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Abstract

Climate change is a result of greenhouse gases released into the atmosphere. These changes are expected to cause extreme weather conditions, including severe storms. Large amounts of rain will fall in shorter periods of time, leading to heavy runoff, and increasing the severity of drought conditions within the soil (Zeglin et al. 2013).

Native grasslands occupy almost a quarter of the earth's land surface and are valuable ecological resources. They contain soils with high concentrations of organic matter and play a key role in mitigating greenhouse gas emissions through carbon sequestration. There are a variety of grassland management techniques including annual burning, patch burning, and cattle grazing. These management techniques can be beneficial for ecosystems, but can also alter soil compositions (Jerome et al. 2014). Microbial communities in the soil influence many ecosystem processes such as nutrient acquisition, carbon and nitrogen cycling, and soil formation (Heijden et al. 2008). Changes in precipitation patterns can effect microbes in these grasslands by causing shifts in community composition, and changes in nutrient cycling and decomposition processes. Many microbial activities can be directly correlated with water availability, and drought conditions may be detrimental to these grazed grassland ecosystems (Gray et al. 2011). Summer months and differences in time lead to changes in temperatures and rainfall patters, similarly having the potential to alter activity and structure of microbial communities.

This study was conducted at the Konza Prairie Biological Station in eastern Kansas, USA. Soil samples were collected to compare June versus July and moist versus dry treatments. Findings from this study concluded that seasonal changes through June and July alter microbial communities in Konza Prairie soil. Total PLFA concentrations significantly increased, with the largest increase occurring in fungi. This change caused a decrease in relative abundance of gram positive and gram negative bacteria, and also an increase in the ratio of fungi to bacteria. Drought conditions caused no significant change in microbial communities, suggesting the microbes in the soil have a high tolerance for lack of moisture.

Introduction

Climate change is a result of greenhouse gases released into the atmosphere. The current changes are expected to worsen in the future and are predicted to significantly alter the water cycle. Extreme weather conditions, including severe storms, are also expected to occur. Large amounts of rain will fall in shorter periods of time with longer dry periods in between (Zeglin et al. 2013). This will lead to heavy runoff, leaching, and an increase in the severity of drought conditions within the soil (Cregger et al. 2014). Trends have shown that higher precipitation and water availability correlates with higher productivity (Hartmann et al. 2012). Changes in precipitation patterns are predicted to decrease plant photosynthetic rates and aboveground productivity (Zeglin et al. 2013). Water availability is a major abiotic factor influencing gas exchange, soil respiration, nutrient cycling, and the ability for ecosystems to thrive (Jumpponen et al. 2014).

Grasslands occupy almost a quarter of the earth's land surface and are valuable ecological resources. These lands are used for livestock production and largely contribute to biodiversity. Grassland soils are known for their high content of organic matter (Jerome et al. 2014), containing nearly 37% of the terrestrial organic carbon (Jumpponen et al. 2014). With such high concentrations, they play a key role in mitigating greenhouse gas emissions through carbon sequestration (Jerome et al. 2014). Grassland functions can be altered using management techniques such as burning and grazing. Burning, as a management technique, can consist of annual burning or patch burning. Annual and patch burning of grasslands can be beneficial for ecosystems by stimulating photosynthesis, increasing stem density, and creating higher aboveground productivity (Toma et al. 2010). Cattle grazing can have similar effects, but both grazing and burning have been observed to alter physical properties of soil such as carbon and nitrogen content and plant community composition (Jerome et al. 2014). These aspects of grazing management along with climate changes could alter grassland ecosystems. Plant function and composition in grasslands are sensitive to changing climate conditions. Precipitation patterns, which determine soil moisture levels, control the structure and function of soil communities (Jumpponen et al. 2014). With higher rain intensity and increased dry periods, stressful conditions will be imposed to these grassland ecosystems (Zeglin et al. 2013).

