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Biogeosciences Discussions

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

Anonymous Referee #1

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In this study the authors investigated changes in microbial communities across four different sites in a karst landscape, ranging from soils outside a gulch to water dripping from stalactites. They specifically focused on bacteria involved in weathering and urease-activity. These groups were identified based on 16S rRNA gene sequence data by searching for taxa that had been described in previous studies to carry out these functions. They set their findings in relation to different environmental parameters such as light penetration across sites and draw some conclusions regarding the role of bacteria in limestone weathering and calcite precipitation. The authors address the interesting question of how microbes are involved in central geological processes in karstic landscapes. However, I have some concerns regarding the interpretation of

the data obtained in this study. The data set used by the authors is rather limited - as far as I understood, samples were obtained at one time point from four different sites without spatial replicates. In addition, the functional role of the microorganisms is solely inferred from their taxonomic affiliation. Here, at least for the "urea-producing bacteria", additional quantification based on functional genes encoding urease would have been helpful. This would allow comparisons of absolute abundances of this group across the different sampling sites and provide a better approximation of the potential impact of the organisms' activity. It was also a bit surprising here that the authors included a soil sample, as this will for sure provide different microbial communities and higher biomass compared to the rock-associated environments. Consequently, it is not a big surprise that the authors found the highest species diversity in the soil sample, or differences in TOC content between their different sampling sites. Some more explanations would be helpful why the authors investigated "sunlight penetration" as one of the key parameters in this manuscript. Why did they expect this factor to influence microbial communities? More explanation is needed why the authors expected to find an effect of light penetration on the growth of heterotrophic microorganisms (l. 360-362). The conclusions drawn regarding this aspect are very speculative, and the connections between different aspects or parameters are not clear (l. 372-374; l. 379-381; l. 389-390). The description of the scientific question in the introduction remains rather broad (l. 92-93) or refers to aspects which were not addressed in this study (l. 93-95). Similarly, also the objective remains rather unspecific (l. 120-121). Here, some more specific objectives or hypotheses would be valuable. What effect of the tested environmental parameters on microbial communities did the authors expect to find? How does that add to our existing knowledge? The discussion in parts remains very speculative (e. g., l. 324-335; l. 351-354) and the numbers on which discussed differences are based are not always convincing (e. g., l. 333: a factor 2 difference can also be due to variation introduced by the molecular analysis pipeline). In several places, the authors should tone down their conclusions in light of the aspects I listed above. Sequence data originating from four sampling sites and one time point provide only limited sup-

port for the two last statements made in the conclusion section (l. 387-390). Here, different experimental approaches would be needed to demonstrate the actual activity of the microorganisms leading to the postulated biogeochemical effects.

Specific comments: l. 81-83: Please add a reference here. l. 89: What is developing orientation? Please explain. l. 105-116: This is a rather detailed description, and parts of this could be moved to the methods section. l. 122 ff: As far as I can see, the authors analyzed data derived from amplicon sequencing in this study. Please avoid the term "genome studies" or "metagenomics" here (and in other places later), because it might be misleading. l. 126-132: This is a very detailed description of the methodological approach which should rather be integrated in the methods section. Please provide information about the key outcomes of your study here instead. l. 136-139: Did the authors take any spatial or temporal replicates? Please explain. l. 168: How did the authors define "weathering-associated bacteria"? Please explain here. l. 178: I wonder if a sequence identity threshold of 95% will be enough to unambiguously identify urease-producing bacteria. Why did the authors not use a different approach by directly targeting functional genes coding for urease? l. 199: What were the exact temperatures here? l. 223-227: This sentence is a bit misleading since the bacterial phyla that are first listed as the four major phyla in all the groups obviously only represent minor fractions in some of the groups. Please rephrase. l. 235-240: This section is not clear, please revise. l. 257-258: How did the authors define "relative impact" here? Is this something that would require activity measurements to be assessed? l. 277-278: which is consistent with its ecological landscape - what does this mean? Please explain. l. 286-287: "geological evolution after their interaction" - what does this mean and was it addressed in this study? l. 290: ...that dominant species in karst samples were affiliated with Actinobacteria and Proteobacteria, please rephrase. l. 293: "extreme diversity" - please provide a comparison to other environments or studies. l. 297-300: This sentence is difficult to understand. Please rephrase. l. 301-303: This sentence is very speculative. l. 305-308: As far as I can see, the authors did not use a metagenomics approach in this study, and the "evolution of microbial communities

and the consequential changes in the environment" have not been studied here in this work. Please rephrase. l. 309, l. 311: How do the authors define "functional bacteria"? Please explain. l. 313-315: Why do the authors address the cutoff question here? What would have been alternative sequence identity cutoffs to use? l. 321-323: This hypothesis does not agree with the objective stated in the introduction. In addition, this seems like a topic that cannot be addressed by a 16S rRNA sequencing approach. Table 1: What is "effective number of species"? Please explain.

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