Liu et al., 2009). Although CO_2 emissions were greater under rainfed and irrigated conditions compared to drought condition, no significant differences were observed between rainfed and irrigated treatments. This could possibly be due to both these treatments receiving nearly similar amounts of water throughout the study period. The rainfall during both these years was enough for soybeans due to which only a little additional amount of water was needed in the irrigated treatments. Also, CO_2 emissions showed significant decrease under drought conditions only after 2-3 weeks of shelter establishment (Figs. 4.2 c and d), revealing the length of time (2-3 weeks) required since the beginning of drought to decrease the soil moisture to adversely affect the microbial activity. Once the shelters were removed and soybeans were harvested in October, three additional gas samplings were conducted biweekly within six weeks, all of which showed similar soil CO_2 efflux among moisture treatments. This is attributed to the similar soil moisture content across the treatment plots resulted from a few rainfall events. Interestingly, during this post-shelter removal period, the CO_2 emissions from all the three moisture treatments were reduced (Figs. 4.2c and d). This could plausibly be explained by lower soil temperatures recorded during the month of November in both the years (7 and 8 °C in 2018 and 2019, respectively) (Fig. 4.1).

Extracellular enzymes play a critical role in soil organic matter decomposition and nutrient cycling. Consistent with the soil CO_2 efflux results, extracellular enzyme activity also decreased under drought (Fig. 4.5). This is attributed to diffusion constraints from moisture limitation, limiting interaction of enzymes with their respective substrates (Steinweg et al., 2012) and slowing down the decomposition (Kardol et al., 2010; Sanaullah et al., 2011). Also, as soils dry, enzymes may be adsorbed to clay minerals more tightly, making them more resistant to proteolytic breakdown (Kandeler, 1990). Allison and Vitousek (2005) also reported that under

drought conditions, enzyme production and activity are reduced as the nutrient requirement for enzyme production exceeds the net increase in nutrient availability for microbes. Interestingly, even though the lowest, I still observed the maintenance of enzyme pool under drought conditions which could possibly be explained by reduced enzyme turnover due to stabilization under moisture limited conditions (George et al., 2007) or by continuous supply of substrates in the form of plant inputs for the enzymes to act on (Schimel and Schaeffer, 2012). The decrease in enzyme activity with depth was observed, which could be due to the decline in the relative contribution of plant derived C (Stone et al., 2014). The reduced enzyme activity observed in drought conditions continued even after the shelters were removed and all the treatments were received multiple rain events. This indicates that microbes under drought conditions may have adapted to the moisture deficit conditions and continued to metabolize at lower rates even when the soil moisture was increased, a potential indication of legacy effect. I measured the enzyme activities and CO_2 respiration for only six weeks after the shelter removal, so I was unable to determine how long the legacy effect of moisture deficit lasted.

Soil moisture changes and active C pools

Active C fractions have been suggested as more sensitive metrics for detecting the impacts of short-term and extreme changes in environmental factors on total soil C pool (de Graaff et al., 2014). This study considered two active C fractions, EOC and MBC. I observed higher concentrations of EOC under drought treatment (Fig. 4.3). Several past studies also found increased EOC under water deficit conditions and attributed that to reduced microbial uptake of EOC (Blankinship et al., 2014; Manzoni et al., 2016; Schaeffer et al., 2017; Xiang et al., 2008; Homyak et al., 2018). Other studies attributed this increased accumulation of EOC under reduced soil moisture conditions to sustained enzyme activity even under reduced substrate diffusivity

(Stursova and Sinsabaugh, 2008; Ladwig et al., 2015; Manzoni et al., 2014). I also found that total enzyme activity under drought treatment was sustained throughout the experiment, despite at a lower rate, compared to rainfed and irrigated treatments. Additionally, constant plant inputs in the form of root exudates and leaf litter throughout the growing season whilst lower microbial activity/uptake due to water stress may also have resulted in EOC accumulation in soils under drought treatment (Keiluweit et al., 2015). The increased EOC accumulation under drought conditions has also been linked to the production of extracellular polymeric substances (EPS) (Holden et al, 1997).

I also found increased MBC, another active C fraction, under drought condition (Fig. 4.4). This is somewhat contrary to the general understanding that moisture deficit conditions impose direct physiological stress on microorganisms (Schimel et al., 2007), eventually leading to decreased microbial biomass (Gorissen et al., 2004). In accordance with my findings, several previous studies have also reported increased microbial biomass under drought conditions (Jin et al., 2011; Schaeffer et al., 2017; Boot et al., 2013; Fuchslueger et al., 2014; Parker and Schimel, 2011). Presence of EPS also helps in explaining the increased MBC under drought conditions as these substances facilitate cellular hydration and delay their desiccation (Holden et al., 1997; Or et al., 2007). There are also evidences that microbial populations become tolerant to drought stress (Lennon et al., 2012) and able to even grow under stress (Manzoni and Katul, 2014), especially fungi (Borken and Matzner, 2009; Clark et al., 2009). Fungi are considered to be more tolerant to moisture stress due to the accumulation of osmoregulatory solutes that do not hinder their metabolism and also due to their filamentous structure that facilitates greater access and utilization of substrates even under drought conditions (Manzoni et al., 2012). To determine if the higher MBC under drought was due to higher microbial biomass or intracellular osmolytes