Microbial communities in the soil of grasslands play an important role in determining the diversity, richness, and abundance of plant life. Microbes influence many ecosystem processes such as nutrient acquisition, carbon and nitrogen cycling, and soil formation. A diverse and abundant microbial community is vital in performing these many different processes which make up an ecosystem (Heijden et al. 2008). Moisture levels in the soil can greatly affect these microbial communities. Different taxonomic and functional groups of microorganisms have different drought tolerance levels. For example, fungi typically benefit from drought conditions while Gram-negative bacteria usually decrease in abundance. This can lead to a shift in community composition, causing changes in nutrient cycling and decomposition processes (Gray et al. 2011). Direct correlations can be seen between soil microbial respiration and water availability (Zeglin et al. 2013). Diffusion rates and activity of microbes have been shown to slow when less moisture is available (Hartmann et al. 2012). Drought conditions can potentially restrict microbial community activities and alter vital ecological processes (Fernandez et al. 2012).

Seasonal changes throughout the year will correlate to different temperatures, amounts of rainfall, vegetation growth rates, and grazing habits. These differences can cause changes within microbial communities. High temperatures have been known to slow growth and activity rate of microbes (Sheik et al. 2011). During high rates of plant growth, microbial activity and abundance is expected to significantly increase. When more grazing occurs, aboveground biomass decreases and root contents increase (Pineiro et al. 2009). This can alter the transfer of nutrients and in turn effect the microbial communities within the soil.

Knowing the effects of drought conditions and time on microbial communities in native grasslands will help prepare for the climate changes predicted to occur. The grassland's rich soil and ability to mitigate greenhouse gases is motivation to better understand these correlations.

Materials and Methods

Site Description and Sampling

The study was conducted in native tall-grass prairie located at the Konza Prairie Biological Station (39°05'N, 96°35'W), a Long-Term Ecological Research site, in the Flint Hills of eastern Kansas, USA. The primary vegetation includes native big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* (L.) Nash.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash.), and switchgrass (*Panicum virgatum* L.) (Zeglin et al. 2013). The landscape is characterized by shallow limestone soils. Both soil type and depth vary by landscape position. In general, the soils are silty clay loams.

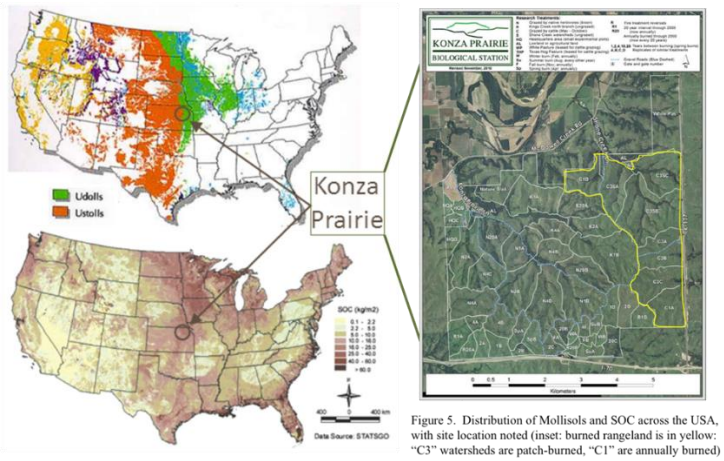

Figure 5. Distribution of Mollisols and SOC across the USA, with site location noted (inset: burned rangeland is in yellow: "C3" watersheds are patch-burned, "C1" are annually burned)

Figure 1: Konza Prairie Map

On June 9, 2015, a total of 15 samples were taken from plots C1A, C3A, and C3B within the cattle grazed Konza Prairie Biological Station. Plot C1A is burned annually while plots C3A and C3B are patch burned every three years. Random auger cores were taken from within a 5 foot radius of each sample location. The top 5 cm of soil from each core was placed into a bag and homogenized. Each sample consisted of approximately 500g of soil. These samples were analyzed for pH, extractable nitrogen, gravimetric soil water content, CO_2 respiration, and phospholipid fatty acids (PLFA).

