Reviewer #2:

In this study, the authors sampled several rivers primarily distributed in the U.S.A, with one additional river in Germany and another one in Israel. They generated an extensive dataset including DOM molecular composition, metagenome assembled genome, inorganic ions, and organic carbon concentration. Whereas the dataset and the data themselves are of high quality, the study is not adding any novelty compared to the literature. Moreover, this study is not a global study. It is mostly a study of rivers in the U.S.A. and therefore, I am not convinced by the global aspect. I think the authors have to carefully rethink their study as I personally do not recommend publication in its current form but believe it can become a nice manuscript.

Thank you very much for taking the time to review the manuscript. We appreciate these concerns, and we will address them in more detail as they arise below.

We will change "global" to "spatially resolved" (or equivalent wording).

Please work a bit more on the abstract. It contains quite a lot of jargon and is not down to the point.

We will attempt to reduce jargon in the abstract.

The introduction is not down to the point and does not introduce the topic well enough. The second and third paragraphs are just a list of studies, mostly from the authors themselves, without any link or any rationale behind it. They just tell us that people have studied microbes and DOM in rivers before. After reading the introduction, I was not convinced that this study was needed, nor that a meta-metabolome ecology was needed. The introduction forces us to question the choice made by the authors instead of helping us understand the study. For instance, why the author did not use a network analysis like previously done (Zhou et al, 2024)? A network approach is best suited "to characterize the microbes that interact with and alter river corridor DOM" "and to observe connections between DOM and the microbes that consume it". Why the author did not use more traditional multivariate analysis as typically done (e.g. Ezzat et al, 2025)? Most of their results are derived from multivariate analysis anyway. So the meta-metabolome was not needed here. Why the authors used ultra-high resolution mass spectrometry when other measurements suffice (e.g. Kohler et al 2024)? I recommend the authors to drastically revise their introduction to ensure the goal, relevance, and novelty of the study are all clear. Similarly, I would recommend the authors avoid over-citing themselves. Currently more than a quarter of the references are from the authors themselves, this is not normal, even if the authors want to claim they are leader in their fields.

Regarding the 2nd and 3rd paragraphs: We believe this confusion arises due to a lack of transitional sentence on the second paragraph. We will add a transitional sentence (e.g., "*Extending our microbial analyses to intentionally include broad non-targeted DOM*

characterization will provide greater insight into putative microbial mechanisms.") to alleviate this concern.

Regarding choices of methodology: We will attempt to better convey our idea through word choice modification, though we do already have a paragraph stating why we used meta-metabolome ecology on Lines 92-111 (e.g., it enables transferrable biogeochemical observations); this is an inherent quality of meta-community ecology from which meta-metabolome ecology arises. We also provide justification for meta-metabolome ecology's usefulness by way of practical examples: 1) it was integral in revealing thermodynamic redundancy (Line 99), 2) it demonstrated that microbes and DOM experienced different levels of selection (Line 103), and 3) it has shown that global change will likely diversify DOM (Line 106). Despite these discoveries, we still have significant gaps in knowledge – particularly with how microbial communities and DOM develop in concert. Given the success of meta-metabolome ecology in the past, we argue that it will help us uncover patterns. The methods you mentioned, while powerful, often have limitations in transferability (e.g., networks are correlative and often do not provide concrete results) or we have already integrated them (e.g., multivariate analyses as you stated). We did enjoy the Zhou et al. manuscript and found it highly relevant to this study – we will be integrating aspects into our introduction and elsewhere when relevant.

Regarding the citations from author: We have a large author list on a manuscript that is compiling data collected from 2018 from sites that have a history of being intensely studied across diverse institutions. Given this context, it is expected that many citations would come from within our group regardless of expertise.

In the methods section, I am puzzled by the reasoning of the author to convert the FT-ICR-MS data to presence/absence only whereas they consider the abundance of MAGs. The same limitation exists with FT-ICR-MS data than with metagenome data. It is not logical to convert one dataset into presence/absence only and not the other one. This is particularly concerning because it is unclear whether the results would remain the same if the author used richness instead of Shannon for the genomic data or if the author used Shannon instead of richness for the FT-ICR-MS data.

The limitations that exist for FTICR-MS data are divergent from those that exist for metagenomics, though they are similar. Metagenomics would be "sum constrained" whereby the depth of sequencing (e.g., how many nucleotides we identify) and the abundance of organisms have a dual effect on the likelihood of a given sequence being observed. Despite this limitation, we can statistically account for this sum constraint by using mathematical transformations like Gene-length corrected trimmed mean of M-values (GeTMM). FTICR-MS instead has more physical and chemical conditions that limit the ability to detect peaks (and eventually molecular formulas) and evaluate their intensities. The environment (often called the "matrix" within the field and includes carbon, metal, etc. concentrations) is known to heavily impact peak detection and intensity. Some of this can arise from charge competition which will mask peak detection, but other missed peaks are

more stochastic. This phenomenon doesn't factor in specific analytical choices that also impact the likelihood of detecting a peak or identifying meaningful intensities of a peak (e.g., ionization choice, ionization efficiency, extractions vs. non-extractions, etc.). This results in a situation where we can statistically control for the limitations of metagenomics, but not for FTICR-MS derived data. Largely, it is useful to maximize data in analyses; in this case means leveraging abundance values for metagenomic data but only presence/absence value for FTICR-MS data.

To specifically address your concerns of differences in Shannon diversity versus richness, Kew et al., 2024 (https://doi.org/10.5194/bg-21-4665-2024) have demonstrated that intensity values are minimally informative (e.g., Shannon's diversity calculated from real intensity values compared to diversity calculated from "false" intensity values yielded similar results). Given the challenges that intensity values present, we decided to take a conservative approach to minimize inaccuracies.