accumulation in microbial cells, soil fungal hyphal lengths were determined. Accordingly, my results showed increased fungal hyphae abundance under drought conditions (Fig. 4.4). Though I found a significant increase in fungal hyphal length under drought compared to rainfed and irrigated treatments, there could be other mechanisms contributing to higher MBC under moisture deficient conditions. For example, the death rates of microbial population may be lower under drier conditions as the movement of microbial predators (such as protozoa, nematodes, phage) through soil water is reduced (Geisen et al., 2014; Schimel, 2018). As a result, microbial biomass can be increased in drought conditions not because of higher growth rates but slower death rates. Accumulation of intracellular osmolytes may also contribute be higher MBC (Warren, 2016), which was not determined in this study.

Soil moisture changes and microbial metabolic status

The enzyme activity expressed per unit MBC, known as specific enzyme activity, represents the metabolic status of the soil microbial community (Landi et al., 2000). This metric that normalizes differences in MBC allows a reliable comparison of enzyme activities under different soil moisture treatments. These results showed that specific enzyme activity was consistently low under drought condition compared to rainfed and irrigated conditions at the top two layers of soil (Figs. 4.6a, b, c, and d). Lower specific enzyme activity under drought treatment could be due to higher MBC (Fig. 4.4) and/or due to immobilization of the extant pool of enzymes by forming complexes with soil particles (Lagomarsino et al., 2011). On the contrary, greater specific enzyme activity in rainfed and irrigated treatments indicates more metabolically active microorganism that decrease MBC by enhanced microbial turnover (Mukhopadhyay and Joy, 2010) or increase synthesis, production and release of more enzymes (Bastida et al., 2008). My observation of higher CO_2 respiration under rainfed and irrigated

treatments aligns well with specific enzyme activity and supports my hypothesis of higher microbial activity when moisture is not limited, which also leads to greater microbial turnover. Even though the extracellular enzyme activity shows same trends (Fig. 4.5), it is not possible to deduce if the higher enzyme activity in rainfed and irrigated treatments is due to alleviation of diffusion constraints by increased soil moisture or due to higher microbial activity or both. Specific enzyme activity results elucidated higher metabolic status of microorganisms under rainfed and irrigated conditions. These results revealed that under drought conditions, the active fraction of microbes is less despite the higher amount of total microbial biomass.

Conclusions

Two years of results from an *in-situ* drought simulation experiment showed reduced CO_2 efflux and enzyme activity compared to rainfed and irrigated conditions with higher soil moisture content. However, total MBC and EOC were increased under drought treatment. Concomitant with the increase in MBC, fungal hyphae abundance was also increased in the drought treatment. Results also showed that changes in soil moisture availability had more drastic changes in microbial cycling of C in the top 15 cm of the soil. I also observed that despite higher MBC, microbes are less metabolically active under drought conditions. In addition, I found indication for potential legacy effects of drought on microbial activity. Findings from this *in situ* moisture manipulation study that reflects soil-plant-microbial-climatic interaction under field conditions is useful for understanding the extent of limitations that water stress conditions impose on C dynamics and the possible mechanisms of such limitations.