On July 7, 2015, four soil samples were taken from within the C1A plot of Konza Prairie Biological Station. Samples were collected as described above, and each sample consisted of approximately 500g of soil. The samples were divided into three treatment groups: time zero, moist, and dry. Time zero samples were immediately analyzed for gravimetric water content and PLFA. The moist samples were placed in mason jars and covered while dry samples were spread on a tray and left to dry in open air. All moist and dry samples were left in the incubator for 6 days. At the conclusion of the 6 day period, samples were analyzed for gravimetric water content and phospholipid fatty acids (PLFA).

Figure 2: A) Dry treatment. B) Moist treatment. C) Soil sample collection.

Soil Sample Diagnostics

To determine the gravimetric soil water content, approximately 10g of soil was measured into a foil tin and placed in the oven for drying. Samples were dried for 48 hours at a temperature of 105°C and then weighed again to determine the amount water lost. Extractable inorganic nitrogen (N) and pH were determined by the soil testing lab at Kansas State University. Carbon dioxide (CO_2) respiration was analyzed by placing 25g of fresh soil into a sealed mason jar and storing in the incubator for 24 hours. At the conclusion of the 24 hour period, the amount of CO_2 respired was analyzed using gas chromatography.

Phospholipid Fatty Acid Analysis

The total lipids were extracted from the frozen, lyophilized soil using a modification of the Bligh and Dyer (1959) extraction (White and Ringelberg, 1998). The phospholipid fatty acids (PLFA) were then separated from the total lipid extract using silicic acid chromatography, the fatty acids cleaved from the glycerol backbone using KOH saponification, and the harvested fatty acids methylated to form fatty acid methyl esters (FAME) (White and Ringelberg, 1998; Allison and Miller, 2005). The resulting FAME were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30m x 250 µm i.d. x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, USA). FAME peaks were identified by comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Tentative assignments of FAME peaks not present in the BAME mix were made by mass spectral interpretation. Peak concentration was quantified using the internal standard nonadecanoate.

The nomenclature used to describe the identified fatty acids is as follows (Bossio and Scow, 1998): total number of C atoms : number of double bonds, the position of the double bonds, cis or trans isomers identified by c or t. Prefixes of a, i, and Me indicate anteiso branching, iso branching, and methylation, respectively. Fatty acids were grouped into Gram positive (Gm+) bacteria (i15:0, a15:0, i15:0, i17:0, and a17:0), Gram negative (Gm−) bacteria (19:0:delta9,10, 17:0:delta9,10, C10:0:2-OH, C12:0:2-OH, C12:0:3-OH, C14:0:2-OH, C14:0:3-OH, C16:1:0:cis, C16:0:2-OH), actinomycetes (10Me16:0 and 10Me18:0), AMF (C16:1:11), and fungi (C18:2:9,12) (McKinley et al., 2005) (White et al. 2009).

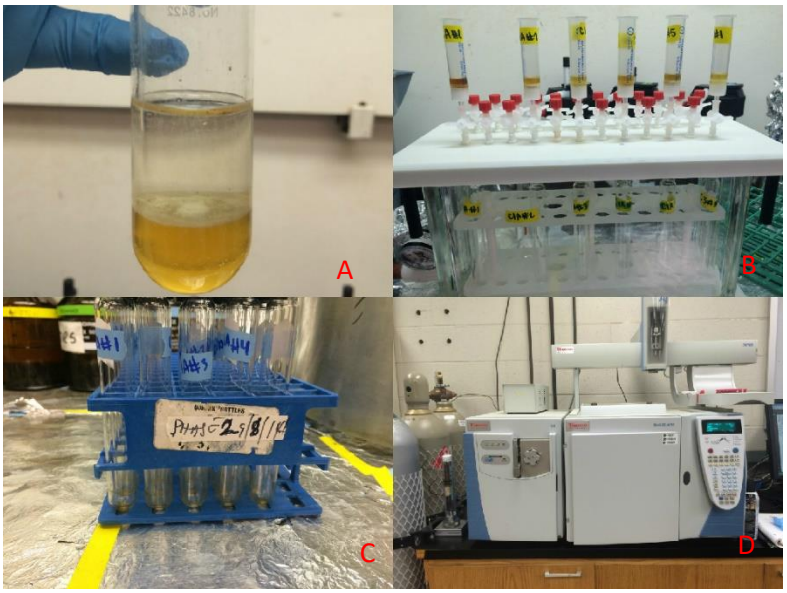

Figure 3: A) Extraction of lipids (lipids in top layer). B) Silicic Acid Chromatography. C) Resulting FAME from lipid methylation. D) Thermo Scientific Trace GC-ISQ Mass Spectrometer

