Running title: Precipitation change alters microbial communities

Soil microbial community response to precipitation change in a semi-arid ecosystem

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Abstract

Microbial communities regulate many belowground carbon cycling processes, thus the impact of climate change on the structure and function of soil microbial communities could in turn impact the release or storage of carbon in soils. Here we used a large-scale precipitation manipulation (+18%, -50%, or ambient) in a piñon-juniper woodland (Pinus edulis-Juniperus monosperma) to investigate how changes in precipitation amounts altered soil microbial communities as well as what role seasonal variation in rainfall and plant composition played in the microbial community response. Seasonal variability in precipitation had a larger role in determining the composition of soil microbial communities in 2008 than the direct effect of the experimental precipitation treatments. Bacterial and fungal communities in the dry, relatively moisture limited pre-monsoon season were compositionally distinct relative to communities in the monsoon season, when soil moisture levels and periodicity varied more widely across treatments. Fungal abundance in the drought plots during the dry pre-monsoon season was particularly low, and was $4.7 \times$ greater upon soil wet up in the monsoon season suggesting that soil fungi were water limited in the driest plots which may result in a decrease in fungal degradation of carbon substrates. Additionally, we found both bacterial and fungal communities beneath piñon pine versus juniper were distinct; suggesting that microbial function beneath these trees is different. We conclude that predicting the response of microbial communities to climate change is highly dependent on seasonal dynamics, background climatic variability, and the composition of the associated aboveground community.

Key Words

Climate change, drought, microbial community, quantitative PCR, semi-arid woodland, TRFLP
Introduction

Soil and litter microbial communities are responsible for the majority of decomposition and nutrient mineralization in terrestrial ecosystems (2) and their abundance, community structure, and activity is often directly influenced by abiotic factors such as temperature and precipitation (1, 12, 48, 56). Because global climate change may have significant impacts on the global hydrologic cycle (36), understanding how changes in precipitation shape soil microbial communities and their function is important for predicting carbon feedbacks to global climate change (32). Changes in precipitation regimes can alter soil microbial communities by causing shifts in community composition through the local extinction of certain operational taxonomic units (OTUs) (10, 18) or by shifting the abundance of bacteria and fungi in favor of one group over those in another group (10, 20, 40, 54). Soil microbial communities may be more resilient to environmental change relative to their aboveground plant counterparts, and changes to soil microbial communities may only occur when abiotic variables are outside the range normally experienced by the communities (14).

In addition to the direct effect of precipitation change on soil microbial community abundance and diversity, soil microbial communities are influenced by changes in plant community abundance and composition (10, 57). Plant inputs via exudates or litter are substrates for soil microbial communities, which then use those inputs to mineralize nutrients (8, 16, 28). These associations can be tightly coupled. In many studies, distinct microbial communities develop beneath individual plant species and function differently when placed beneath a new plant species (6, 30, 38, 57). These associations can be particularly important when considering the responses of symbiotic relationships like mycorrhizae to projected changes in plant...
distribution (4, 24). It is thus important to consider how climate change might alter both plant
distribution and the distribution of the associated soil microbial community.

Seasonal and temporal shifts in rainfall, especially in ecosystems where organisms may
be at or near their physiological tolerance limits, can have a large impact on the diversity,
abundance, and responsiveness of soil microbial communities (29, 33, 47, 55). Environments
that have greater seasonal variation in rainfall may ameliorate the direct effects of climate change
on soil microbial communities because a wide range of physiological tolerances may already
exist within the community (14, 27). Alternatively climate change may increase the severity of
this variation resulting in new dynamics within the microbial community such as changes in
species richness or composition (27, 55). Therefore, measuring the responses of soil microbial
communities across seasons and years enables researchers to better predict microbial responses
to climate change.

