Point-by-point answers to reviewer's comments on "Combining microbial, isotopic and residence time data to characterize groundwater dynamics in a multi-layered aquifer system in Kurikka, western Finland" by Purkamo et al.

Reviewer1:

The manuscript reports on a study that added microbial community analyses (amplicon sequencing of 16S rRNA genes for bacteria and archaea and ITS for fungi) to more conventional methods for dating and tracing groundwater (isotopes, CFCs, SF6). Samples included water from sediments of various depths (~6-76 m) and a deep (162 m) rock-hosted aquifer. The findings follow patterns in which shallower, younger waters have greater microbial diversity and older, deep waters have less diversity but contain microbial groups (e.g. sulfate reducers) typical of older water with longer rock-water interactions. This supports the idea of including microbial community analysis for groundwater dating and flow path analysis, but I would like to have seen more of a big-picture wrap-up in which flow paths are modeled, possibly circling back to figure 1.

The authors would like to thank the reviewer for the constructive criticism and good suggestions for improving the manuscript. We have now modified it to be a characterization of the aquifer system and a baseline study for future monitoring when large-scale drinking water abstraction from the system is initiated.

Specific comments:

1. Title: Microbial communities may be novel tracers, but isotopes are not. Also, did they actually determine flow paths?

Fair point. While there are no speculations on the exact flow paths, there is assessment on potential mixing of waters in the discussion. From the limited amount of sampling locations/depths, it is challenging to reach more definitive conclusions on this issue. There is one publication currently under review from the same study area that describes the conceptual model of the hydrology of the aquifer that will hopefully clarify the flow paths more. We have changed the title to more relevant "Combining microbial, isotopic and residence time data to characterize groundwater dynamics in a multi-layered aquifer system in Kurikka, western Finland" and removed the flow path assessment from the study aims.

2. Lines 115-118: This is a long, but incomplete sentence. Maybe delete "which"?

We reformulated this sentence to following: In its most recent developmental stages, this region situated beneath the central sector of the Fennoscandian Ice Sheet (FIS) during the Late Weichselian Glaciation (23,000–10,500 years BP; Stroeven et al., 2016)—which covered southern Finland and facilitated the preservation of pre-existing sediments through limited glacial erosion (Putkinen et al., in review, 2025, Åberg et al. 2026)—remained largely unaffected. l.121->

3. Lines 157, 159, and 167: I probably should know what “uc.” denotes with regard to these acids, but I don’t. Explain?

Ultraclean, we replaced this with a full word “ultrapure”. E.g., l. 164, 174, 176

4. Lines 235-236: If this chloride value is obviously wrong, then I suggest leaving it out altogether. Chloride concentrations are unlikely to change in stored samples. Could this be measured in the lab on a stored sample if one exists?

The chloride value was not considered in the clustering. We removed it from the Table 2 here as there is a relevant measurement done in the laboratory, results are given in the Supplementary Table 1. The text was also corrected accordingly, see lines 280-293.

5. Line 264: I may be misunderstanding here, but it seems to me that the Cl values in the text don’t match the values in the table above.

These chloride values were measured in the laboratory, as stated above. We added a clear reference to the text about this, l. 289.

6. Also in Table 2, two of the samples have very high dissolved oxygen values, supersaturated, in fact. Are these correct? If so, then what are the implications?

Good point and well spotted, thank you for noticing this. We had mistakenly used the oxygen saturation percentage values for the two “supersaturated” readings of dissolved oxygen in Table2. The concentration (mg/l) values are for KUU19 **5.65** and for HÄJY11 **3.14**. The confusion is from the fact that the readings from the YSI meter are displayed both as the concentration and as saturation percentage values for dissolved oxygen and from these two sites, the DO% was marked down to field notes. The Table 2. has been corrected accordingly.

7. Line 322: Insert “Nearly” at the beginning of this sentence.

This section has been modified, see l. 341-357.

8. Lines 343-344, 373-373, and 385: Evidently three samples failed to yield bacterial amplicon sequences and four samples failed to yield archaeal or fungal amplicon sequences. I think this is not discussed anywhere. What if any explanation is there for this? Very low biomass? Interfering substances? What are the implications?