Statistical Analysis

Results from plot C1A in June and July were compared to determine if time would affect microbial communities. Results from treatment groups, time zero, moist, and dry, were compared to determine how drought conditions would affect microbial communities. Statistical Analysis System (SAS) was used to analyze results using an analysis of variance (ANOVA) method. The remaining data, taken from plots C1A, C3A, and C3B, was averaged and used to classify soil properties of Konza Prairie.

Results

Plot C1A, C3A, and C3B Soil Properties

Konza Prairie Plots	Average % Moisture
C1A	26.45
C3A	24.55
C3B	32.95

Table 1: Gravimetric soil water Content from Plots C1A, C3A, and C3B within the Konza Prairie Biological Station.

Konza Prairie Plot	Average pH	Average NH_4-N (ppm)	Average $N0_3$-N (ppm)	Average CO_2 (ppm)
C1A	6.20	0.57	0.09	4036.48144
C3A	6.31	0.41	0.06	3951.69974
C3B	6.47	0.57	0.05	4942.49312

Table 2: Average pH, Ammonium (NH_4-N), Nitrates (NO_3-N), and Carbon Dioxide (CO_2) from Plots C1A, C3A, and C3B within the Konza Prairie Biological Station.

Study 1- Time (June VS. July)

Time	Average % Moisture
June	26.45
July	24.55

Table 3: Average % moisture content from plot C1A in June and July.