Semi-arid piñon-juniper woodlands provide a model test case to understand and possibly
predict how both seasonal dynamics and how the dominant plant community may influence the
response of soil microbial communities to climate change. Piñon-juniper woodlands are defined
by a long dry season followed by monsoonal rains that can saturate the soil system (21), thus soil
microbial communities in these woodlands likely have a large range of physiological tolerances
to high and low moisture regimes (49). In addition, piñon-juniper woodlands in the southwestern
US have experienced multiple years of severe drought since 2000, which is leading to a
significant shift in the plant community (39). Junipers, that are more drought resistant, are able to
persist, while piñon pine populations are declining (5). Given climate models predict that the
frequency and severity of drought will continue to increase in the southwestern US (13, 22, 23,
41, 45) where these woodlands cover >17 million hectares (39), understanding how climate
change may directly or indirectly alter soil microbial communities and the processes they regulate is important.

Large-scale manipulation of climate variables can inform scientists how ecosystems, and their associated communities, will respond in the future (34). We took advantage of a precipitation manipulation in a piñon-juniper woodland to investigate how precipitation, increases and decreases, might alter soil microbial community composition and abundance beneath piñon and juniper trees across seasons. We predicted that: (1) soil microbial community composition and abundance would vary with moisture availability due to changes in precipitation both seasonally and across experimental precipitation treatments, (2) that soils beneath piñon and juniper would harbor distinct microbial communities, and (3) that relative to juniper, soil microbial communities beneath piñon would be more responsive to increases and decreases in precipitation because previous work at our site shows that piñon is more stressed than juniper by drought (42, 43).

Materials and Methods

Site and experimental description

To assess how precipitation change, including both increases and decreases, tree species, and season altered the soil microbial community, we examined microbial community composition and abundance beneath piñon and juniper at a precipitation manipulation experiment in Central New Mexico (see Pangle et al. 2012 for extensive experimental design details). The experiment is located in a piñon-juniper woodland at the Sevilleta National Wildlife Refuge in central New Mexico (1900 m elevation), where the Sevilleta long term ecological research (LTER) program is located (32º 20’ N, 106º50’ W). Climate records from the Sevilleta LTER meteorological
station (Cerro Montoso #42; http://sev.lternet.edu/) indicate the mean annual temperature around
our study site is 13 °C and the mean annual precipitation is 368 mm. The largest amount of
precipitation comes during the monsoon season (July, August, September). Monsoon
precipitation accounts for over half of the total annual precipitation; but high evapotranspiration
rates prevent monsoon rain events from recharging soil moisture to depth (21).

12 experimental plots (each 1600 m²) were established in mid-summer 2007 across three
blocks that varied in slope %, aspect, and soil depth. These plots consist of a decreased
precipitation treatment (~50% reduction), an increased precipitation treatment (~18% addition), a
precipitation removal control treatment (cover control), and an ambient control treatment (n = 3).
Precipitation was reduced using a throughfall displacement design (42, 46). Troughs were
constructed of clear UV-coated acrylic sheets and installed in each drought plot at a height of
approximately 1 m. The cover controls were constructed by inverting the troughs. To increase
precipitation, sprinklers were installed in each precipitation addition plot, where 57 mm of
precipitation was added in three separate 19 mm rain events in 2008. The ambient control
treatments were unmanipulated plots located within the experimental plot matrix. These plots
serve as the control for the water addition treatment and as an unmanipulated control for the
precipitation exclosure plots. Volumetric water content (VWC) is monitored at -5 cm depth using
EC-20 ECH₂O probes (Decagon, Pullman, WA; 42).

Soil sampling and DNA extraction

We collected soil samples in the pre-monsoon and during the monsoon season because we
expected that seasonal variability in rainfall might alter soil microbial community characteristics.
Combined with drought, the dry pre-monsoon season may represent a threshold for changes in
microbial community structure not evident during the rainy monsoon season. We collected soil
cores (10 cm depth, 5 cm diameter) in June (pre-monsoon) and August (monsoon) of 2008 from
three randomly chosen locations beneath one piñon (Pinus edulis) and one juniper (Juniperus
monosperma) crown in each treatment plot. Soils were collected within the drip line of the tree
near to the trunk, but not necessarily within the rhizosphere. We combined and homogenized the
soil by cover type within each treatment and flash froze a soil subsample from each in liquid
nitrogen for subsequent molecular analyses. Subsamples were placed on dry ice, and stored at -
80 °C in the laboratory until DNA was extracted. The remainder of the soil was homogenized,
sieved to 2 mm, and used to assess gravimetric water content.