This is very likely due to low biomass and therefore, very low DNA yields in some samples. Typically, bacteria are more abundant than archaea in most environments, so it is likely that it is possible to retrieve enough bacterial biomass for DNA sequencing but miss the archaea. There is not much information about the concentrations of fungal cells in groundwaters, so it might be that these are not very abundant either. In addition, extraction of fungal DNA might require some extra steps to break down the cells, so this could be one reason for not retrieving enough DNA for fungal sequencing.

Another possible explanation might be the inefficient elution of the biomass from the filter that leads to low DNA yield. The filter is not designed to this specific purpose, so it may behave differently compared to the normal use, although this method has been in use in for example tracking the food-borne epidemics from drinking water with typically very low concentration of microbes.

There was a brownish color emerging to some of the filters while pumping the groundwater through, so this might be pointing towards some organic compounds that may have some interference in DNA extraction.

These explanations are now part of the discussion, l. 640-649.

9. Lines 409-413: This is repetition from the Introduction and can be skipped.

Indeed, but as an introduction to the discussion it maybe holds its place.

10. Sections 4.2.1 and 4.2.2: I suggest being more specific about the chemical species of S and Fe, rather than simply saying “sulphur” or “iron”.

Thanks for the comment. We decided to combine the different metabolisms around each element in the subtitle level in the Discussion, but we agree that there is inaccuracy in the description of sulfur metabolisms, as we mostly discuss the sulfate-reducing microbes below the sulfur title. However, as there is discussion also about the sulfur isotope, the interest lies in the sulphate-sulfur, since the analysis method used for analyzing the isotopic composition of sulfur converts all the sulfur components into sulfate (as described in https://doi.org/10.1016/j.chemgeo.2013.02.022) and sulfide was not measured/analyzed separately. Furthermore, with prevailing conditions of low organic matter, DO present, and at least in places high ORP (Oxidation Reduction Potential), sulfur in groundwater occurs mainly as sulfate.

With all this in mind, we kept the subtitles but added an explanation of the sulfur isotope method and correct the “sulfur-reducing” to sulfate-reducing microbes, l. 550->. Similarly, we tried to sharpen the text describing the different iron metabolisms using the actual species. However, as we don’t have exact measurements of different species, this was done carefully. See lines 570-576.

11. Line 540: I think that “Omnitropha” should be “Omnitrophales.”

The nomenclature has been corrected in order to refer to either Candidatus Omnitrophus or Omnitrophales, e.g., l. 586, 589, 593.

12. Lines 570-594: It's good to discuss the limitations of a study, but I would like to see some discussion of the strengths as well (unless the aim is to dissuade others from this approach, but I think this is not the intent here).

This is a good point, and we see that there are valuable insights that this study provides on characteristics of the aquifer system. We have rewritten the section to highlight the usability of water stable isotopes and strontium isotopes for provision of a baseline for tracing changes influenced by future pumping activities, as well as identification of end-members of different groundwater types. We stated that tritium from the tested residence time indicators was most promising while SF6 was not reliable in this type of geological setting, and mention some microbial indicator taxa that could support the geochemical and isotope tracer methods. See chapter 4.3.

13. Lines 611-612: Incomplete sentence.

We have rewritten this, see l. 717-720.

Reviewer 2:

Summary

The manuscript presents a multi-tracer investigation of a buried-valley aquifer system in Kurikka (Finland), combining hydrochemistry, environmental isotopes, noble-gas-based age tracers, and microbial community analyses across several wells. The overall aim appears to be a characterization of the multi-layered aquifer using complementary methods (i.e., combining tracer hydrology and microbiology). Recent studies have already demonstrated the potential of combining microbiological and environmental tracers to examine groundwater systems. Situating the study more explicitly within this existing frame of work would help contextualize the approach and clarify its contribution.

We thank the reviewer for insightful comments and suggestions for the manuscript, and hope our revised work is now more appropriate in the context of interdisciplinary characterization of this complex aquifer.

1. Lack of research question, hypothesis, and conceptual focus

We have revised the manuscript to clearly articulate the larger goal of the research initiative that this study is part of. Specifically, we now state that the study aims to determine the natural state of the aquifer, providing the baseline hydrogeochemical and microbiological characteristics of the aquifer system prior to large-scale water abstraction.