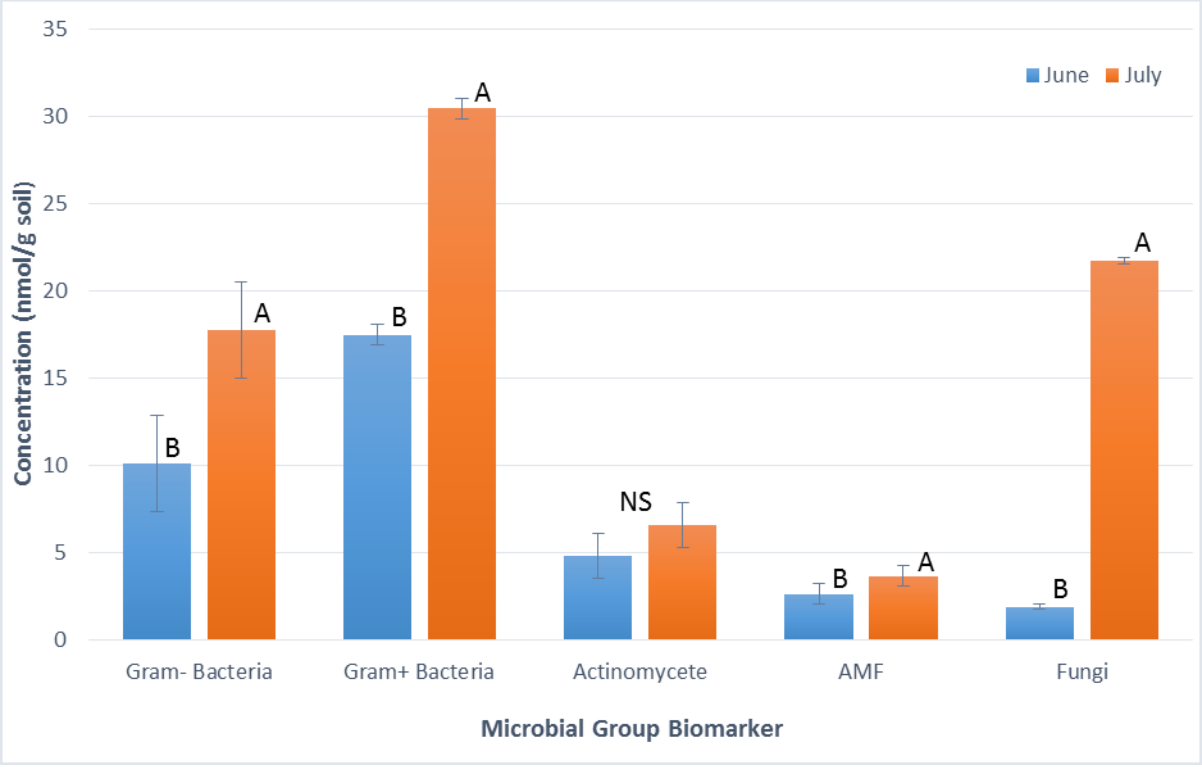

Figure 4: Microbial biomarker concentrations for June and July. Error bars represent standard error of concentration. Different letters (A and B) above bars indicate significant differences ($p<.05$). NS indicates no significant difference ($p>.05$).

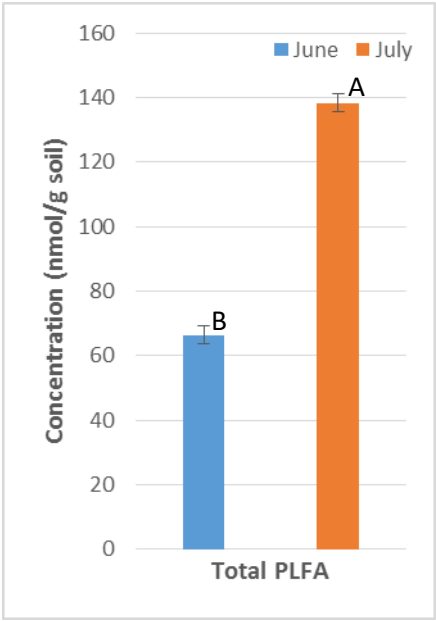

Figure 5: Total PLFA concentration for June and July. Error bars represent standard error of concentration. Different letters (A and B) above bars indicate significant differences ($p<.05$).

