Response to review Reviewer 2
Barry-Sosa et al., "Effects of surface water interactions with karst groundwater on microbial biomass, metabolism, and production"

R2.C1: "*In Table 1, some instances of "N.d." were written as "N.d" in the row for "DIC".*

Author's response: Thank you for pointing out this typographical error. We will correct these discrepancies in Table 1 of the revised manuscript.

R2.C2: "*the note below Table 1 for "Sp. Cond" does not match the values in the table*."

Author's response: We are a little confused by this comment. If this refers to "Sp. Cond." in the table appearing as "Sp. Cond" in the legend, we will add a period to the latter in the revision.

R2.C3: "*Table 2 contains two variations of "Not applicable", mentioned as "N.a" or "N.a.""*.

Author's response: Thank you for pointing out this discrepancy, which we can easily correct in the revision.

R2.C4: "*The figures appear blurry, and the color of the graph's axes could be made darker to enhance clarity for readers*."

Author's response: We agree with this suggestion and will also use higher resolution images for the figures in the revised manuscript.

R2.C5: "*In line 351-352, please provide accurate data or a figure to support the statement regarding "ATP-based biomass concentrations in samples from River Rise at low flow being significantly higher than values at high flow and approximately 3-fold lower than those observed for River Sink at low flow.""*.

Author's response: In the initial submission, we neglected to point out that this statement is supported by the data in Fig. 3 c, which we intend to include in the revised text.

R2.C6: *Line 357-359 refers to "Fig. 2c," but there is no such figure mentioned in the article.*

Author's response: Thank you for pointing out this oversight, which will be corrected in the revision.

R2.C7: "*In line 390-392 and line 407-408, the authors state that the incorporation rate of 3H-thymidine was much higher from Madison Blue Spring. Therefore, it may be more appropriate to use the 3H-thymidine incorporation rate when evaluating doubling times at Madison Blue Spring.*".

Author's response: More rapid generation times were inferred from the ^{3}H-thymidine at all sites, but for Madison Blue Spring, they were much more rapid (7 to 30 h versus 232 to 1028 h for the ^{3}H-leucine incorporation data; Barry Sosa, PhD thesis, University of Florida, 2023). Additionally, we observed very low ratios of leucine to thymidine incorporation at Madison Blue Spring, which were ~10-fold lower than those observed at the other sites (Table 2). This coupled with low BGE values implied uncoupling between catabolism and anabolism and/or that the community was investing most of its energy flow in maintenance metabolism rather than growth (see discussion in lines 575-578). For these reasons and those described in lines

403-405, we believe the ^{3}H-leucine data are the most appropriate for estimating doubling times and bacterial production to make comparisons among sites. Please note that ^{3}H-thymidine incorporation rates are provided in the supplementary materials (Figure S7), which allow anyone seeking to makes direct comparisons to calculate bacterial production and doubling rates from these data.

R2.C8: "*In line 460, please attempt to explain why ATP contents are high at Devil's Eye Spring.*"

Author's response: The very high per cell ATP contents inferred in groundwater from Devil's Eye Spring are surprising and remain unexplained. The simplest possible explanations are that 1) the groundwater community at Devil's Eye has higher amounts of biomass/ATP, 2) is producing ATP relatively more rapidly than the communities at other sites, or 3) the excesses are due to low anabolic rates that consume ATP slowly and allow produced ATP to accumulate in the cells. Given that the first two options are not supported by our data (see lines 462-464), the latter explanation (#3) is the most likely. We propose to add a few sentences in the revision that explore these possibilities and state this working hypothesis.