We extracted DNA from 1 g of soil in each of our samples using the UltraClean Soil
DNA Isolation kit (MoBio Laboratories, Carlsbad, CA). DNA concentration and purity were
evaluated spectrophotometrically using a Synergy HT microplate reader (Biotek Instruments,
Winooski, VT). DNA concentrations ranged from 10-150 ng/μl. OD260/OD280 ratios were used to
assess DNA quality. Ratios ranged from approximately 1-2; therefore sample DNA was diluted
1:10 in sterile water before PCR reactions to avoid inhibition of PCR.

Microbial community composition

Bacterial and fungal community fingerprints were obtained using terminal-restriction fragment
length polymorphism (TRFLP) following a modified protocol outlined by Singh and Thomas
2006. Due to decreases in fluorescence when multiplexed (data not shown), we modified the
protocol by performing the analyses for the bacterial and fungal communities in separate
reactions. Polymerase chain reaction (PCR) was performed to amplify the 16S rRNA gene from
bacteria using primers 63f (35): 5'- AGGCCTAACACATGCAAGTC -3' and 1087r (25): 5'

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PCR was performed to amplify the ribosomal ITS region from fungi using primers ITS1f (19): 5' (6-FAM) – CTTGGTCATTTAGAGGAAGTAA -3' and ITS4r (51): 5' – TCCTCCGCTTATTGATATGC -3'. PCR mixtures contained 5 μl 10x KCL reaction buffer, 2 μl 50 mM MgCl₂, 5 μl 10 mM dNTPs (Bioline, Tauton, MA), 1 μl 20 mg/ml BSA (Roche, location), 0.5 μl (2.5 Units) Taq DNA polymerase (Bioline, Tauton, MA), either 1 μl of each bacterial primer or 2 μl of each fungal primer (Labeled primers - Invitrogen, by Life Technologies, Grand Island, NY, unlabeled primers – Integrated DNA Technologies, Coralville, IA) and 2 μl of sample DNA diluted 1:10 with sterile water. All PCR reactions were performed on 96-well Tgradient thermocycler (Biometra, Goettingen, Germany). Amplification of DNA consisted of an initial step of 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min. The last cycle was followed by extension at 72 °C for 10 minutes. At completion, PCR product quality was assessed with 1% agarose gel electrophoresis.

PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia, CA). After cleanup, PCR products were quantified using a Synergy HT microplate reader (Biotek, Winooski, Vermont, USA). All PCR products were digested individually with MspI. Reactions contained 14 μl PCR product, 2 μl 10X buffer B, 2 μl MSP1 (Fisher Scientific, USA), and 2 μl 10 mg/ml acetylated BSA (Promega, Madison, WI). Reactions were brought up to a final volume of 20 μl with sterile water. Samples were incubated at 37 °C for 3 hours followed by a deactivation step at 95 °C for 10 min. After digestion, a cocktail was made containing 0.5 μl LIZ labeled GeneScan 1200 internal size standard (Applied Biosystems, Grand Island, NY), 12.5 μl Hi-Di formamide (Applied Biosystems, Grand Island, NY), and 1 μl of digested product. Samples were centrifuged then incubated at 94 °C for 4 min followed by incubation at 4 °C for 5
Fragments were analyzed on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Grand Island, NY).

**Microbial abundance**

To assess bacterial and fungal gene copy number as a proxy for abundance (53), we ran quantitative polymerase chain reaction (qPCR) on each individual sample of DNA in conjunction with primers Eub 338 (ACT CCT ACG GGA GGC AGC AG) and Eub 518 (ATT ACC GCG GCT GCT GG) for bacterial 16S ribosomal DNA and nuSSU1196F (GGA AAC TCA CCA GGT CCA GA) and nuSSU1536R (ATT GCA ATG CYC TAT CCC CA) for fungal 18S ribosomal DNA (7).

