Response to Reviewer 1
Barry-Sosa et al., "Effects of surface water interactions with karst groundwater on microbial biomass, metabolism, and production"

R1.C1: *"You mention regional groundwater in L266 but I don't think its mentioned again. Because all the springs & river samples provide this gradient of SW-GW mixing (nicely introduced in L9-L10 of abstract) across the region, it would be nice to include data more prominently from a true regional groundwater sample as a sort of end member comparison. In addition, having a surface water sample as the opposite end member (maybe the Santa Fe Sink & Rise system during high river discharge) could be cool. If you had data from these, you could use your conductivity data as a "conservative tracer" to quantify actual SW-GW mixing at each of your springs which would provide a nice backdrop for all of the presented data. See "Figures" comments and others regarding the necessity for displaying the gradient in SW-GW mixing more prominently throughout, and this could be a cool way to do so."*

> **Author's response:** Based on this comment, we realize that "regional groundwater" is not the best word choice for this description. Hence, we propose replacing it with "matrix water" (i.e. water stored and circulating through the aquifer rock porosity).
>
> Using a conservative tracer to quantify the degree of SW-GW mixing is an excellent idea. However, the main challenge in applying this approach to our data is that we lack a true groundwater endmember for the River Sink-Rise system, which has at least two distinct groundwater sources that are difficult to discern from each other. This together with surface water mixing causes wide variability in the matrix water geochemistry as indicated by measurements at monitoring wells in the area (See Table 1, Moore et al., 2009, J. Hydrol., 376, 443–455, https://doi.org/10.1016/j.jhydrol.2009.07.052). In addition, separating these three endmembers would require more solute data than we have for this project.

R1.C2: *"Though the introduction is very well-written (I felt like I could easily understand the importance & relevance as someone that studies SW-GW mixing but not in karst systems), I feel that the last paragraph needs to be reworked – maybe into 2 separate paragraphs – so that the reader can fully grasp previous work and the importance of this work in the UFA system. I say a new paragraph because there seems to be a lot of previous research on the UFA that is briefly mentioned in L61-65. Because of the amount of work done here, I think it would help to have a paragraph prior to this discussion the previous research done here and what is known about these springs. I'd especially touch on the differing discharges and what is known about how this alters organic matter (mentioned in L61) because this is directly relevant to this manuscript. I'd then end the introduction with a final paragraph that succinctly states what you are doing that is new, your hypothesis, how you addressed it, and briefly what you found. I make these recommendations because I feel that this manuscript relies heavily on the readers understanding of the sites (which might be able to be addressed, see other major comments) and I leave the introduction not feeling like I have the full background to follow the significance of the dataset through the results & discussion."*

> **Author's response:** We agree with this suggestion and propose to divide the final introduction paragraph as recommended. While the site description section provides explicit detail on existing information for each site, we intend to briefly highlight the most relevant information in the introductory paragraph of the revised manuscript. We propose to describe how prior research conducted at many of our sites has shown residence time in the aquifer and surface-groundwater interactions are connected to the levels of dissolved oxygen, organic carbon, and microbes in the groundwater, but that the effects of groundwater hydrogeochemistry on microbial physiology and

production has not been studied. In the revision, we will also edit the final paragraph of the introduction to succinctly state our experimental hypotheses and the significance of our results.

R1.C3: *"I feel that underlying differences in GW-SW mixing between sites need to be much more prominently displayed in the figures and that the presenting of data needs to be changed for readers to be able to follow the main story without jumping back to Section 2.1. The entire relevance of the different sites is the altered GW-SW regimes but this is completely lost in how the data is currently presented. Overall, editing the figures so that the readers can more easily understand the takeaway from the dataset would help in following the overarching findings that shifts in GW-SW mixing in these karst systems impacts biogeochemistry and greatly strengthen the manuscript!."*