We have clarified that the research question focuses on whether microbial community structure, i.e. “microbial fingerprint”, combined with selected chemical and isotopic tracers, can provide insights into groundwater origin, age, and connectivity within a complex buried valley aquifer system. The hypothesis is that microbial community structures, when integrated with multitracer hydrogeochemical approaches, offer a novel tool for characterizing flow dynamics and aquifer connectivity. These additions explain why this specific suite of tracers and microbiological approaches was selected: to evaluate their applicability and compatibility for aquifer characterization and to support sustainable groundwater management. We believe these revisions address the concern that the study previously appeared as a catalogue of measurements and strengthen the interpretation and relevance of the results. See lines 37-45, 106-113.

2. Insufficient treatment and interpretation of noble gas, CFC/SF6, and 3H/3He data

The tracer dataset contains internal inconsistencies that remain unexplained:

- Ne concentrations exceed air-saturated water (ASW) values by 121–427%, which is far above typical excess-air ranges expected for such aquifers.

Yes, this is an unusual feature. 7 of 10 He samples were measured in duplicates. Duplicates result in identical data. Because of the large 4He concentrations the amount of gas was split in the measurement system to achieve the proper range for calibration of the signals. This resplitting reduces also the amount of Ne in the device and results in slightly larger errors. While typical error for Ne is about 1% for concentrations near equilibrium, the errors increase to about 1% of value received for the He concentration. I.e., sample "R56 bedrock borehole" with the highest 4He concentration of 1E-1ccSTP/kg created an error for Ne of about 1E-4ccSTP/kg which is exceed by far any plausible concentration. Larger errors for Ne the He concentration beyond these indicate some problems with sampling. The Ne concentrations should not be overrated. See modified text l. 351-355, Figure 4., and the whole modified chapter 3.4.

- Some CFC and SF6 values also exceed ASW by large margins.

For the interpretation of CFC and SF6 concentration the amount of excess air is critical. This is usually derived from Ne concentration. Here, this method fails completely and CFC and SF6 data should not be used. Also, SF6 is in general produced in the rock matrix with large rates and should be applied rated here. We have added these notions to the text, 341-350 and the whole modified chapter 3.4, and discussion l. 516-527.

- Dissolved O2 values reach several hundred percent of ASW (23–401%), including values incompatible with waters sampled at depths up to 160 m.

There were errors in the Table 2. We had mistakenly used the oxygen saturation percentage values for the two “supersaturated” readings of dissolved oxygen. The concentration (mg/l) values are for KUU19 **5.65** and for HÄJY11 **3.14**. The confusion is from the fact that the readings from the YSI meter are displayed both as the concentration and as saturation percentage values for dissolved oxygen and from these two sites, the DO% was marked down to field notes. See also discussion about the high Ne excess. The Table 2. has been corrected accordingly.

> • Tritium values are inconsistently reported, and negative 3He* values appear to have been omitted without comment. When applying the unfractionated air (UA) model to the published data, similar 3H/3He results can be reproduced; however, this approach also yields negative 3He* values, which seem to have been excluded from the table without explanation.

Tritiogenic 3He cannot be clearly separated from other 3He sources if 4He concentrations are as large as 5E-4ccSTP/kg. Here data are neglected. Only for samples NOPPA15, KUU19 and HARJA10 3H-3He-age could be derived. We have added a clarifying Figure 4 on the relationship between the 3He/4He and the 20Ne/4He ratios in groundwater from the study site in the manuscript.

> • Such large oversaturations in O2, Ne, CFC, and SF6 could stem from sampling artefacts, air contamination, or analytical issues. If real, they require detailed mechanistic discussion. As presented, the tracer dataset cannot be reliably interpreted.

See the above discussion. We agree with the reviewer that there were major issues with residence time indicators, and we now give explanations to these in the manuscript and advise on careful assessment before using these in the future, see l. 516-524, 667-673.

3. Lack of methodological transparency

More detailed information has been added to the Materials and Methods section (l. 149-152, 192-216) regarding the field sampling, as well as the calculation of tritiogenic and terrigenic Helium.