PCR mixtures for both 16S rRNA and 18S rRNA gene amplification contained 15 μl of SYBR green master mix (Invitrogen, Life Technologies, Grand Island, NY), 5 μmol of each primer (Eurofins mwg operon, Huntsville, AL), and 1 μl of sample DNA diluted 1:10 with sterile water. All reactions were brought up to a final volume of 30 μl with sterile water. Amplification protocol for 16S rRNA gene consisted of an initial denaturing cycle of 95 °C for three minutes. This cycle was followed by 39 cycles of 95 °C for 15 s, 53 °C for 15 s, and 72 °C for 1 minute. Amplification of 18S rRNA gene consisted of an initial denaturing cycle of 95 °C for three minutes. This cycle was followed by 39 cycles of 95 °C for 15 s, 53 °C for 15 s, and 70 °C for 30 s. Abundance was quantified by comparing unknown samples to serial dilutions of 16S and 18S rDNA from *Escherichia coli* and *Saccharomyces cerevisiae* respectively in each PCR run.

For both bacterial and fungal assays, the $R^2$ value for the linear regression of threshold (Ct) value and standard abundance was greater than 0.95 indicating that the assays were quantitative across the range of DNA concentrations tested. After completion, for both ribosomal genes, a melting
curve analysis was conducted to ensure purity of the amplification product. All products showed the same overlapping melting peak indicating the specificity of the primers (44). PCR amplification was performed on a 96-well Chromo4 thermocycler (Bio-Rad Laboratories, Hercules, CA).

Data analysis

TRFLP profiles were produced using the GeneMapper software (Applied Biosystems, Grand Island, NY). Only terminal restriction fragments (TRFs) at positions beyond 55 bp were considered in order to avoid TRFs caused by primer-dimers. The relative abundance of a TRF in a TRFLP profile was calculated by dividing the peak height of the TRF by the total peak height of all TRFs in the profile (51). Four samples were omitted from this analysis because PCR amplification did not work after repeated attempts. Community analyses of fragments were conducted using Primer 6 (Primer-E Ltd, United Kingdom). All data were square root transformed before subsequent analyses. Summing the bacterial and fungal matrices prior to analysis assessed total microbial community composition. A 3-way interactive PERMANOVA was conducted to assess differences in the total microbial, bacterial, and fungal community by tree type, treatment, and season. When significant effects were found, subsequent pair wise comparisons were assessed. Community data were used for ordination by nonmetric multidimensional scaling. Data were also analyzed using a binary system of presence/absence instead of relative abundance to examine the robustness of the patterns.

Prior to analysis, volumetric water content (VWC), gravimetric water content (GWC), and microbial abundance data were tested for normality and log transformed to meet analysis of variance (ANOVA) assumptions. We used a 3-way ANOVA with a split-plot design with the
main effects of season, precipitation, plant species, and the interactive effects of season × precipitation, season × plant species, precipitation × plant species, and season × precipitation × plant species on GWC, VWC, fungal abundance, bacterial abundance, and the fungal:bacterial ratio. A Tukey HSD test was used to differentiate between treatment means when more than 2 levels were present (i.e. precipitation treatment differences). All data were analyzed using JMP 8 (Cary Institute, Cary, NC).

To understand how changing soil moisture, regardless of treatment or season, altered microbial abundance we ran linear regressions between these variables and the average monthly volumetric water content in the month soil samples were taken. This allowed us to assess the effect of a continuous measurement of water availability on microbial abundance. Additionally, we graphed these results and looked for non-linearities in the data and were unable to detect any significant trends or thresholds for changes in microbial community structure.

Results

**Soil moisture.** As expected, soil GWC fluctuated seasonally with higher levels of GWC during the monsoon season (Table 1; $F = 128.10, p < 0.01$). GWC was approximately $5.5 \times$ higher in August (during the monsoon season) compared to June (pre-monsoon). Surprisingly, GWC did not vary between the precipitation treatments on the day soil samples were taken. VWC in the water reduction treatments during the monsoon season was significantly different from all other plots during both seasons. (Table 1; $F = 3.89, p = 0.02$). During the monsoon season, VWC was significantly decreased relative to all other plots in that same season. Overall, VWC was $1.6 \times$ less in the water reduction plots relative to all other plots across both seasons (Table 1; $F = 2.53,$
Microbial community composition. Aspects of microbial community composition responded significantly to the precipitation treatments, season, and the aboveground plant community when both relative abundances were used and presence/absence data were used. Because the patterns were similar, we present only the relative abundance results. Results of the presence/absence analysis can be found in Appendix 1. There was a significant interactive effect of precipitation treatment and season on fungal community composition (Table 1; pseudo F = 1.32, p = 0.03).