Author's response: Thank you for this suggestion. We propose to rearrange the site order in Table 2 and Figs. 2 to 5 and supplementary figures S1, S2, S5 and S7 so that the three categories of springs are clearly indicated and the sites are maintained in the same order for consistency of presentation. As an example, we show the new presentation for a revision of Fig. 5:
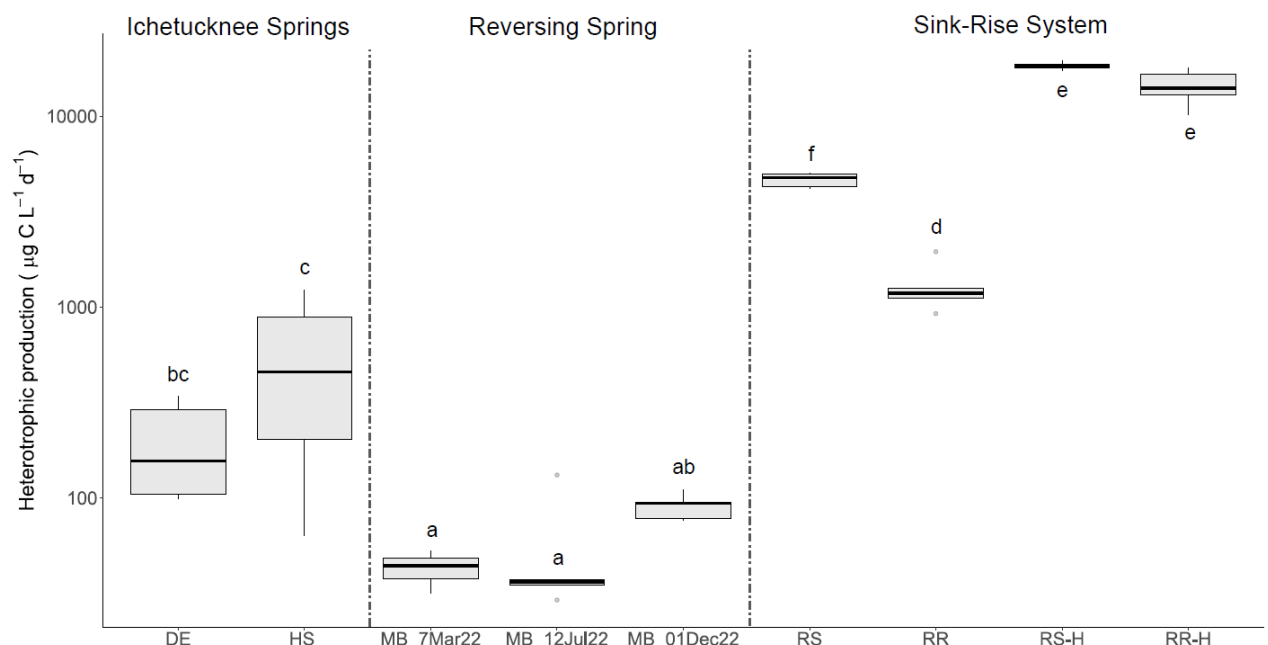
R1.C4: *"I I feel that more information needs to be added to the figures as presented. For example, the axes of all figures (besides the site figure, Fig. 1) need to include more than just the units (ex: Fig. 2a needs to say POC concentration (mg C L-1))."*

Author's response: We agree with this suggestion and propose to add the following descriptive information to the y-axis labels in the revision:
Figure 2 (a): "POC (mg C L^{-1} d^{-1})"
Figure 3 (b): "Cell-based biomass (ng C L^{-1})"
Figure 3 (c): "ATP-based biomass (ng C L^{-1})"
Figure 3 (d): "ATP/cell (zmol)"
Figure 4 (a): "O_2 consumption rate (mg O_2 L^{-1} d^{-1})"
Figure 4 (b): "DIC production rate (µg C g^{-1} d^{-1})"
Figure 5: "Heterotrophic production (µg C g^{-1} d^{-1})"

R1.C5: *"I also think the manuscript needs to be more consistent with the use of acronyms or not. Each figure uses the site acronyms (which I get as the site names can be lengthy), but the text largely uses the full site names. Make sure the manuscript is consistent throughout: either exclusively use acronyms or exclusively use the full site names, across the figures and text."*

Author's response: When drafting the manuscript and figures, we had an active debate on whether or not to use acronyms through the manuscript. Ultimately, we decided that the acronym heavy text would be extremely unfriendly to the reader. We drafted figures that replaced the acronyms with full names, but the limited space available in the multi-sample plots did not allow the full names to be adequately displayed. To address this comment, we are proposing to use the full spring names in the text, keep the acronyms in the figure axes, but define the acronyms for each figure in the legend and/or figure caption. We believe this will make the text easily readable without an acronym key and that the addition information provided in the legend will make deciphering the sample designations easier. Please also see our response to R1.C3, which will also assist with differentiating among the site types.

