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A watering manipulation in a semiarid grassland induced changes in fungal but not bacterial community composition

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ABSTRACT

Monsoon precipitation in the arid southwestern United States is an important driver of ecosystem productivity, delivering up to 50% of annual precipitation during the summer months. These sporadic rainfall events typify drying-rewetting cycles and impose a physiological stress on the soil microbial communities responsible for carbon and nutrient cycling. As one aspect of climate change is an intensification of the hydrologic cycle, understanding how soil microbial communities and the processes they mediate are impacted by moisture fluctuations is increasingly important. We performed a month-long watering manipulation in the field and characterized bacterial and fungal communities across five time points using high-throughput sequencing. Watering treatment had a significant impact on fungal community composition, and there was a trend toward decreased fungal diversity and OTU richness in watered plots. In contrast, no significant differences were observed in bacterial communities between watered and control plots nor among sampling times. These findings suggest that fungi are more sensitive than bacteria to changes in soil moisture.

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1. Introduction

Sporadic precipitation with varied temporal distribution dictates productivity in arid regions (Noy-Meir, 1973). For the southwestern United States, a shift from dry westerly winds to wet southeasterly winds results in a transition from an extremely dry spring to a wet summer. This wind shift, known as the North American monsoon, transports pulses of moisture from the tropical eastern Pacific Ocean, Gulf of California, and Gulf of Mexico (Hales, 1972; Brenner, 1974). Arizona and New Mexico receive up to half of their annual precipitation during the summer monsoon season (Climate Assessment for the Southwest, 2012), making this rain event crucial for ecosystem productivity and dynamics. As alterations in precipitation patterns are predicted due to climate change, it is critical to uncover how resilient ecosystem processes are to fluctuations in precipitation. Microbial processes, including organic matter decomposition and nitrogen cycling, are important contributors to ecosystem productivity, and yet the mechanisms behind microbial population dynamics in response to major changes in precipitation levels remain poorly understood.

In a descriptive study of soil microbial population dynamics in a semiarid grassland during the monsoon period in northern Arizona, select bacterial populations, including Actinobacteria and Firmicutes, responded immediately to an increase in soil moisture. However, overall bacterial community composition did not change significantly in response to increased soil moisture (McHugh et al., 2014). In contrast, fungal community composition shifted late in the season after the rains ceased, possibly in response to plant growth. These compositional shifts were driven primarily by increased abundances of Glomeromycota and Zygomycota. In general, the relative abundances of fungal phyla were more variable through time and with soil moisture fluctuations than those of bacterial phyla, suggesting the fungal community was more dynamic during the rainy period (McHugh et al., 2014). Many environmental parameters, including temperature, soil moisture, relative humidity, and photoperiod change during the monsoon season. In order to understand the mechanism behind microbial population dynamics, it is imperative to conduct manipulation experiments in which the impacts of altered environmental parameters on microbial community composition are investigated.

In a field manipulation with decreased precipitation and/or removal of the plant community, we measured the direct and

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interactive effects of these changes on bacterial and fungal communities at five time points during a summer season in a semiarid grassland site (McHugh and Schwartz, 2014). We found that soil microbial communities were sensitive to alterations in precipitation and plant assemblages, though the observed changes in community composition were not pronounced. These findings are significant in light of the growing recognition that the composition of microbial communities influences important ecosystem processes across a variety of spatial and temporal scales (Strickland et al., 2009; Allison et al., 2013).

In the present study, we report the results of a watering experiment in a semiarid grassland where we altered both the timing and frequency of precipitation by applying an entire monsoon period's moisture to soil during the dry season, prior to the advent of the summer rains. Using high-throughput sequencing, we describe the responses of bacterial and fungal communities to extreme moisture fluctuations. We hypothesized that abundant bacterial populations, including Actinobacteria and Firmicutes, would be immediately impacted by increased soil moisture, while the composite fungal community would change slowly in response to the watering manipulation.

2. Material and methods

2.1. Site description and experimental design

This study was conducted in a semiarid, high-desert grassland north of Flagstaff, Arizona (35°34′20′N, 111°34′4′W, 1760 m above sea level, 230 mm of rain annually). Vegetation at the site consists mainly of perennial grasses (*Bouteloua eriopoda*,*Bouteloua gracilis*, *Sporobulus cryptandrus*, and *Pleuraphis jamesii*) with few shrubs (*Ericameria nauseousa*, *Gutierrezia sarothrae*). Soils are cindery and are classified in the U.S. Department of Agriculture Soil Taxonomic Subgroup of Typic Haplustolls. A Campbell Scientific (Logan, UT) weather station continuously collects precipitation and temperature data, along with other environmental parameters.