Specifically, fungal community composition in the water reduction plots was distinct from control plots during the dry pre-monsoon season but not during the wet monsoon season (Pairwise comparison; t = 1.22, p = 0.10). On average across both seasons, fungal community composition in the water reduction plots was significantly different from the cover control plots (Figure 1B NMDS; Pairwise comparison; t = 1.25, p = 0.04 by PERMANOVA) and marginally different from control plots (Figure 1B NMDS; Pairwise comparison; t = 1.23, p = 0.06 by PERMANOVA). Total microbial community composition in the water addition plots was also distinct from the cover control plots (Figure 1A NMDS; Pairwise comparison; t = 1.29, p = 0.04 by PERMANOVA). We were unable to detect any effect of our precipitation treatments on bacterial community composition (Figure 1C NMDS; pseudo F = 1.11, p = 0.35 by PERMANOVA).

Season also played a large role in structuring soil microbial communities in this semi-arid woodland. Microbial communities in the dry pre-monsoon season were compositionally different from those in the wet monsoon season (Figure 2A NMDS; pseudo F = 15.89, p < 0.01 by PERMANOVA).
PERMANOVA). This pattern held for both fungal and bacterial communities, where fungal composition (Figure 2B NMDS; pseudo F = 4.52, p < 0.01 by PERMANOVA) and bacterial composition (Figure 2C NMDS; pseudo F = 30.39, p < 0.01 by PERMANOVA) were different in the pre-monsoon and monsoon season.

Overall, there were significant differences in microbial community composition between the two tree species. Specifically, soil microbial community composition beneath juniper was distinct from composition beneath piñon crowns (Figure 3A NDMS; pseudo F = 3.11, p < 0.01 by PERMANOVA). There were significantly distinct communities of both fungi (Figure 3B NMDS; pseudo F = 2.40, p < 0.01 by PERMANOVA) and bacteria (Figure 3C NMDS; pseudo F = 3.53, p < 0.01 by PERMANOVA) beneath piñon and juniper trees.

Microbial abundance. Consistent with our composition results, we found that season, treatment, and tree type had strong individual and interactive effects on the ratio of fungi and bacteria in the soil. The fungal:bacterial ratio was significantly greater beneath piñon in the cover control plots during the monsoon season relative to piñon in the + water plots and juniper in all treatment plots in the pre-monsoon season (Table 1; F = 3.05, p = 0.05). The fungal:bacterial ratio was also higher beneath piñon in the cover control plots relative to all other treatment plots (Figure 4A; F = 3.48, p 0.03). We did not find a significant main effect of our precipitation treatments on the fungal:bacterial ratio (Figure 5A; F = 0.66, p = 0.60), but we did find that across all treatments and seasons, the fungal:bacterial ratio increased with increasing volumetric water content (Table 2; F = 7.23, p = 0.01, r² = 0.14).

There were significant, sometimes interactive, effects of precipitation treatment, season, and tree type on fungal and bacterial abundance. Fungal abundance was 4.7 × greater during the
monsoon season in the water removal plots relative to the pre-monsoon season in these same plots (Figure 5B; F = 4.36, p = 0.01). There was a marginal increase in bacterial abundance beneath piñon in the water addition plots relative to all other plots (Figure 4C; F = 5.04, p = 0.01). Surprisingly, across all treatments and seasons, bacterial abundance decreased with increasing volumetric water content (Table 2; F = 5.41, p = 0.03, r² = 0.11).

