

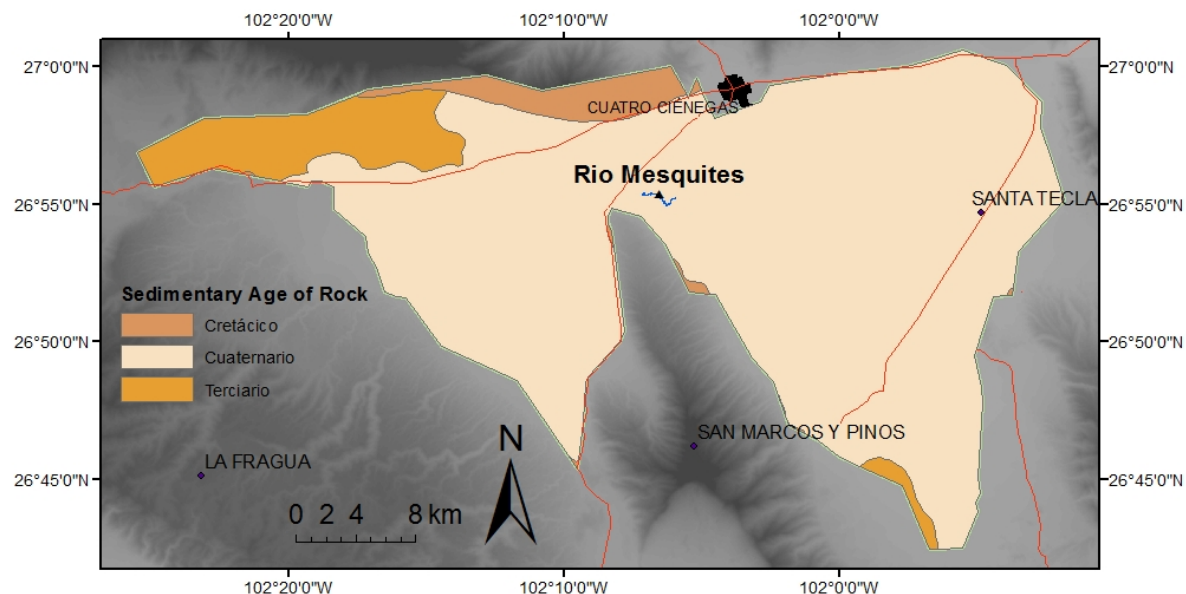
## Nutrient limitation as a factor for microbialite formation in Cuatro Ciénegas, México

### Project Report

#### Introduction

Stromatolites are layered, biogenic structures formed either directly or indirectly by microbial processes or “organomineralization” (Burne and Moore 1987). Stromatolites are found through much of the fossil record and are thought to have played a crucial role in biogeochemical cycling throughout Earth’s history (Dupraz and Visscher 2005). While studies of the fossil species have provided important information on their occurrence and diversity, studies of living, lithifying microbialites (non-layered, biogenic lithified structures) provide an opportunity to explore how geochemical and microbial processes promote organomineralization. Indeed, the degree to which different microbial mechanisms influence the mineralization of calcium carbonate ( $\text{CaCO}_3$ ), the mineral structure of many stromatolites and microbialites, remains of the most hotly debated topics in carbonate geochemistry (Kandianis et al. 2008).

Through my research on the living microbialites of Cuatro Ciénegas, México (Fig. 1), I have found evidence to support the hypothesis that calcification leads to phosphorus-limitation. Cuatro Ciénegas is one of the few aquatic environments that hosts living microbialites. Previous work from genetic analyses (Souza et al. 2006, Breitbart et al. 2009) and through experimental manipulation ((Elser et al. 2005)Corman et al) suggests microbes in Cuatro Ciénegas are starved for phosphorus. Building from these observations, and the knowledge that  $\text{CaCO}_3$  deposition can co-precipitate phosphorus (Otsuki and Wetzel 1972), I set out to determine (1) if calcification attenuates phosphorus availability to microbialites and (2) if microbial communities interact with  $\text{CaCO}_3$  deposition to improve access to phosphorus.



**Figure 1. Geologic map of the Cuatro Ciénegas basin, Coahuila, México.** The sedimentary rock of the valley is colored by geologic age; the town of Cuatro Ciénegas is in black, while roads are in red. Río Mesquites, shown in blue, is a meandering river/wetland system near the base of the San Marcos y Pinos Mountains.