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Appendix IV

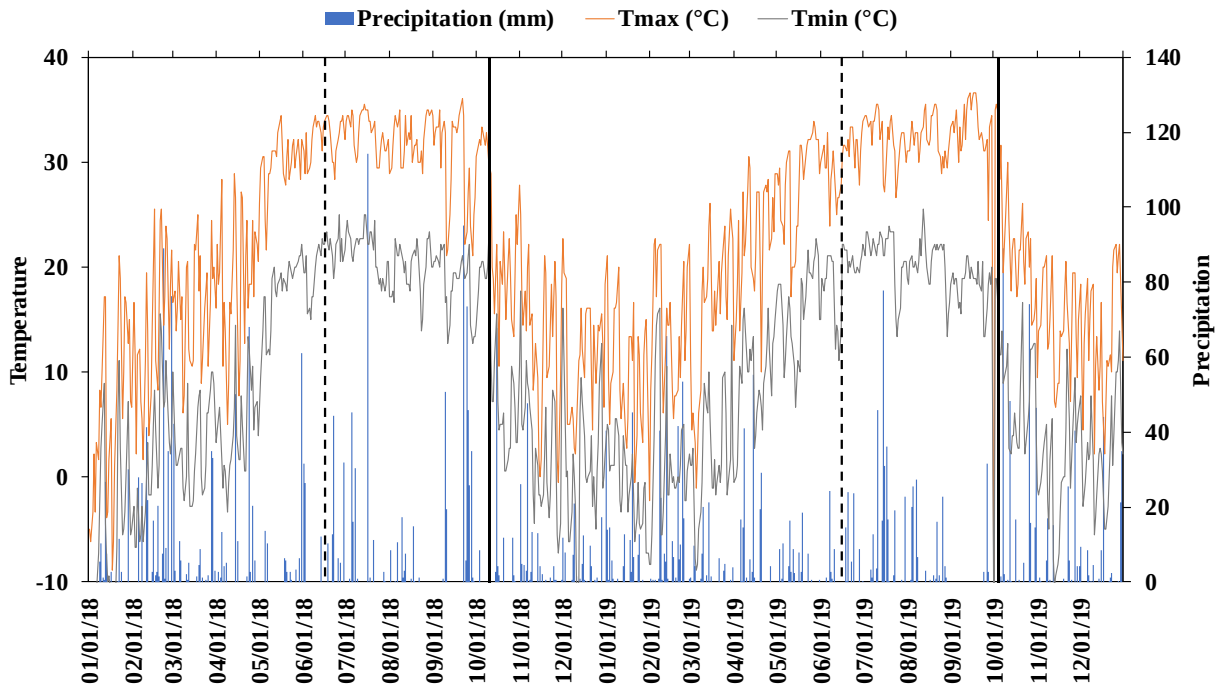


Figure 4.1. Daily air maximum and minimum temperature and precipitation during 2018 and 2019.

Tmax, maximum air temperature; Tmin, minimum air temperature. The dashed line and the solid line show the rainshelter establishment and removal, respectively, during 2018 and 2019.

