

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

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I'd like to thank Reviewer 2 for their review and comments.

Subsurface microbial community dynamics are poorly characterized, especially in the coastal regions of Florida. The microbial community dynamics of other systems, including meromictic lakes, were used as a comparison of what could be occurring in the subsurface. The seasons referred to are "wet" and "dry", and were chosen based on the comparison to the literature of other systems. It was expected that rainfall, which would cause changes in hydrology, would impact the microbial communities and would therefore show a distinct wet and dry season microbial community in each layer, as was

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seen in Davis et al. 2020 (PLOS ONE, doi: 10.1371/journal.pone.0232742). It was unknown whether the changes in hydrology would cause similar or different patterns in the microbial community assemblages. Hospital Hole is unique in that it is a spring, and conduits from the aquifer discharge into the sinkhole. Davis and Garey 2018 (Water, doi: 10.3390/w10080972) suggested this sinkhole could be analogous to the surrounding aquifer. These items can be clarified in the introduction before publication, which may help elucidate the novelty of this study.

The microbial groups that were dominant were described in Davis and Garey 2018, which provided a comparison of the different microbial communities in each layer, and this study focused on how these groups may change in relation to each other.

Would Reviewer 2 please clarify what is meant by a graphical display of seasonal hydrochemistry, and other ways to analyze the data?

The specific comments with line numbers are addressed below, and can also be clarified before publication: 76 - the filter brand can be added to identify the filter 82-83 - a reference dataset was made for the mock community to calculate the error rate 84 - Davis and Garey 2018 established the differences in the microbial communities in this sinkhole. Due to the large dataset, it was difficult to analyze all of these samples together despite using a cluster network. It was decided, based on the 2018 study, to separate these out by layer to see how the patterns of change compared to each other. 96-98 - if the GenBank queries were less than 80% similar, the taxa was considered "unidentified" 99-105 - PICRUST and other predictive functioning profiles were considered, but these tools may not accurately characterize the extremophiles present within the subsurface. This discussion can be added to the manuscript before publication. 112 - ug/L was used because low concentrations (e.g. 11 ug/L) were present, but can be converted to mg/L before publication 129-131 and 133-134 - these sentences will be clarified before publication 151 - The introductions of nutrients, rather than the hydrology, may be driving microbial community changes in the Weeki Wachee River system. Local management agencies have identified that this springshed has elevated

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nutrients. The introduction of nutrients to the subsurface, including those from aquifer storage and recovery programs, may cause changes in the microbial communities in the aquifer. This sentence can be expanded to highlight this discussion.

I thank the reviewer for noting the typo in Table 1. The "nitrogen oxidizers" and "nitrogen reducers" include multiple potential metabolic functions, as noted on lines 99-105.

I'd like to thank Reviewer 2 again for their comments, and appreciate their recommendations which can be included before the publication of this manuscript.

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