Using Real-Time Quantitative PCR to Examine the Seasonal Dynamics of Soil Fungi and Bacteria in Rangeland Soils

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Introduction

• Recent work has revealed that the soils of southwestern rangelands represent strong seasonal sources and sinks of atmospheric trace gases, and that the activities of drought-tolerant soil fungi contribute strongly to C and N cycling during periods of extreme dryness (McLain and Martens, 2006a; Collins et al., 2008).

• We are using real-time quantitative PCR to examine the influences of soil moisture status, livestock grazing, and mesquite management on fungal and bacterial markers in an Arizona rangeland soil. Knowledge of seasonal dynamics in fungal and bacterial biomass, and the environmental factors controlling shifts in fungal and bacterial activities, will aid in understanding the global impacts of C and N cycling in semiarid soils.

Southwestern US Rangelands

• Our study site is in the Santa Rita Experimental Range (SRER) southwest of Tucson, Arizona (Figure 1). Such rangelands cover more than 200 million ha of land area in the Western US (Mitchell, 2000).

• Yearly precipitation in the SRER averages 350 mm, 50% of which falls during the summer monsoon season (Jul – Sep).

• The SRER is a Sonoran Desert Grassland with a strong presence of velvet mesquite (*Prosopis velutina*), a native leguminous tree. Increased soil C and N beneath mesquite has been shown to contribute to significant increases in carbon dioxide and nitrous oxide production during the monsoon season (Peterjohn and Schlesinger, 1991; McLain and Martens, 2006b).

• Our research was conducted in Pasture 2S of the SRER (Figure 2a), where animal stocking has been maintained at 0.03 animals y^{-1} since the 1970s. Additional sites were located within an area that has been fenced to prevent grazing for nearly 100 years (Figure 2b).

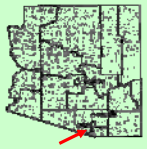


Figure 1. Location of the Santa Rita Experimental Range in southeastern Arizona.

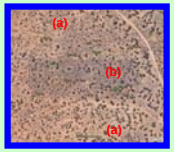


Figure 2. Aerial photograph of Pasture 2S of the SRER. Note the visible differences in surface vegetation and soils between the areas outside (a) and inside (b) the grazing exclosure fencing.

Soil Collection and DNA Extraction

• Soils were collected in June (pre-monsoon, "dry soils") and July (5 days after onset of monsoon, "wet soils") in sites beneath a mature mesquite (Photo 1) and an open site dominated by grasses (Photo 2).

• Sites for each vegetation type were duplicated, with one set located in the grazed area (Figure 2a), and the other located within the grazing exclosure (Figure 2b).

• Soils were collected using sterile trowels, at depths of 0-5 cm and 5-10 cm. Samples were collected from each site in duplicate, from the north and south sides of the site.

• Soils were placed on ice during transport to the laboratory, and were maintained at 4°C until DNA extraction (less than 1 week after collection).

• DNA extraction was performed on 0.5-g subsamples of each soil in triplicate, using the ZR Soil DNA Extraction Kit (Zymo Research, Orange, CA).

Photo 1: Mesquite Site

Photo 2: Open Site

Molecular Methods

• Real-time PCR (ABI-7000 Sequence Detection System, Applied Biosystems, Foster City, CA) was used to assess the abundance of bacterial and fungal biomarkers using SYBR Green technology and the primers shown in Table 1.

• Quantitative standards (Figure 3) were created by cloning purified PCR product into plasmid vectors (TOPO TA Cloning Kit, Invitrogen Life Technologies, Carlsbad, CA).

Table 1. PCR Primers Used to Quantify Bacterial and Fungal Markers.