Across all the factors measured, there were significant main effects of season and tree type on microbial abundance. The fungal:bacterial ratio was 2.02 beneath piñon and 1.23 beneath juniper across all treatments (Figure 5A; F = 7.10, p = 0.01). In addition, fungal abundance was 2 × greater beneath piñon relative to juniper (Figure 5B; F = 10.21, p < 0.01). Season also had an effect on microbial abundance. The fungal:bacterial ratio increased from 0.82 in the pre-monsoon season to 2.4 during the monsoon season (Figure 5A; F = 18.61, p < 0.01). Contrary to this, fungal abundance did not vary significantly by season, although there was a trend for increased fungal abundance during the monsoon season (Figure 5B; F = 2.71, p = 0.11). Additionally, bacterial abundance was greater in the pre-monsoon season relative to the monsoon season (Figure 5C; F = 5.59, p = 0.03).

**Discussion**

Precipitation change due to global climate change can alter the composition and abundance of belowground microbial communities directly by changing soil water availability or indirectly by altering plant community composition, production, and allocation (5, 7, 10, 20). Consistent with this, we found that seasonal fluctuations in rainfall, precipitation treatments, and tree type interactively and independently altered microbial community composition and abundance. Long-term changes in microbial communities due to changes in precipitation or the plant community
may have large implications for the future trajectory of this ecosystem with climate change and the functioning of this ecosystem.

Seasonal variation in rainfall may result in a microbial community that is acclimated to fluctuations in precipitation thus resulting in a diminished response to the precipitation manipulation (17, 18, 52). Consistent with this, we found that soil microbial community structure and abundance were more responsive to fluctuations in seasonal rainfall than to our relatively constant precipitation treatments. Throughout the year, the microbial community at this site is subjected to excessive drought followed by rapid changes in rainfall during the monsoon season (21). This seasonal variability greatly exceeds that provided by our precipitation manipulation. For example, during the monsoon season of 2008, we added 57 mm of precipitation in the water addition plots. This addition is $3 \times$ less than the precipitation received from June to the end of August in 2008 (42). Other studies have demonstrated that when microbial communities are acclimated to multiple dry-wet episodes, their response (measured by microbial respiration rates or changes in community composition) is diminished with each repeated event, and the magnitude of this response is dependent upon precipitation history and the associated aboveground community (18, 52).

Biotic mechanisms, like competition, predation, and niche differentiation, may be relatively more important for the structure and abundance of soil microbial communities in semi-arid ecosystems when water is not limiting. Unexpectedly, soil fungal and bacterial abundance did not always increase with increasing water availability. Contrary to other studies, we found a significant decline in bacterial abundance in association with increasing water availability (3, 50). This shift in bacterial abundance may be driven by competitive interactions between soil fungi and soil bacteria, or predator-prey dynamics between soil microorganisms and soil.
protozoa or arthropods. As soil fungi increase with increasing water availability, they may
exclude soil bacteria thus reducing their abundance (37). Alternatively, increases in soil protozoa
or arthropods preying on bacteria may increase when water is no longer limiting resulting in a
reduction in soil bacteria during wet periods (9, 11, 31). Although we did not measure protozoa
or arthropod abundance, other studies have shown that these organisms do indeed increase
during periods of increased water availability (9, 11, 31) and may decrease bacterial abundance.

Given piñon are dying more quickly than juniper with drought events in this ecosystem
(43), differences in the microbial communities found in soils beneath their crowns could scale to
alter the function of this ecosystem over time (5, 39). Distinct fungal communities beneath piñon
and juniper crowns with varying richness and abundance may be attributable to their differences
in mycorrhizal association; piñon associate with ecto-mycorrhizal (EM) fungi, while juniper
associate with arbuscular mycorrhizal (AM) fungi (24), although we did not test for this directly.
As piñon die in this ecosystem due to drought, their associated fungal symbionts may also
decline, decreasing the ability of piñon to re-establish in this ecosystem and aiding in the
transition to a juniper dominated ecosystem. Further, differences between both bacterial and
fungal communities beneath piñon and juniper crowns may indicate a difference in the function
of these two communities (Cregger et al, in review). As piñon die with drought, a distinct
microbial community may be lost from this ecosystem resulting in a functional shift.