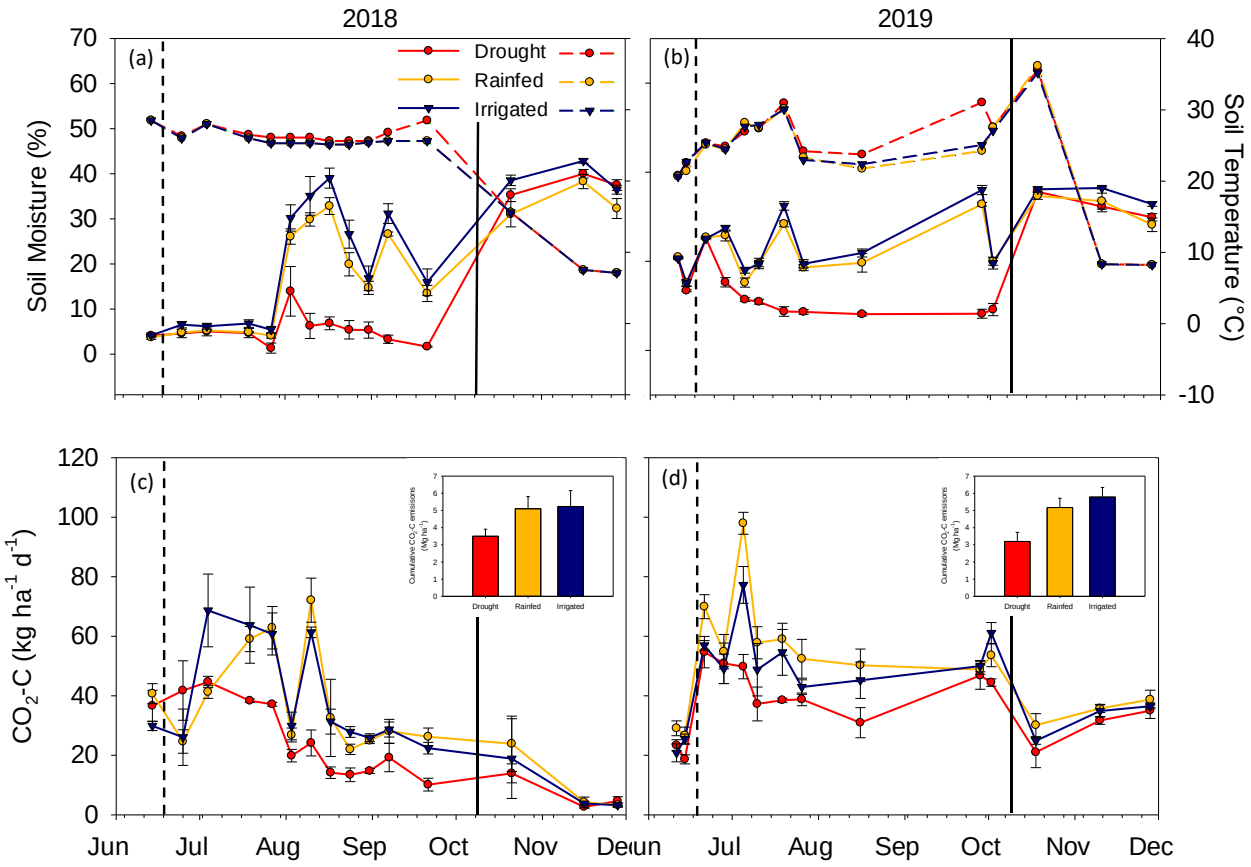


Figure 4.2. Soil temperature and soil moisture (0-5 cm) as influenced by moisture manipulation in 2018 (a) and in 2019 (b). Dashed line in the legend represents the soil temperature and solid line represents the soil moisture. Daily CO_2 fluxes during the growing season in 2018 (c) and 2019 (d).
Legend for daily CO_2 fluxes is same as soil moisture. Vertical dashed line and solid line represents shelter establishment and removal dates, respectively. Inset: cumulative CO_2 emissions during the growing season. Error bars represent standard error (n = 3).

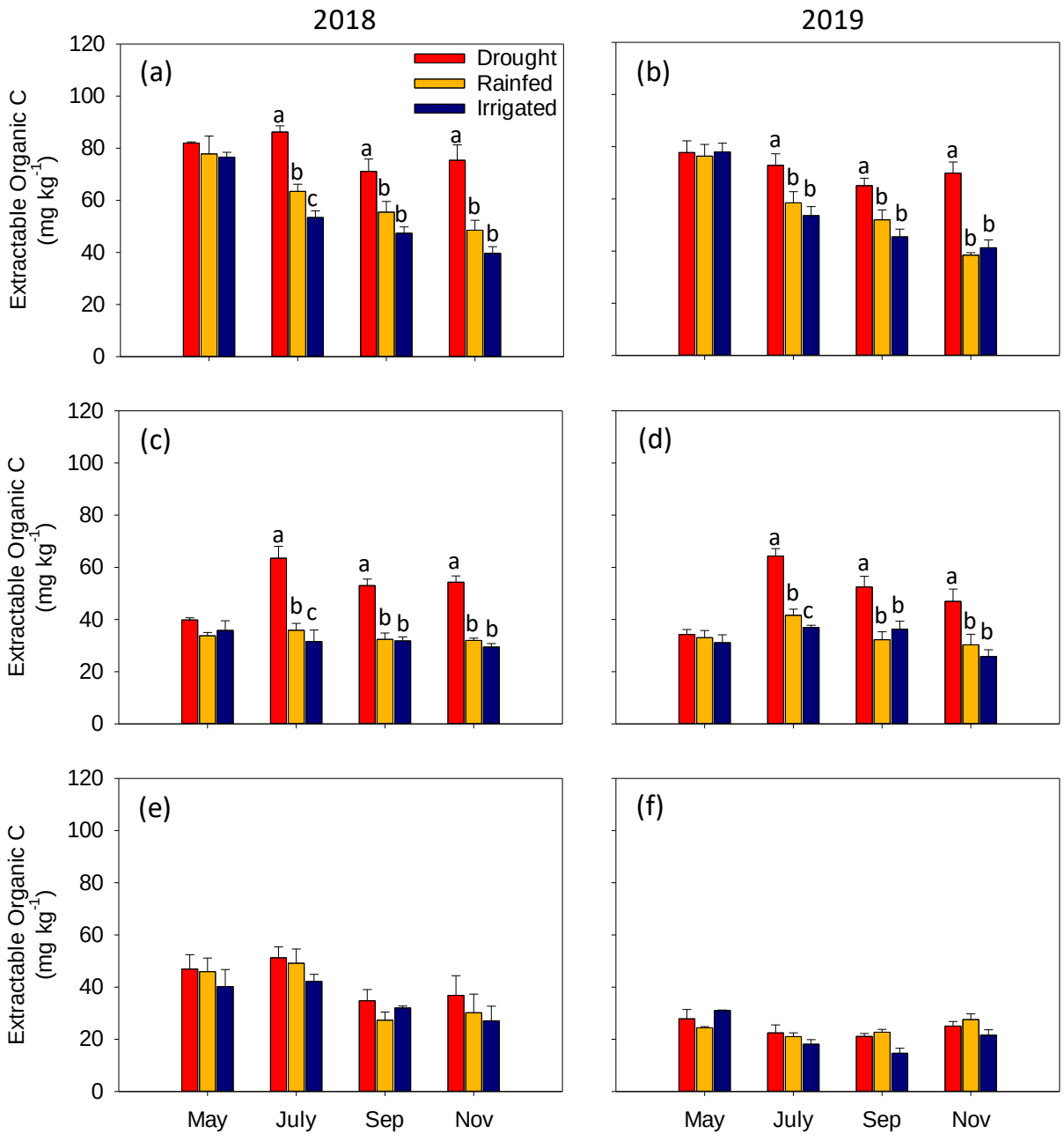


Figure 4.3. Extractable organic carbon concentrations under drought, rainfed, and irrigated treatments at 0-5 (a, b), 5-15 (c, d) and 15-30 (e, f) cm depths during 2018 and 2019. Different letters represent statistical significance at $p \leq 0.05$ at each time point. No letters mean no statistical significance. Error bars represent standard error (n=3). Sampling in May represents baseline and in November represents post shelter removal.