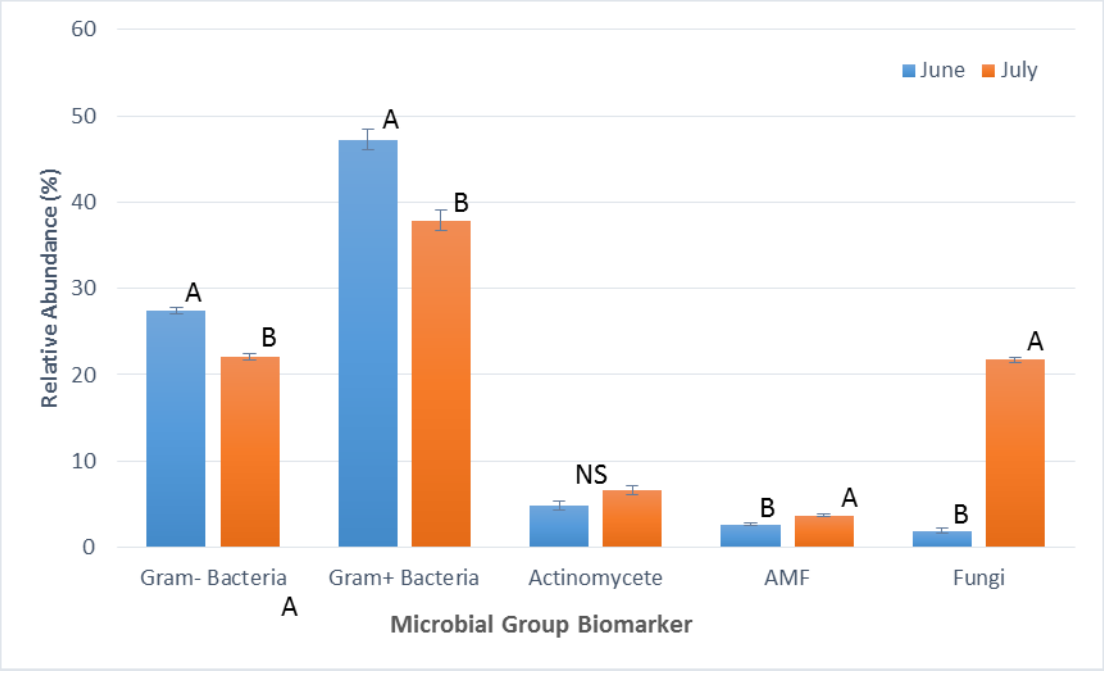

Figure 6: Microbial biomarker relative abundance for June and July. Error bars represent standard error of concentration. Different letters (A and B) above bars indicate significant differences ($p<.05$). NS indicates no significant difference ($p>.05$).

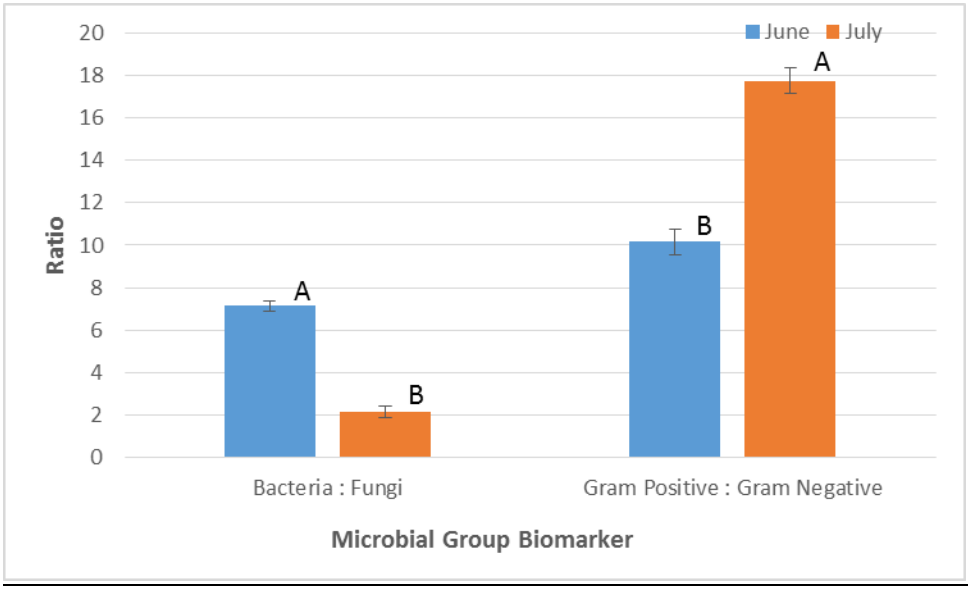

Figure 7: Ratio of bacteria to fungi and gram+ to gram- for June and July. Error bars represent standard error of concentration. Different letters (A and B) above bars indicate significant differences ($p<.05$).

Treatment Groups

Biomarker	Concentration	Relative Abundance
Gram- Bacteria	0.31	0.28
Gram+ Bacteria	0.62	0.51
Actinomycete	0.29	0.77
AMF	0.50	0.59
Fungi	0.74	0.18

Table 4: ANOVA p values of concentration and relative abundance of microbial group biomarkers. P values greater than .05 mean no significant difference.

Treatments	Average % Moisture
Time Zero	24.55
Moist	28.34
Dry	5.07

Table 5: Gravimetric Soil Water Content of Treatment Groups (Time Zero, Moist, and Dry) from Plot C1A within the Konza Prairie Biological Station.

Discussion

The data collected from plots C1A, C3A, and C3B, shown in Tables 1 and 2, was not significantly different between each plot. This gives general information on the soil type and topography at Konza Prairie Biological Station.