When taken together, our results indicate that the response of microbial communities to
climate change is complex and highly dependent upon the underlying seasonal variability and
associated plant community. These changes have important effects on how we design microbial
community assessments in such systems, as a one-time, snap shot look at the microbial
community to predict their response to climate change will not reflect the overall structure of
these systems and their dynamic nature. Seasonal and temporal variation as well as plant community compositional changes will play a large role in the response of these communities and should be incorporated into future climate change experimental manipulations especially with advances in pyrosequence based analysis of both rRNA and metagenomes that will allow higher throughput of community samples and more detailed circumscription of the microbial species-level responses.

Acknowledgments

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References


Table 1. Precipitation change, plant species, and season had significant effects on microbial composition and abundance. F and p values show the main and interactive effect of these variables on soil GWC, VWC, microbial community composition, fungal community composition, bacterial community composition, fungal abundance, bacterial abundance, and fungal:bacterial ratio. PERMANOVA analysis was used for the community composition data, thus full model F and p values were not available.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Full model</th>
<th>Precip.</th>
<th>Plant species</th>
<th>Season</th>
<th>Precip. × plant</th>
<th>Precip. × season</th>
<th>Plant × season</th>
<th>Precip. × plant × season</th>
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</thead>
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<td>GWC</td>
<td>6.25, &lt;0.01</td>
<td>1.39, 0.27</td>
<td>0.35, 0.56</td>
<td>128.10, &lt;0.01</td>
<td>0.28, 0.84</td>
<td>1.33, 0.29</td>
<td>0.44, 0.51</td>
<td>0.23, 0.87</td>
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<td>VWC*</td>
<td>16.05, &lt; 0.01</td>
<td>2.53, 0.13</td>
<td>2.66, 0.12</td>
<td>268.49, &lt; 0.01</td>
<td>1.34, 0.29</td>
<td>3.89, 0.02</td>
<td>0.66, 0.43</td>
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<td>15.89, &lt;0.01</td>
<td>0.28, 0.84</td>
<td>1.33, 0.29</td>
<td>0.44, 0.51</td>
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<td>Bacterial community composition</td>
<td>NA</td>
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<td>1.38, 0.14</td>
<td>0.89, 0.52</td>
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<td>Fungal abundance</td>
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<td>0.63, 0.61</td>
<td>10.21, &lt;0.01</td>
<td>2.71, 0.11</td>
<td>1.21, 0.33</td>
<td>4.36, 0.01</td>
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<td>2.14, 0.04</td>
<td>0.31, 0.82</td>
<td>0.06, 0.80</td>
<td>5.59, 0.03</td>
<td>5.04, 0.01</td>
<td>1.92, 0.15</td>
<td>1.12, 0.30</td>
<td>2.65, 0.07</td>
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<td>Fungal:bacterial ratio</td>
<td>2.46, 0.02</td>
<td>0.56, 0.66</td>
<td>7.10, 0.01</td>
<td>18.61, &lt;0.01</td>
<td>3.48, 0.03</td>
<td>0.56, 0.65</td>
<td>0.32, 0.57</td>
<td>3.05, 0.05</td>
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*Data published in Pangle et al., 2012
Table 2. VWC, GWC, and microbial abundance mean (± standard error), across all treatments and beneath piñon and juniper crowns in the pre-monsoon and monsoon season of 2008.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>- water</th>
<th>Cover control</th>
<th>+ water</th>
<th>Control</th>
<th>- water</th>
<th>Cover control</th>
<th>+ water</th>
<th>Control</th>
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<td>VWC*</td>
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<td></td>
<td></td>
<td></td>
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<td>0.02 (0.00)</td>
<td>0.01 (0.00)</td>
<td>0.01 (0.00)</td>
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<tr>
<td>GWC</td>
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<td>0.03 (0.00)</td>
<td>0.02 (0.00)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.00)</td>
<td>0.02 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.00)</td>
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<tr>
<td>Fungal:bacterial</td>
<td>0.25 (0.05)</td>
<td>0.45 (0.09)</td>
<td>0.69 (0.19)</td>
<td>0.44 (0.05)</td>
<td>0.82 (0.30)</td>
<td>0.94 (0.16)</td>
<td>1.26 (0.54)</td>
<td>1.73 (0.46)</td>
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<td>Fungal abundance</td>
<td>2.2 x 10^7 (6.7 x 10^6)</td>
<td>4.2 x 10^7 (6.7 x 10^6)</td>
<td>5.0 x 10^7 (8.2 x 10^6)</td>
<td>4.2 x 10^7 (9.3 x 10^6)</td>
<td>3.6 x 10^7 (2.0 x 10^7)</td>
<td>6.7 x 10^7 (1.7 x 10^7)</td>
<td>1.1 x 10^8 (3.2 x 10^7)</td>
<td>1.8 x 10^8 (4.1 x 10^7)</td>
</tr>
<tr>
<td>Bacterial abundance</td>
<td>8.7 x 10^7 (1.6 x 10^7)</td>
<td>9.6 x 10^7 (1.7 x 10^7)</td>
<td>7.8 x 10^7 (9.7 x 10^6)</td>
<td>9.2 x 10^7</td>
<td>5.9 x 10^7</td>
<td>8.1 x 10^7</td>
<td>9.5 x 10^7</td>
<td>1.1 x 10^8</td>
</tr>
<tr>
<td>Fungal abundance</td>
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<td>4.2 x 10^7 (6.7 x 10^6)</td>
<td>5.0 x 10^7 (8.2 x 10^6)</td>
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<td>1.1 x 10^8 (3.2 x 10^7)</td>
<td>1.8 x 10^8 (4.1 x 10^7)</td>
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<td>Bacterial abundance</td>
<td>8.7 x 10^7 (1.6 x 10^7)</td>
<td>9.6 x 10^7 (1.7 x 10^7)</td>
<td>7.8 x 10^7 (9.7 x 10^6)</td>
<td>9.2 x 10^7</td>
<td>5.9 x 10^7</td>
<td>8.1 x 10^7</td>
<td>9.5 x 10^7</td>
<td>1.1 x 10^8</td>
</tr>
</tbody>
</table>