We performed a month-long watering experiment during June 2012, prior to the natural monsoon rains, when temperatures were elevated (daily mean 25.6 °C) and soil moisture content was extremely low (1.94% on average). Plants were physiologically dormant at this time and vegetation cover was patchy. No natural precipitation occurred during the experimental period, which extended from 1 to 30 June. A random block design was employed, with four blocks as replicates and 1.0 m spaces between blocks. Each block contained two 1.0 m x 1.0 m plots randomly designated as treatment plots or controls, and spaces of 1.0 m were left between plots in each block. Long-term monsoon precipitation averages for this grassland were used to design the watering regime (Fig. 1). A total of 78 mm of water was delivered to each treatment plot over a 28-day period. The approach mimicked the natural dry-wet cycles typical of monsoon precipitation (McHugh et al., 2014), with the greatest amount of moisture being delivered in the middle of the experiment.

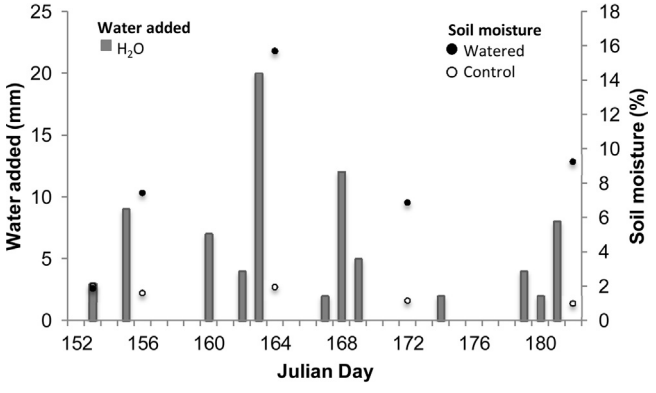

Fig. 1. Watering scheme and soil moisture content during the watering experiment. Error bars are standard error for means (n = 4).

2.2. Soil collection and moisture measurements

A Hydrosense II soil-water sensor with 12 cm rods (Campbell Scientific, Logan, UT, USA) was used to measure volumetric water content on each watering day, prior to irrigation. At five time points (experimental days 1, 4, 12, 20, and 30), one 5 cm (depth and diameter) soil core was collected from bare soil between plant patches within each plot, for a total of 40 cores. Soil cores were sieved (<2 mm), homogenized, and subsampled to determine gravimetric water content.

2.3. NO_3^- and NH_4^+

Soil NO_3^- and NH_4^+ were extracted from 10 g soil samples in 40 mL 2 M KCl solution in the field. Samples were transported back to the laboratory, shaken for 1 h, filtered through Whatman No. 1 filter paper and stored at −20 °C until further processing. Ammonium and NO_3^- extracts were analyzed colorimetrically with a Lachat analyzer.

2.4. Soil respiration

On the five soil collection days, a 40 g subsample of fresh soil from each core was weighed into a specimen cup, placed in a 1 L Mason jar, and sealed with a lid containing a rubber septum. CO_2 concentrations were measured for each sample with a LI-COR 6262. CO_2/H_2O gas analyzer (LI-COR Biosciences Inc., Lincoln, NE, USA). Gas samples were taken shortly after field collection and two days following collection to obtain a rate of CO_2 production. All jars were incubated in the dark at 23 °C. Remaining soil was stored at −40 °C until further processing.

2.5. Nucleic acid extraction and sequencing

Total genomic DNA was extracted from 0.5 g of soil in each sample using PowerLyzer PowerSoil DNA Isolation Kit according to the manufacturer's instructions, with an initial 10-min incubation at 70 °C followed by bead beating for 90 s (MO BIO Laboratories, Inc., Carlsbad, CA). High-throughput short amplicon sequencing on an Illumina MiSeq platform was performed at Northern Arizona University's Environmental Genetics and Genomics Laboratory. The 16S rRNA gene was amplified using primers 515f and 806r, which target the hypervariable V4 region (Caporaso et al., 2012). The fungal ITS region was amplified using primers ITS4_Fun and 5.8S_Fun, which target ITS2 (Taylor, 2014).

Bacterial and fungal samples were prepared for sequencing using an approach described previously (McHugh and Schwartz, 2014). All samples were quantified by PicoGreen (Molecular Probes, Eugene, OR) fluorescence and normalized to 1 ng/μL before amplification. Samples were processed in two barcoded primer PCR steps (Berry et al., 2011). First, each sample was amplified in triplicate, 8 μL-PCRs containing 1 mM of each primer, 0.01U/μL Phusion HotStart II Polymerase (Thermo Fisher Scientific, Waltham, MA), 1X Phusion HF buffer (Thermo Fisher Scientific), 3.0 mM $MgCl_2$, 6% glycerol, and 200 μM dNTPs. PCR conditions were as follows: 95 °C for 2 min, 15 cycles of 30 s at 95 °C (25 cycles for ITS), 30 s at 55 °C, and 4 min at 60 °C. Initial PCR products were pooled, diluted 10-fold, and used as template in the subsequent tailing reactions with region-specific primers. These reactions were identical to initial PCR reagent concentrations and cycling