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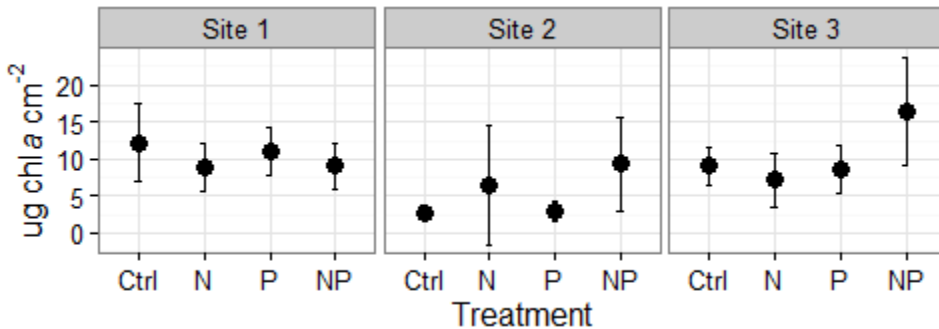
**Methods and Results**

To determine if calcification attenuates phosphorus availability in Cuatro Ciénegas, I first placed replicate artificial substrates in the streambed of Río Mesquites, a river in Cuatro Ciénegas that hosts spherical, stromatolites communities (“oncoids”). I used two types of substrates: copper and stainless steel to prevent or allow microbial growth, respectively (Fig. 2). Unfortunately, microbes were able to grow on both substrates indiscriminately, thus I could not use this experiment to test my hypothesis. Therefore, I used an experiment common in stream ecology to test nutrient limitation: growing microbes on nutrient-amended agar (or nutrient-diffusing substrata, “NDS”) that is incubated in the stream (Hauer and Lamberti 2011). As microbes growing on the agar would not necessarily be associated with oncoids, I would be able to determine whether or not all microbes in Río Mesquites are phosphorus-limited. I placed NDS in three sites in Río Mesquites with Site 1 as the most upstream.



**Figure 2. Artificial substrates.** Microbial growth can be seen on copper (left) and stainless steel (right) pieces placed in Río Mesquites.

Non-oncoid associated microbes were not strongly nutrient limited (Fig. 3). There was a trend towards nitrogen and phosphorus co-limitation going downstream (Treatment effect at Site 3:  $F_{3,12} = 0.3187$ ,  $P = 0.0763$ ). When compared to earlier work testing



**Figure 3. Nutrient limitation to non-lithifying microbial communities.** Nutrient limitation, as seen by an increased in chlorophyll *a* (chl *a*) relative to the control (Ctrl), trends towards nitrogen-phosphorus (NP) co-limitation from upstream (Site 1) to downstream (Site 3).

phosphorus-limitation to oncoid-associated microbes (Elser et al. 2005, Corman et al. *in prep*), the lack of a strong response to phosphorus supports the hypothesis that calcification attenuates phosphorus availability.

For my next objective, to determine how microbes interact with CaCO<sub>3</sub> deposition, I used a mesocosm experiment with manipulations to rates of CaCO<sub>3</sub> deposition (Fig. 4). Individual oncoids were placed in plastic containers with Río Mesquites water; water was changed every other day. Temperature and light were manipulated to mimic stream conditions; submersible water pumps in each mesocosms provided flow. CaCO<sub>3</sub> deposition rates were manipulated biotically (by decreasing light to prevent growth of cyanobacteria) and abiotically (by removing calcium ions from the stream water using a custom-made water softener and by adding strontium, which prevents the formation of CaCO<sub>3</sub> crystals (Wasylenki et al. 2005). When calcium is removed, I expect that the microbes will no longer need to compete with calcification to access P. Therefore, I expect the ratio of

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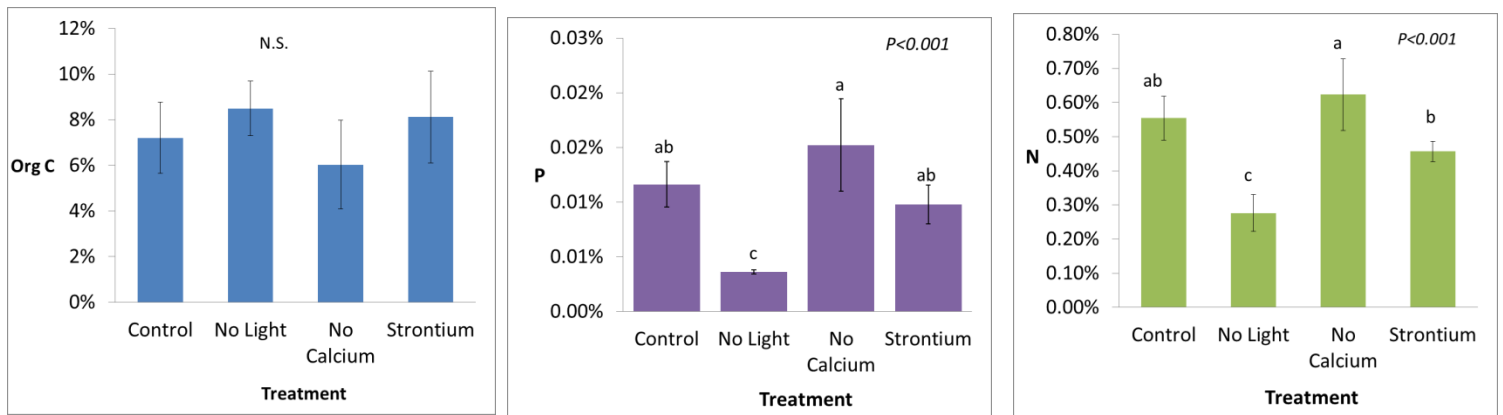


