

Interactive comment on “Temporal and spatial mediated changes in subsurface microbial community assemblages and functions” by Madison C. Davis

Anonymous Referee #2

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In this study, the author analyzed microbial communities from a sinkhole from five different hydrochemical zones and nine time points during a two-year-period. Although hydrochemical parameters showed seasonal patterns, the author did not observe such patterns for the microbial communities. In general, this is an interesting question and a comprehensive data set. However, I have some concerns regarding the analysis, presentation and interpretation of the data. The motivation of this work is not fully clear from the introduction. The introduction is very general and does not provide sufficient information about microbial communities or microbial community dynamics in the subsurface, which is the subject of this study. Moreover, it is not always clear for which environment previous observations of community dynamics are reported, and to what

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extent they can be compared to or are relevant for the subsurface system investigated in this study. Since weather extremes are also of interest in this context, they should also be introduced in the introduction. I also wonder if the number of sampling time points is high enough to identify seasonal patterns. It is also not clear what kind of seasonal pattern the author expected to find. In what way were microbial communities expected to change, and what could be key drivers in this sink hole system? This should be worked out in more detail in the introduction. Moreover, the author states that seasonal patterns existed for hydrochemical parameters, however, these data are only provided as tables. Here, a graphical display confirming the seasonal patterns would be helpful. Despite the large set of microbial data that seems to be available, the results section is rather short, and there might be more potential in the data than the author makes use of. It would also be interesting to learn more about which microbial groups are dominant in these sinkhole communities. Finally, the author should make more clear what is the novelty of this study. Some of the (hydrochemical) patterns presented appear to be similar to stratified lakes. What is special about the sink hole system? The conclusions are very general and broad and are not directly derived from the results of this work.

Specific comments: The title is unclear: should this rather be "temporal and spatial changes. ..."? The information in the last two sentences of the abstract is slightly redundant. Please consider rephrasing.

l. 76: what filter material was used? Please add the information. l. 82-83: This is unclear. Was this reference data set used for the community data from the sinkhole or for the mock community? l. 84: Why were sequence data from each zone analyzed separately with the Mothur pipeline? I would suggest analyzing all sequence data together and then only run statistical analyses with selected data for within-zone comparisons. Comparison of data across the different zones could add additional value to the results. l. 96-98: This is unclear. Does the author mean that sequences with less than 80% similarity were termed "unidentified"? l. 99-105: This approach seems a bit compli-

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cated given the fact that programs such as PICRUSt are available that would predict functions based on 16S rRNA gene sequences using comprehensive databases. Why did the author not try such an approach? l. 112: Why are sulfide concentrations not expressed as mg/L? l. 129-131: Please add information here which hydrochemical parameters appeared to be important in the different zones. l. 133-134: This sentence is unclear. How is percent abundance linked to potential metabolic functions? l. 151: It is not clear how this analogy would work. The first Table 2 should be table 1. Table 2: The terms "nitrogen oxidizers" and "nitrogen reducers" are not common. Please use more specific categories such as nitrate reducers, nitrifiers etc.

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