In the Results and Discussion section, it is disturbing that the results are not presented using the past tense. It gives the impression that a specific result is a general truth. Furthermore, much like in the introduction, the authors focus mostly on their previous work. Consequently, it feels like there is nothing novel. Except for some river parameters, I fail to see novelty in those results or their interpretation. The Results and Discussion section need more discussion. I am pretty sure this study can advance our knowledge of riverine microbes and their interaction with DOM, but it is up to the authors to tell us how it does it.

Regarding tense: We will ensure that the tense is consistent throughout.

Regarding the novelty: We agree that our results were not necessarily presented in a way that emphasizes the novelty of our observations but were presented as providing context to a developing comprehensive conceptual model within the field. However, despite this limitation, we believe that we already have noteworthy observations that may have been lost in the text. Specifically, the observed glycoside hydrolase niche conservation, interactions between environmental conditions and DOM assembly, and relationships between organic matter richness and glycoside hydrolase diversity were all previously unreported. Importantly, each of these observations have potential implications when attempting to predict organic matter dynamics within and across watersheds.

We will plan to improve our language and rearrange text to better emphasize our novel observations while balancing the limitations inherent to these large-scale metagenomic/DOM characterization studies.

Minor comment and typos:

There were many typos and grammatical mistake in the text. I only reported those that obviously stood out, down to the beginning of the Results and Discussion section.

Line 38: Why Berlin, Germany and not Israel or Middle East? On the map, the westernmost rivers are in Oregon/Washington, and the easternmost river is the Jordan river in Israel. Having Berlin here is a bit odd.

We will change Berlin to the Jordan River

Line 39: Define geochemistry. Too much jargon for an abstract.

Geochemistry refers to the study of minerals and the chemical components of the earth. We will attempt to clarify the abstract through word choice modification.

Line 41: Which scale?

In this case, we are referring to spatial scale and we will add that to the sentence

Line 44: in across the rivers --> across the rivers

We will drop "in"

Line 45: for recovered metagenomically assembled genomes --> from recovered metagenomically assembled genomes

We will change this

Line 92: reveal --> revealed

We will change "reveal" to "revealed"

Line 116-117: What do you mean by "and shotgun metagenomic sequencing partnered to ecological analyses". Please specify what ecological analyses and how they partnered with the metagenome data.

We will change "ecological analyses" to "meta-metabolomic ecological analyses"

Line 127: the seven rivers --> seven rivers

We will change this.

Line 158: What was the volume of surface water collected?

20mL; this is on Line 173.

Line 159: In total, 690 total samples. Remove one "total".

We will change this to “In total, 690 samples”

Line 167-169: No clogging of the filter at any site? Even with Sterivex filters, clogging does occur. Please specify if less water was passed through the filters at some sites and what was done if clogging occurred. Specifically, how the replicates were handled.

No clogging occurred.

Line 170-171: Please provide reference of the probe use for temperature, pH, etc.

We will add a reference to the probes used.

Line 171-172: Please specify the vials were pre-combusted before injecting the DOM samples.

We use new vials that are approved for volatile organic analysis (https://www.mtc-usa.com/Microsolv_Brand_Ordering) so we do not pre-combust our vials.

Line 198: Reference of the PPL cartridges? And what does PPL stand for?

PPL stands for “Priority PolLutant” but that is the Agilent’s product name for their styrene-divinylbenzene polymer; we will add the Agilent Bond Elut reference and mention the polymer.

Line 209: “or had an isotopic signature” does that mean authors removed all peaks that contained a e.g. 13C? It is quite unclear as written here.

That is correct - we will add 13C assigned formulas were removed.

Line 274: on the NovaSEQ6000 platform on a S4 flow cell --> on a NovaSEQ6000 platform using a S4 flow cell

We will change this.

Line 302: and using --> using

We will change this.

Line 351: It is unclear what is meant by “we see that each river has characteristic geochemical parameter values”. Please develop further and define what “characteristic geochemical parameter values” mean.

This meant that each river had a geochemical parameter that appeared to be associated with it over other rivers – for example, Erpe was associated with high DOM and the Altamaha was

associated high iron. We will change the text to mirror this intention (e.g., “We see that rivers were characterized by elevated values of different geochemical parameters.”).

Line 363-364: Examining carbon data for combined pore and surface water samples à Examining carbon data from combined pore and surface water samples

We will change this.

Line 624: A network is better suited to identify those relationship than the speculation proposed by the authors.

While we appreciate the utility of network analyses having used them in the past, we do not believe that they would lead to decreased speculation. This is because networks intrinsically rely on correlations and cannot ascribe direct causation which will lead to speculation. Our methodology, which is not a replacement for network analysis, treats molecular formula as discrete ecological units and allows us to start discussing fundamental processes that give rise to observed phenomena. In the specific example cited (e.g., microbial community trends), a network analysis would likely not provide greater context in this domain (e.g., observations that link DOM assemblages to microbial communities). Network analyses may reveal putative correlations between gene abundance and specific molecular formulas but given the uncertainties in formula assignment and the absence of metatranscriptomic/metaproteomic data, we would have similar levels of speculation.

References:

Zhou L, Wu Y, Zhou Y, Zhang Y, Xu H, Jang KS, Dolfing J, Spencer RG, Jeppesen E. Terrestrial dissolved organic matter inputs drive the temporal dynamics of riverine bacterial ecological networks and assembly processes. Water Research. 2024 Feb 1;249:120955.

Ezzat L, Peter H, Bourquin M, Busi SB, Michoud G, Fodelianakis S, Kohler TJ, Lamy T, Geers A, Pramateftaki P, Baier F. Diversity and biogeography of the bacterial microbiome in glacier-fed streams. Nature. 2025 Jan 1:1-9.

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