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Discussions

Interactive comment on "Role of Microbial Communities in the Weathering and Stalactite Formation in Karst Topography" *by* Tung-Yi Huang et al.

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Anonymous Referee #2 The paper 'Role of Microbial Communities in the Weathering and Stalactite Formation in Karst Topography' attempts to connect microbial metabolic activity to both dissolutional and depositional processes with landscape-scale processes in the evolution of karst. Unfortunately, the manuscript suffered from several major problems, the greatest of which was a complete disregard for the vast body of research on hydrogeological processes in shaping karst.

This error was compounded by: 1. The very small number of sample sites

Response: For collecting the samples in the surface of limestone walls and soil, sterile cotton swabs were used to wipe the surfaces of sampled spots, which were randomly selected areas of 15 cm square from each habitat. For the water samples, sterilized bottles (250 ml) were used to collect the water dripping from the stalactite. A total of 4 samples were sent to the laboratory for DNA extraction for total bacterial community prediction in each habitat and quantification of bacteria of interested. Our intention was to propose a novel idea of microbiological role in the development of karst surface and shape change by using the data from NGS platform. As we considered that an empirical testing on the propose will make this study a valuable contribution to scientific research, we are taking further step to complete the work.

2. A lack of cause-and-effect studies to demonstrate a direct role for microorganisms in the describe processes

Response: In response to reviewer#2's question regarding the lack of cause-and-effect studies to demonstrate a direct role for microorganisms in the described processes, we are currently working on it by replicating 2 batches of samples for testing the urease-genes at different time points. To conduct the spatial and temple replicates for testing our theory, samples were gathered from randomly selected areas of 15 cm2 square. In the surveillance of urease-gene in habitat, quantitative PCR is carried out to quantify the yields of the genes at each habitat. As we are testing the optimal condition for measuring the possible candidates of heterogenous urease-genes as well as other photobacteria. We would like to have another 2 month extension in conducting the confirming tests.

3. A lack of understanding of general calcification processes (ureolysis is not the sole mechanism of calcification)

Response: We agree with the comment of reviewer #2 and we will add the description of general calcification processes in the revised manuscript.

4. No description of the source of urea that could drive the putative calcification pro-

cesses

Response: Previous studies showed that the urease-producing bacteria in the presence of ammonium ions in the alkaline and calcium-rich environment. Our study showed relatively large proportion of urease-producing bacteria in the dark environment. We will use quantitative PCR for verification the metagenomics outcome.

5. A 95% identity to a ureolytic species has no bearing on whether the identified phylotype is also ureolytic. I would recommend that the authors work with a geologist and/or geomicrobiologist to better understand the processes they are examining, dramatically expand the sample sites being tested, and develop assays that can directly test whether the microbial activities they are examining are tied to the geologic processes they observe.

Response: The 95% threshold should be good for the prediction of relative abundance of urease-producing bacteria and give the reasonable basis for this study. We agree with reviewer 2 in using a different approach to confirm the quantity of encoded functional genes in each habitat. The empirical test is now undergoing.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-12/bg-2019-12-AC2-supplement.pdf

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