

Effects of surface water interactions with karst groundwater on microbial biomass, metabolism, and production

Adrian Barry-Sosa, Madison K. Flint, Justin C. Ellena, Jonathan B. Martin, Brent C. Christner

Review

Summary and general comments

The manuscript from Barry-Sosa et al. represents a comprehensive analysis of subsurface biogeochemistry within the karstic Upper Floridan Aquifer that includes sites across a range of surface water and groundwater mixing. The dataset is extremely comprehensive (& cool!), including a wide variety of analysis techniques that expansively assess water chemistry (temperature, pH, conductivity, DO, OM quality using EEMs, etc.), microbiome characteristics (cell counts, biomass), and microbiome activity (respiration calculations via incubations, heterotrophic production with ³H-leucine and -thymidine). The vast variety of tools used makes this dataset incredibly unique. Overall, I believe that the data are solid and that this is an impactful contribution to the research community for assessing how surface water-groundwater interactions in karst systems influence biogeochemistry. However, with all this data, I struggle following the broader story and tracking whether the data aligns with the hypothesis stated in the introduction and think there are some major improvements that must be made to refine the manuscript for publication.

Major comments

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.

Introduction: 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.

Figures: There is a **lot** of data presented in this manuscript and I had trouble understanding or following what the broader story was, especially in the results section which is very lengthy. I tend to go to manuscript figures when I feel I can't follow the overarching story, but the figures are not super helpful if you don't fully understand the relevance of the different sites. Because of this, I had to keep jumping back to Section 2.1 to remind myself of the differences between the sites. I understand that the relevance here is the backdrop of different groundwater-surface water mixing between the different springs & river sink-rise system, but those differences are only primarily presented in the Section 2.1 and Section 3.1 (& yes, sprinkled in here & there). 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!

A few potential suggestions below:

-Adding more information to all boxplot figures, perhaps grouping and labeling sites by discharge (e.g., Peacock Springs, Madison Blue Spring, Little River Spring, & Gilchrist Blue Springs all labeled as reversing springs and placed together on boxplots).

-I think you have discharge information for each site (at least the majority?, Table S1), for some of the data it would be interesting to plot against discharge with points shaped by site.

Another note on figures: In addition to thinking of other ways to display your data to include the different GW-SW regimes, 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)). 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.

Results: 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ value for POC was observed at MB that had a [higher/lower?] influence of GW than these other sites with [less/more] GW influence).

Discussion: I love Section 4.1! Super helpful in framing the discussion and reaffirming the significance to the reader. 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. Again, there is just a lot here and I struggled coming away understanding the key findings of this work!

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.

Minor comments

L40: Remove extra Jin in citation. (Jin et al., 2014)

L70: Define what a river sink-rise system is. I work in alpine streams and have never heard this term!

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.

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!

L179: Define ATP as its the first time its used!

L253-L55: No suggestion just that this is cool!! And a good presentation of the data with the included background of the hydrology here!

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.

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

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).

L523: This correlative relationship would be nice to include in a figure.