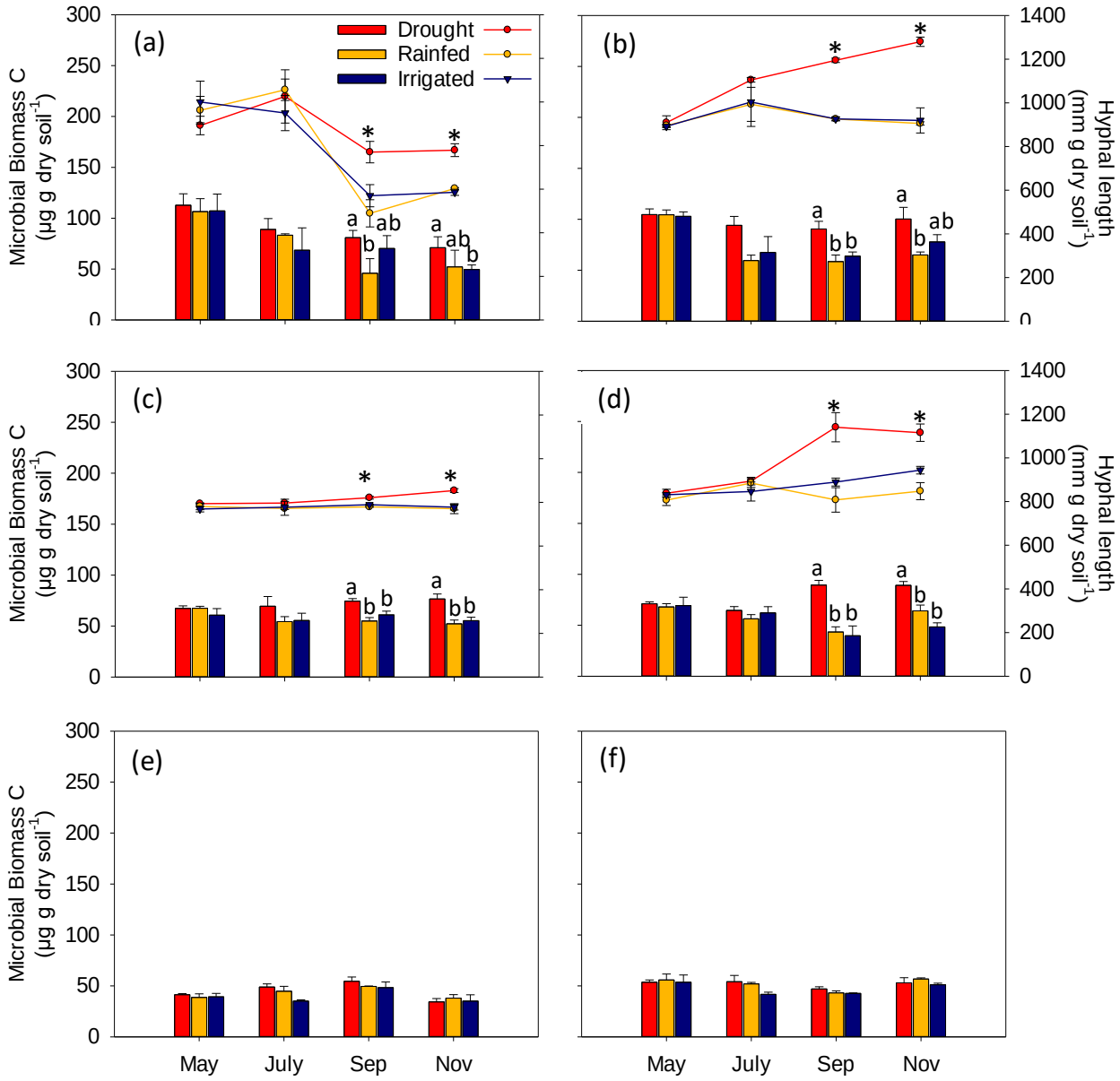


Figure 4.4. Microbial biomass carbon concentrations (bar graphs) under drought, rainfed, and irrigated treatments at 0-5 (a, b), 5-15 (c, d) and 15-30 (e, f) cm depths during 2018 and 2019. Different letters represent statistical significance at $p \leq 0.05$ at each time point. No letters mean no statistical significance. The secondary axis represents the hyphal length (line graph) in each treatment at each time point. Asterisk represents statistical significance at $p \leq 0.05$ at each time point. Error bars represent standard error (n=3). Sampling in May represents baseline and in November represents post shelter removal.