4. Insufficient integration between microbiological and tracer datasets

The redox conditions as well as all samples being hypoxic (there were two incorrect values for DO in the Table 2.) at each sampling site are now incorporated into the microbiology discussion, with reflection to the potential cycling of specific elements (C, S, Fe, N). However, as there is no knowledge on the speciation of iron and only total sulphur and SO4 have been measured (sulphite concentrations are lacking), in addition to the limits on making conclusions on microbial metabolic activity from the taxonomy, this somewhat hinders the discussion. Nevertheless, we have now combined the microbial diversity data (Shannon H') with the microbial community structure figures (Fig. 5) and plotted the relative abundances of known sulphate reducers in samples with the sulphur isotope data (new Fig. 7) to show the relationship between the samples that hosted significant proportions of sulphate-reducers (e.g., Desulfovibrio, Desulfosporosinus, Desulfurivibrio) and the enrichment of the $\delta^{34}S$ values.

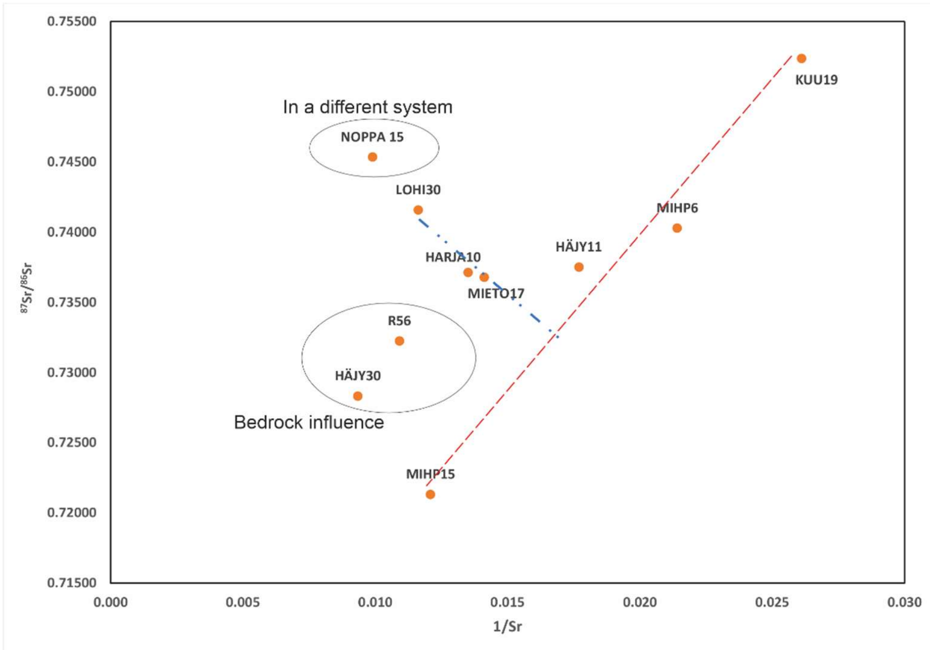
The reviewer's notion on the air contamination can be somewhat relevant when considering the DO measurements. These were done in field conditions, pumping the fluids from the aquifer to the surface, and the measurement, although done immediately from the pumped fluids, some oxygen from atmosphere may have dissolved to the fluid. However, as the DO values show hypoxic conditions and most samples are also on the reductive side, we concluded that the groundwater sampled here is most likely oxygen-depleted. Regarding the notion in the reviewer's Figure 3 about the anaerobic fungi, there are several publications that describe diverse fungal communities in hypoxic or anoxic groundwaters in deep terrestrial subsurface as well as subseafloor crust and sediments (Sohlberg et al. 2015, Drake and Ivarsson 2018, Purkamo et al. 2018, Inkinen et al. 2019, Velez et al. 2022). Nevertheless, despite the limited understanding of fungal metabolic capabilities in groundwater systems (Retter et al. 2024), most taxa we identified belong to saprotrophic lineages that are typically adapted to oligotrophic conditions, may be originating from surface as they are distinct from the deeper bedrock groundwater communities such as those described for example in Sohlberg et al. (2015) and Purkamo et al. (2018).