Comparing data between times June and July, the average % moisture was not significantly different. PLFA biomarkers did show a significant difference between June and July. Total PLFA concentration more than doubled in July, with a significant increase in every microbial biomarker except actinomycete (Fig. 4 and 5). Fungi showed the largest increase between June and July, which in turn altered the relative abundance of each biomarker (Fig. 6). With a massive increase in concentration of fungi, gram positive and gram negative bacteria decreased in relative abundance. In June, the ratio of fungi to bacteria and the ratio of gram positive bacteria to gram negative bacteria was significantly smaller. Since moisture calculations were taken on the day of soil collections and have no significant difference, moisture does not account for the changes in microbial biomarkers shown. There was no major difference in the average amount of precipitation in the 28 days preceding collections. However, the dates preceding the June collection had several small rain events spread throughout the duration. The dates preceding the July collection had few rain events but one large rain event the date prior to collection. The large rain event preceding the July collection may account for the higher PLFA concentration within the sample. An increase in plant growth between June and July could also contribute to the increase in PLFA concentration.

The treatment groups, time zero, moist, and dry, were analyzed to show how microbial communities may change with drought conditions. As expected, the gravimetric soil water content of the dry treatment group had significantly less moisture than both of the other treatment groups (Fig. 8). Comparing the time zero and moist samples, data shows moist samples having a slightly higher moisture content than time zero samples. This could be due to the moisture levels in the incubation room, where the moist samples were placed for 6 days after sampling. The analysis of PLFA data displayed no significant difference between any microbial biomarker in wet and dry treatments (Fig. 9). This implies that the drying of the soil

may not affect the microbial community abundance and concentrations in samples taken from within this field. The bacteria to fungi ratio (not shown in results) from this study showed no significant difference between wet and dry treatments. These findings were similar to a study by Bachar et al. 2010 who found that bacteria richness did not change based on precipitation. However, a study conducted by Zeglin et al. 2012 found fungal to bacteria ratios to be higher at low soil water contents. The same study also found microbial biomass to increase after rewetting dry soil, meaning moist soil would have a higher abundance of microbes. On the date of sample collection, the high temperature was 22.2°C and the low was 15.92°C. The average temperature for July is 26.6°C. These lower temperatures observed may have influenced our results.

Though pH, CO_2 respiration, and extractable nitrogen were not analyzed for the treatment groups, the changes within these variables under drought conditions can be predicted. Soil nitrogen (in forms of ammonium and nitrate) at the Sevilleta National Wildlife Refuge in New Mexico has been observed to be highest in low moisture soil, with nitrogen content decreasing as water availability increases. This suggest that precipitation changes will have a direct effect on levels of extractable nitrogen in the soil (Cregger et al. 2014). Another study on the Canterbury Plains of New Zealand found that nitrogen mineralization was lower under dry conditions, meaning less ammonium and nitrates would be found in the dry soil (Harrison-Kirk et al. 2014). This shows the complexity of drought conditions on nitrogen in the soil, and proves there could be many factors affecting this variable including soil type, temperatures, and geographic location. Several studies have concluded that CO_2 flux is reduced under decreased amounts of rainfall. Additionally, carbon cycling has been observed to slow when there is an increase in variability of soil moisture. The reduction of carbon cycling and CO_2 respiration is likely mediated by a decrease in root and soil respiration due to a lack of water availability (Harper et al. 2005). Another study, conducted at the Konza Prairie Biological Station, measured CO_2 flux over a period of time and identified respiration decreasing as the duration of low-moisture in the soil increased (Zeglin et al. 2013). These studies indicate that CO_2 respiration will decrease when precipitation patterns change and drought conditions occur. Acidification caused by drought was observed in a study by Xiang et al. 2012 where pH values

decreased from an average of 5.67 to 4.84 as moisture decreased. This suggests that drought conditions will alter pH levels found in soil. It is generally understood that soil water content is positively correlated with microbial activity, but correlations can be extremely complex and many factors can contribute to these results (Zeglin et al. 2013).

Conclusion

In Konza Prairie soil, seasonal changes through June and July alter microbial communities. Total PLFA concentrations significantly increase, with the largest increase occurring in fungi. This change causes a decrease in relative abundance of gram positive and gram negative bacteria, and also an increase in the ratio of fungi to bacteria. Drought conditions were observed to cause no significant change in microbial communities, suggesting the microbes in the soil have a high tolerance for lack of soil moisture.

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