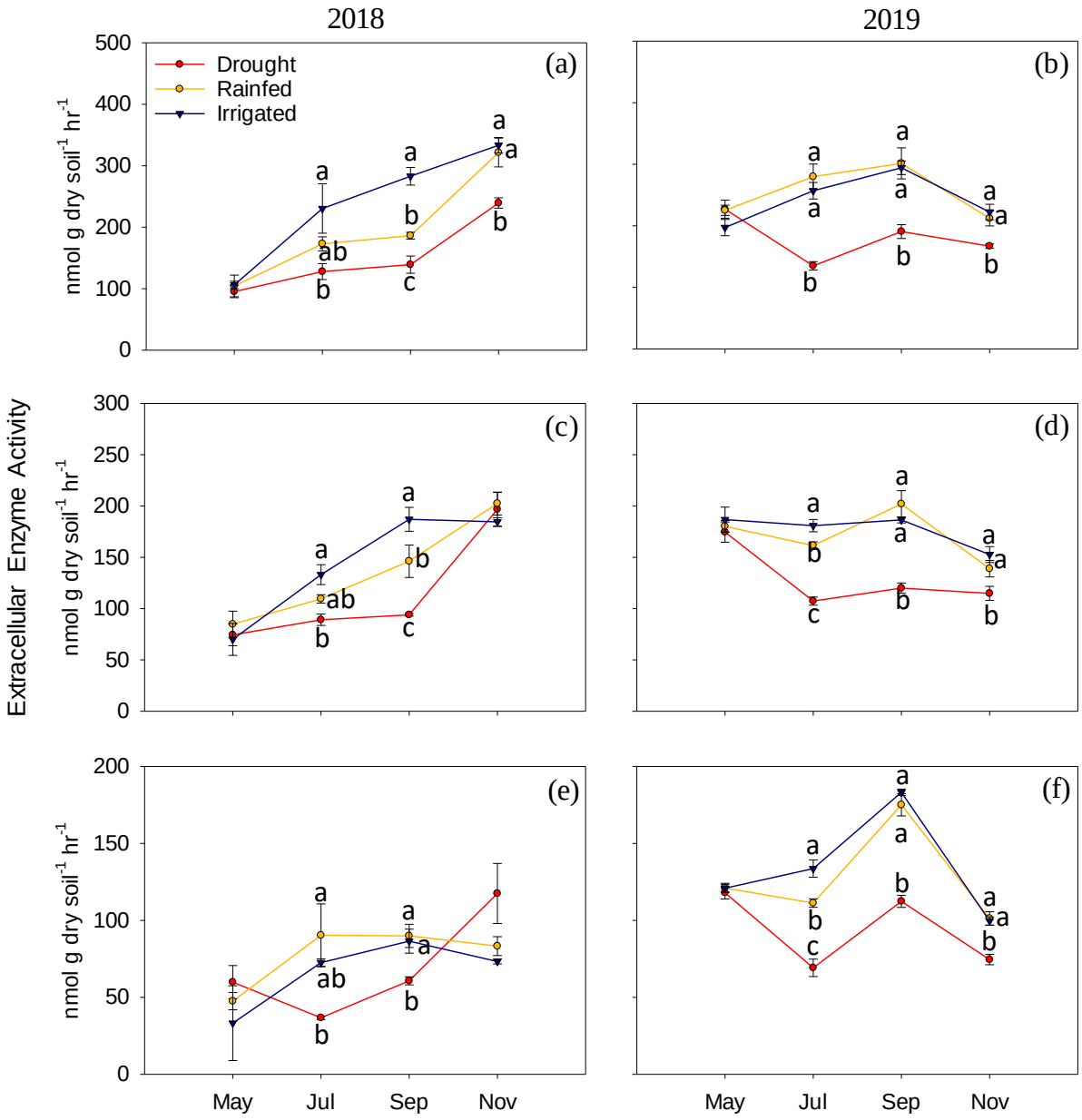


Figure 4.5. Extracellular enzyme activity under drought, rainfed, and irrigated treatments at 0-5 (a, b), 5-15 (c, d) and 15-30 (e, f) cm depths during 2018 and 2019.
Different letters represent statistical significance at $p \leq 0.05$ at each time point. No letters mean no statistical significance. Error bars represent standard error (n=3). Sampling in May represents baseline and in November represents post shelter removal.