5. Limited site characterization

Interpreting groundwater flow patterns in this complex, multi-layered aquifer system—partly leaky and partly confined—is challenging outside the recharge zones, which are controlled by bedrock topography. There are two publications describing the conceptual model of the aquifer system referenced (Rashid 2022 and another that has just been published, Åberg et al. 2026 that we have now to the manuscript, l. 45, 124, 130), and two more on the pipeline that provide more detailed information on the site. We have now incorporated details on the suspected flow patterns to the Figure 1 and added the references to the papers to the site description part. These have also been incorporated to the discussion in more detail, l.486…515.

6. Figures and tables require substantial revision

We thank the reviewer for providing the guiding figures as we have now a more comprehensive discussion on the end-members based on strontium analyses and the binary mixing trends. The modified Figure 6 represents assumed mixing trends.



The sample point NOPPA15 is located north of the buried valley site, and the water sample represents a different geochemical environment that just happens to look like a continuation of the blue mixing line. However, it is most unlikely that there is a hydraulic connection between NOPPA15 and the other sampling sites. R56 and HÄJY30 show non-conservative behaviour outside the two mixing lines that suggest three endmembers. The two “outliers” are most likely explained by bedrock groundwater influence in HÄJY30, a groundwater well that has no screen, and R56 being a bedrock groundwater well. This seems to create an additional endmember to the three portrayed in the diagram. In a diagram with the reciprocal of the Sr concentration a binary mixing line draws as a straight line. Here the two dotted lines (red and blue) represent binary mixing trends. The samples near the red dotted mixing line (MIHP15, HÄJY11, MIHP6 and KUU19), represent influence of the Paloluoma buried valley modern/young groundwater. The sample points MIHP15 and MIHP6 are located fairly near the Kyrönjoki valley, but the flow of groundwater in the Paloluoma valley to the north has a volume that overrides the influence from the Kyrönjoki direction (Fig .1 in the manuscript). The remaining samples on the blue mixing line (LOHI30, HARJA10 and MIETO17) represent the Kyrönjoki valley, south of the buried valley system where there is northbound groundwater flow (figure 1). The mixing line does not clearly represent this south-north direction, due to hydraulic connections west of LOHI30.

As this study was conducted with the purpose of extracting information about the aquifer system prior to extensive groundwater extraction, all the isotope geochemistry results can be considered as end-members.

We have also modified figures 1, 5 and tables 1, 2, 4.

7. Interpretation remains descriptive

Without a guiding question or conceptual framework, the manuscript does not progress beyond description. A hydrogeological model relating the measured variables to flow, recharge, mixing, or redox evolution would greatly improve interpretive strength. Some figures and questions are added here which might stimulate some interesting scientific discussion.

We thank the reviewer for raising this important point. While building a full numerical or analytical hydrogeological model is beyond the scope of the present study, we agree that the manuscript benefits from a clearer conceptual framework. We have therefore improved the description of aims of the study in the introduction: establishing baseline hydrogeochemical and microbiological properties for future monitoring (l. 41->), determining water origin and potential mixing using isotopes, and assessing groundwater residence time and recharge periods (l.54->). We also included the need to evaluate the potential of multitracer approach, including the microbial community profiling as one tool to the introduction (l. 74-75, 106-113). We have modified the Figure 1 as mentioned above, reorganized and rewritten the results and discussion to improve the interpretation and incorporated some additional figures.

Additional suggestion

We have now had additional help for interpretation and the practices in describing the methods from the expert that made the noble gas analyses in the first place, Jürgen Sültenfuss from University of Bremen. He is listed now as a coauthor.

Reviewer 3:

The paper by Purkamo et al. is an extensive study of deep granite groundwater in Kurikka, Finland. The study is notable for its analysis of a large number of hydrochemical, geochemical and biological parameters, which distinguishes it from comparable investigations. This comprehensive data collection has the potential to provide valuable insights into the system.

However, the manuscript appears to be in the early stages of development and could benefit from streamlining and more concise data representation. While the data is valuable for characterizing subsurface ecosystems, I would advise against publishing it in its current form.