**Figure 4. Mesocosms.** Oncoid microbialites were suspended in

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microbial biomass carbon to phosphorus, C:P, to decrease when calcification is decreased.

Unexpectedly, there was not a strong reduction in C:P ratios across all treatments (Fig. 5). Organic carbon did not change significantly; this suggests that total microbial biomass did not change due to the experimental manipulations. Nitrogen and phosphorus content, however, did. In the “No Light” treatment, both N and P decreased significantly compared to the control. As the “No Light” treatment would have reduced the capacity for photosynthetic organisms to carry out photosynthesis (as the light they need to drive the photosynthetic reaction was removed), the lowered N and P concentrations suggests that active cyanobacterial growth is an important mechanism to bring N and P into the oncoid ecosystems. The calcium removal treatment, “No Calcium,” did result in a slight increase in both N and P in the microbial biomass. Likewise, the C:P ratio decreased from a mean of



**Figure 5. Response of oncoid microbial biomass to decreases in calcification.** While oncoid microbial biomass showed no significant change in organic carbon (Org C) concentrations, phosphorus (P) and nitrogen (N) concentrations both responded significantly to reductions in light (“No Light”).

1890:1 in the control to a mean of 1690:1 in the “No Calcium” treatment. The increase in N likely reflects an increase in nutrient uptake as a result of increased microbial growth. However, when compared the C:P ratio of the microbial biomass is compared to the C:P ratio in the Redfield Ratio of 106:1 (used to indicate potential nutrient limitation in an organism (Redfield 1958)), the C:P ratio of the microbial biomass is still quite high.

**Conclusions**

While my results suggest a clear distinction in nutrient limitation between microbialite-associated microbes and non-microbialite-associated microbes, they do not lend strong support for the hypothesis that calcification is the mechanism for phosphorus limitation. However, in the marine stromatolites of the Exuma Cays, Bahamas, CaCO<sub>3</sub> precipitation occurs as a result of sulfate reduction, as well as aerobic photosynthesis (Visscher et al. 1998). If sulfate reduction is a mechanism by which most or all of CaCO<sub>3</sub> precipitates in the Río Mesquites microbialites, then my experimental manipulations that assumed aerobic photosynthesis was the mechanism behind CaCO<sub>3</sub> (Garcia-Pichel et al. 2004) may not have been effective at lowering rates of CaCO<sub>3</sub> deposition. Indeed, there is other evidence to support the role of sulfate reduction in the formation of Río Mesquites microbialites: abundant concentrations of sulfate and dissolved organic matter in the water, which are necessary reactants for sulfate reduction (Elser et al.

Jessica CORMAN

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2005); smell of hydrogen sulfide in the stream water, which is a byproduct of sulfate reduction (*pers. obs.*); and presence of genes associated with sulfate reduction in the Río Mesquites oncoid metagenome (Breitbart et al. 2009). If sulfate reduction is occurring in the Río Mesquites oncoids, this, to my knowledge, would be the first documented case of lithification by sulfate reduction in freshwater microbialites. This would extend our understanding of the environmental conditions by which microbialite formation can occur, and perhaps, has occurred in ancient environments. I will pursue this observation in future work.

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APPENDIX. Photographs from fieldwork in Cuatro Ciénegas, MX. Photos by Jessica Corman unless otherwise noted.



Photo 1. Experimental set-up of 4 replicate blocks for testing the influence of calcification on Río Mesquites microbialites.



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Photo 2. Jessica Corman snorkeling in Río Mesquites to collect oncoid microbialites for experimentation. A bed of oncoid microbialites can be seen in the bottom on the river. Photo by Jorge Ramos.



Photo 3. Río Mesquites with San Marcos y Piños Mountains in the background.

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Photo 4. Close-up of the oncolite microbialites in Río Mesquites, MX. Wristwatch is shown for scale.