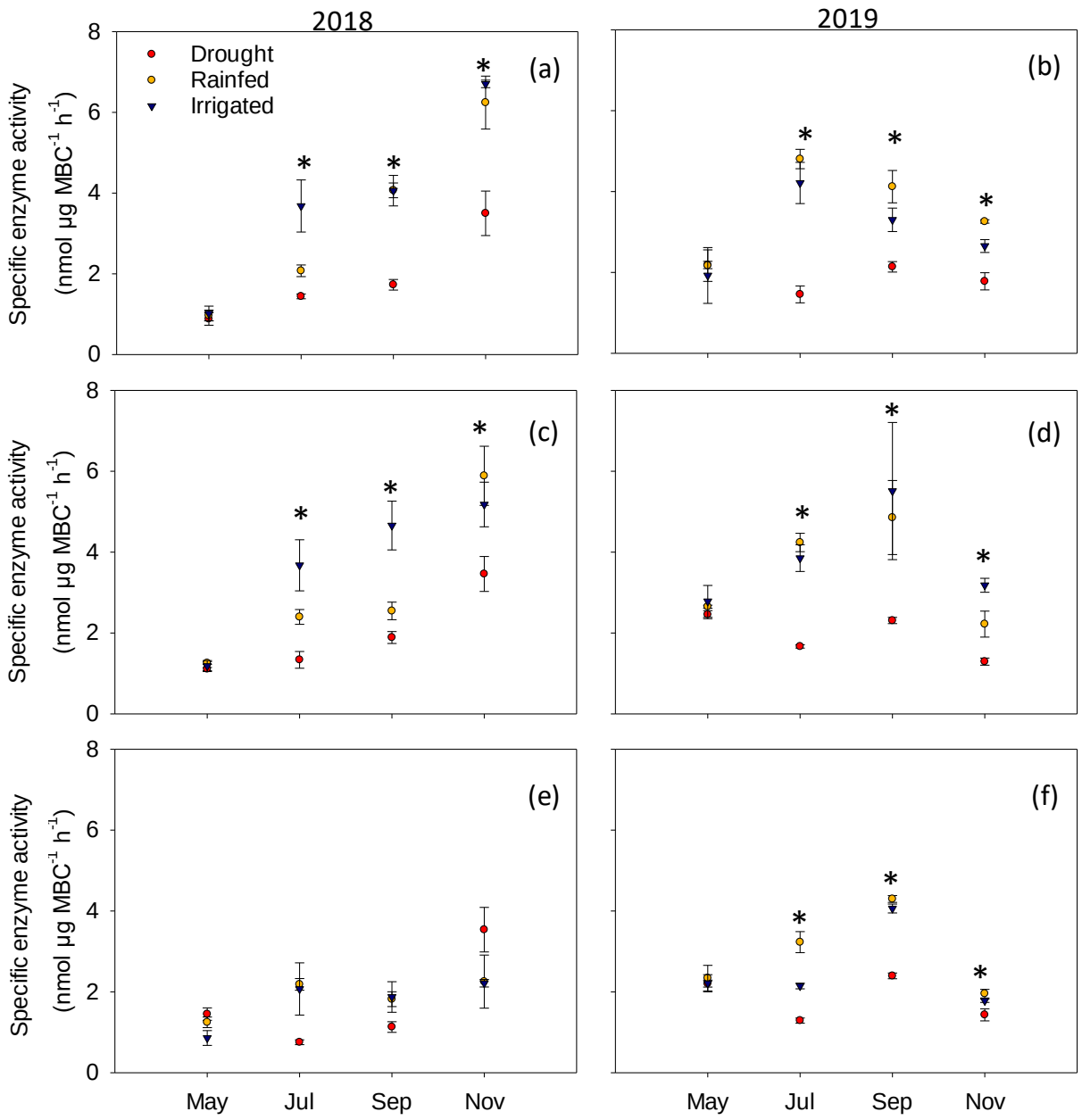


Figure 4.6. Specific enzyme activity under drought, rainfed, and irrigated treatments at 0-5 (a, b), 5-15 (c, d) and 15-30 (e, f) cm depths during 2018 and 2019.
Asterisk represents statistical significance at $p \leq 0.05$ at each time point. No asterisks mean no statistical significance. Error bars represent standard error (n=3). Sampling in May represents baseline and in November represents post shelter removal.

Table 4.1. The amount of water added to each treatment during the growing season in 2018 and 2019.

Treatment	Amount of water added (cm)	
	2018	2019
Drought	0	0
Rainfed	103	83
Irrigated	114	91

Table 4.2. Repeated measures ANOVA statistics (p value) for soil moisture and sampling time effects on various measured parameters.