R1.C6: "*The results section is very long – which I get as there is a lot of data here – but I recommend condensing. Of course, still comprehensively present the data but the manuscript doesn't need to explicitly state every data point! I'd also try to rework so that again the data is presented with the backdrop of hydrology regimes. The first paragraph of Section 3.1 is awesome and super helpful but with this many sites it's hard to remember all the different hydrology regimes, especially as someone that has never worked in the UFA. For example, in L278-280, the manuscript presents which sites have the heaviest δ13C value for POC and the variation in the dataset. I'd refine this so that it is framed with the hydrology (e.g., "the heaviest δ13C value for POC was observed at MB that had a [higher/lower?] influence of GW than these other sites with [less/more] GW influence)."*

Author's response: This criticism largely concerns style and we believe that a large reduction in the results section would eliminate valuable information that is of interest to many readers. However, this feedback has prompted us to identify areas in the results that can be streamlined to improve flow and the presentation. This includes the section on POC mentioned (lines 278-280) as well as content in lines 335-345, which describes the ATP biomass measurements in explicit detail. We also agree that revising sections of the results to emphasize the influence of surface water and groundwater at the sites discussed would assist the reader in evaluating the effects of hydrology on the various biogeochemical variables discussed.

R1.C7: "*I do feel that the discussion is also very lengthy, and should maybe be reformatted so, instead of it sectioned by the different datasets, format it by the different key findings. Some of the points in the discussion seemed to be more fitting for a results section (e.g., L523-524, the correlation analyses and p-values fit more in a results discussion). I recommend condensing the discussion and focusing mainly on what the data means: does it agree with previously published work on UFA, what are the key findings, continuously connecting findings to hypothesis, include significance of results."*

Author's response: Thank you for this feedback to improve the discussion. We agree that the section headers in the initial submission could be revised to be more informative and appropriate for the discussion. Therefore, we propose modifying the following section headers as follows:

Section heading – initial submission	Section heading - revision
Groundwater and surface water mixing in karst landscapes	Importance of groundwater and surface water mixing in karst landscapes
Abundance of microbes, biomass, and organic carbon	Effects of groundwater residence time on microbes, biomass, and organic carbon

| Microbial respiration | Hydrology and DOC influences on microbial respiration |
| Heterotrophic production and growth | Heterotrophic production and growth do not scale with groundwater age |

Similar to comment R1.6, the criticism on the length of the discussion is about style, but we do agree that the discussion can be improved by better highlighting the key findings and their significance, as well as eliminating text that is more suitable for the results such as lines 523-524.

R1.C8: "Conclusion: *Super well-written, but I feel that some of this could instead be in the discussion. In my experience, the discussion should include significance of findings and the conclusion includes an overview of what you did & found with suggestions for future work."*

Author's response: To address this comment, we will move and integrate the text from lines 593-597 into the first paragraph of the discussion (lines 438-448).

R1.C9: "*L40: Remove extra Jin in citation. (Jin et al., 2014)"*

Author's response: Thank you for pointing out this typographical error, which we have noted to correct in the revised manuscript.

R1.C10: *"L70: Define what a river sink-rise system is."*

Author's response: We agree with this suggestion and propose to parenthetically add the following definition to this sentence: "(i.e., a swallet or opening where a river recharges water-filled caves and discharges to the surface at a point downstream)".

R1.C11: *"L72-L75: Mentioned in major comments, be consistent with acronyms throughout. I think this is the only time acronyms are used in the text, but are always used in the figures."*

Author's response: Please see our response to R1.C5.

R1.C12: *"L145: Add a clarifier to what entails "humic characteristics", especially as folks have moved away from the terms "humics" and "humification" in the OM world. Something like: "The Humification Index (HIX) indicates degree of polycondensation where higher HIX values are more indicative of lower H/C ratios and higher molecular weights". "Humic characteristics" isn't super informational or specific!"*

Author's response: We agree and will revise this section using the phrasing suggested.

R1.C13: *"L179: Define ATP as its the first time its used!"*

Author's response: Agreed.

R1.C14: *"L266: You mention regional groundwater here, it would be helpful to include throughout the reference of this regional groundwater sample to provide some sort of end member to compare the data! See major comments for more details here."*

Author's response: Please see our response to R1.C1.

R1.C15: "*Table 2: Missing the first row that you have in Table 1 that includes the different groups.*"

Author's response: The revised Table 2 will organize and separate the springs identically to that shown for Table 1.

R1.C16: "*L470: Try to use better descriptors than "quality" here. The use of quality isn't helpful to the reader, you can use quality but include more like: "….had low quality (e.g., higher H/C ratios)."*

Author's response: Thank you for this suggestion. We will revise the text to indicate that low quality carbon is inferred by high HIX and low FI and BIX values.

R1.C17: "*L523: This correlative relationship would be nice to include in a figure***"**

Author's response: Agreed. A plot showing the correlation of heterotrophic production with DOC concentration and quality (HIX, FI, and BIX values) will be added as a supplementary figure to the revised manuscript.