Target Group	Primer Name	Sequence (5' – 3')	Reference
All Bacteria	Eub338 (forward)	ACT CCT ACG GGA GGC AGC AG	Lane, 1991
All Bacteria	Eub518 (reverse)	ATT ACC GCG GCT GCT GG	Muyzer et al., 1993
All Fungi	S.ite (forward)	CGC TGC GTT CTT CAT CG	Vilgalys and Hester, 1990
All Fungi	ITS1f (reverse)	TCC GTA GGT GAA CCT GCG G	Gardes and Bruns, 1993

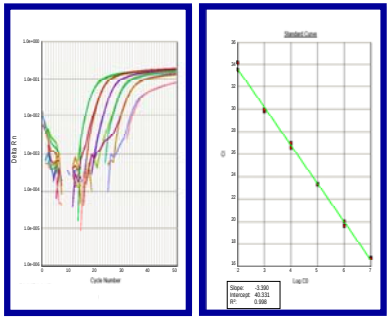


Figure 3. Real-time standard curve.

Results

• Pre-monsoon "dry" soils were collected from all sites on June 13; "wet" soils were collected less than a week after the first significant rainfall, on July 6 (Figure 4). Molecular results are not yet available for an additional set of soils, collected on August 28.

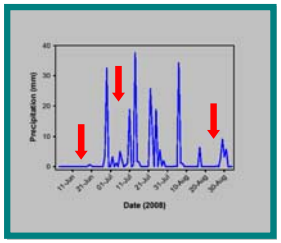


Figure 4 (right). Precipitation patterns during the 2008 monsoon in the SRER. Red arrows show dates of soil collection.

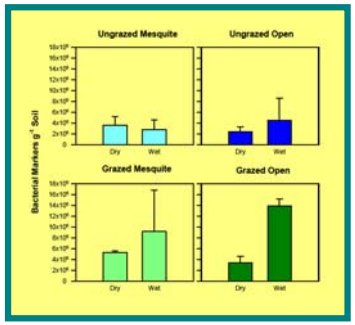


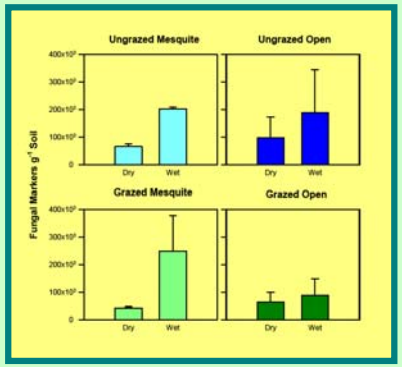
Figure 5. Bacterial markers in 0-10 cm layer of wet and dry soils under two grazing regimes and two vegetation types.

• Bacterial markers increased overall in response to monsoon moisture, showing a 100% increase across all depths and treatments.

• The bacterial response was strongest in the grazed sites (Figure 5), where bacterial markers increased by 165%. Although the increase in the ungrazed open sites averaged 21%, bacterial markers decreased in response to moisture in the ungrazed mesquite sites.

• Bacterial markers increased dramatically (+243%) in the 0-5 cm soil depth across all treatments, but decreased by nearly 50% in 5-10 cm soils.

Results, Continued



• Fungal markers increased dramatically in response to monsoon moisture, showing a 150% increase across all depths and treatments.

• The fungal response was strongest in the mesquite sites (Figure 6), where fungal markers increased by more than 300%. An 70% increase in fungal markers was found in the open sites.

• The increase in fungal markers was nearly equal in the 0-5 and 5-10 cm depths, averaging 177% and 149%, respectively.

Figure 6 (left). Fungal markers in 0-10 cm layer of wet and dry soils under two grazing regimes and two vegetation types.

Additional Work

• It has long been known that the biological community in arid soils responds rapidly to moisture input. The 3-fold increase in fungal markers beneath mesquite is somewhat surprising, given that this increase occurred within a very short period (less than a week) following monsoon onset.

• It is clear from the data presented that both bacteria and fungi in SRER soils show significant response to moisture input. This data set is too limited (2 samplings) for conclusions to be made regarding interactions with vegetation and/or land use.

• We hypothesize that, upon soil drying, fungal markers will remain abundant for a longer period than bacterial markers. Soil collections will continue in the SRER over several seasons to collect additional information on environmental controls on bacterial and fungal markers.

References

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