		2018			2019		
		Trt	Time	Trt x Time	Trt	Time	Trt x Time
0-5 cm	CO_2 Emissions	<0.0001	<0.0001	0.0004	0.0002	<0.0001	0.0002
	Extractable Organic C	<0.0001	0.009	NS*	<0.0001	<0.0001	0.007
	Microbial Biomass C	0.086	0.0001	NS	0.004	<0.0001	0.0002
	Hyphal length	NS	<0.0001	NS	0.002	0.003	0.006
	Enzyme Activity	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0001
	Enzyme Specific Activity	0.0003	<0.0001	0.001	<0.0001	0.006	0.044
5-15 cm	Extractable Organic C	0.0001	0.038	0.004	0.001	0.0001	0.006
	Microbial Biomass C	0.0002	NS	NS	0.001	0.044	0.001
	Hyphal length	NS	NS	NS	0.023	0.0001	0.001
	Enzyme Activity	0.015	<0.0001	0.001	0.0002	<0.0001	0.001
	Enzyme Specific Activity	<0.0001	<0.0001	NS	0.001	0.004	0.259
15-30 cm	Extractable Organic C	NS	<0.0001	NS	0.009	0.0002	0.007
	Microbial Biomass C	NS	0.001	NS	NS	0.016	0.640
	Enzyme Activity	NS	NS	NS	<0.0001	NS	NS
	Enzyme Specific Activity	NS	0.001	0.026	<0.0001	<0.0001	0.0002

*NS, non-significant.

Chapter 5. General conclusions

Despite the strong control of soil moisture on microbial responses, how moisture changes in different textured soils affect soil organic carbon (SOC) decomposition remains relatively uncertain. This dissertation aimed to unravel microbial cycling of SOC to changes in soil moisture in three different textured soils (sandy, loamy, and clayey) through two laboratory experiments and in a loamy soil through a field experiment. Understanding the interaction of soil texture, moisture and microbial respiration is central to accurately predict future climate change

The first laboratory experiment, a 90-day incubation using sandy, loamy and clayey soils under steady state moisture condition, showed that soil texture has a stronger influence than moisture on the microbial activity. Different soils exhibited different moisture optima for highest microbial activity. I also observed a decoupling of soil moisture from microbial respiration in coarse textured soils while this decoupling was weak in fine textured soils. The experimental verification of the empirical soil moisture-respiration relationships reported by Moyano et al. (2012, 2013) was conducted and results showed that sandy and loamy soils followed the empirical relationships closely while clayey soils did not.

The second laboratory experiment, a 140-day incubation under transient moisture changes through alternating wetting and drying cycles including a steady state moisture control aimed to gain fundamental understanding of the Birch effect in different soils. The study investigated three potential mechanisms for the Birch effect including (i) release of cellular metabolites upon rewetting, releasing more C in soils for mineralization, (ii) physical breakdown of macroaggregates by repeated drying and wetting, releasing aggregate protected C in soils for mineralization, and (iii) changes in microbial population under repeated drying and wetting contributes positively to C mineralization. Results showed that different mechanisms played major roles in fueling the Birch effect in different soils. For sandy soil, metabolite accumulation

and bacterial composition change were the key mechanisms for the Birch effect while for loamy and clayey soils, aggregate breakdown and metabolite accumulation mechanisms contributed more to the Birch effect. This study revealed that soil texture is a major controller of microbial activity when soils experience extreme and repeated changes in soil moisture.

The field experiment that involved two years of drought manipulation using rain-out shelters along with rainfed and irrigated treatments in a loamy soil under soybean production system revealed that microbial activity was lowest under drought conditions despite highest microbial biomass C and extractable organic C. The fungal hyphae abundance was also higher under drought conditions, however, the metabolic status of microbes, shown by specific enzyme activity, under drought conditions was low compared to higher moisture treatments. These findings also showed strong influence of moisture on SOC cycling under field conditions where different biotic and abiotic drivers of microbial activity are simultaneously at play.

This research highlights the need for incorporating the soil moisture-texture interactive influence on SOC cycling in ecosystem models for more accurate predictions of global climate change. It implicates the need to further probe into “microbial-centric” view of environmental stressors and its impacts on SOC dynamics. More mesocosm- and field-based studies should be conducted for gaining understanding into community level responses to simultaneous changes in various environmental conditions and incorporate those into microbial mediated ecosystem models to make more realistic projections.

VITA

Shikha Singh was born in the city of Ranchi, Jharkhand, India to Kalendra Bahadur Singh and Kiran Singh. She received her Bachelor's in Agriculture Sciences in 2014 from Punjab Agricultural University, Punjab, India. She joined South Dakota State University for her MS in soil science in Spring 2015 where she looked at impacts of winter manure application on surface runoff and soil quality. She later joined the Department of Biosystems Engineering and Soil Science at the University of Tennessee Knoxville to pursue her Ph.D. under the supervision of Dr. Sindhu Jagadamma. She had presented her research at various regional and national conferences and won awards for the same. She also received several fellowships, and college/university level awards during her graduate studies.