*Data published in Pangle et al., 2011*
Table 3. Volumetric water content had a significant impact on bacterial abundance and the fungal:bacterial ratio. F, P, and $R^2$ values are listed for each variable.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>F</th>
<th>P</th>
<th>$R^2$</th>
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</thead>
<tbody>
<tr>
<td>Fungal:bacterial ratio</td>
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<td>0.01</td>
<td>0.136</td>
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<tr>
<td>Fungal abundance</td>
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<td>0.71</td>
<td>0.003</td>
</tr>
<tr>
<td>Bacterial abundance</td>
<td>5.41</td>
<td>0.03</td>
<td>0.105</td>
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**Figure Legend**

**Figure 1.** Microbial communities were compositionally distinct between precipitation treatments. Non-metric multi-dimensional scaling demonstrates: A. total microbial (bacterial and fungal), B. fungal, and C. bacterial community composition in the water addition (black circles), control (white circles), water reduction (black triangles), and cover control (white triangles) plots. Each point represents a specific community in one of the treatment plots or control plots. Points that are close together are more similar to one another than points that are far apart.

**Figure 2.** Microbial communities were compositionally distinct in the pre-monsoon and monsoon season across all treatments. Non-metric multi-dimensional scaling demonstrates: A. total microbial (bacterial and fungal), B. fungal, and C. bacterial community composition in the pre-monsoon (black circles) and in the monsoon (white circles) of 2008. Each point represents a specific community either in the pre-monsoon or monsoon season. Points that are close together are more similar to one another than points that are far apart.

**Figure 3.** Microbial communities beneath piñon and juniper crowns were compositionally distinct from one another. Non-metric multi-dimensional scaling demonstrates A. total microbial (bacterial and fungal), B. fungal, and C. bacterial community composition beneath juniper (black triangles) and piñon (white triangles) in 2008. Each point represents a specific community beneath piñon or juniper crowns. Points that are close together are more similar to one another than points that are far apart.
Figure 4. Precipitation treatment and tree type interacted to alter: A. fungal:bacterial ratio, B. fungal abundance, and C. bacterial abundance. Different letters denote significant interactive differences determined by Tukey’s HSD.

Figure 5. Season and precipitation treatment interacted to alter A. fungal:bacterial ratio, B. fungal abundance, and C. bacterial abundance. Different letters denote significant interactive differences determined by Tukey’s HSD.