Major comments:

1) The study contains a wealth of data from a variety of locations, but several aspects of the data analysis and presentation require substantial improvement to enable the reader to follow the relationship between sites and measurements and convey key messages. We recommend that the

authors perform statistical analysis to link their data and identify important trends and distinguishing/similar features between sites. Furthermore, Tables and Figures should be improved for readability and clarity.

We would like to thank the reviewer for their valuable comments and suggestions. We feel that the manuscript has indeed improved and matured during the review process. Major modifications to the narrative and flow of the paper have been made and most figures and tables have also now been improved according to reviewers' comments.

2) The central narrative of the manuscript requires reconsideration. The title and conclusions regarding subsurface connectivity and transport processes are not adequately supported by the data because no direct measurements or modeling of groundwater flow paths, hydraulic connectivity between boreholes, or microbial advection were performed. The study is based on single-time-point sampling without replication which is insufficient to infer connectivity or transport between sites.

We have modified the focus of the manuscript and framed the study as the characterization of the groundwater at Kurikka aquifer system with multiple tracer methods to provide a first look and the baseline for the biogeochemical properties and residence times of groundwater at different watersheds in the complex aquifer system. (See Title, l. 37-45, 106-113, Conclusions). Conceptual groundwater flow model and geological development of the aquifer system are described elsewhere (Rashid 2022, Åberg et al., 2026, Putkinen et al. 2026 in press, upcoming paper by Petre et al 2026.) and are referenced in the Site description chapter 1.1.

3) The general writing of the manuscript should be improved and the introduction and discussion require further integration. In the current form many sections are disconnected and include unnecessary details. Many items are introduced but the connection between topics and the rational for inclusion is missing.

We have now worked on linking of the introduction and discussion and the connection between the different sections of the manuscript, and overall streamlining has been made. We hope that the general writing is now more fluent.

Minor comments:

Title

There is no modeling or measurement of flow path in the paper. Its rather the microbial and hydrogeological characterization of the multi-layered aquifer system in Kurikka, Western Finland.

We have now changed the title to be more coherent with the message of the manuscript: “Combining microbial, isotopic and residence time data to characterize groundwater dynamics in a multi-layered aquifer system in Kurikka, western Finland”.

> Figures and Tables
>
> Figure 1: Colors of the cross section and colors of the legend do not match.

The colours in the legend are referencing to the geological map of the study area. The cross-section colours are artificial and only used to distinguish between the different units of the valley section. These are also described in Åberg et al. 2026 (now referenced in the manuscript, l. 130) and mentioned in Table 1.

> Table 1: Please designate if MM.DD.YYYY or DD.MM.YYYY

We have modified sampling dates in Table 1 according to journal’s instructions.

> Figure 4 and 5: Please remove border around the figures. Missing units on the x axis.

We have omitted these figures from the results section, and new improved versions have been added to discussion, with Sr plot showing the mixing lines and description of NOPPA15 being in another system. See Figure 6.

> Introduction
>
> Line 69: Biological isotope fractionation is a passive process. The slight difference in mass influences e.g. bond length and vibrational entropy within the molecule, making the enzymes work more efficiently with the 'more flexible' light isotope. It's not an active 'decision' of the microbe. Please rephrase.

This has now been rephrased from l. 69->: The most important fractionation process in low temperature unconsolidated environments is the microbial reduction of sulphur, where microbes facilitate the removal of the lighter ^{32}S, increasing the ratio in the dissolved sulphur of the water sample (Onac et al. 2011). Sulphide oxidation (reoxidation), in turn, produces lighter isotopic compositions of sulphur in water samples.

> Line 81: Not all groundwater systems are "cool." Also, "cool" is a subjective description of temperature. Please rephrase.

This has now been rephrased, l. 88->: Groundwater as a dark and often oligotrophic environment with relatively stable temperature selects specific microbial communities compared to the aquatic ecosystems aboveground (Lee et al., 2018). Groundwater habitats vary in geological, chemical and hydrological properties.

Line 94: This does not make sense. In order to survive, many groundwater microbes must reduce oxygen, nitrate, sulfate, or iron and oxidize organic carbon (in the case of heterotrophs) or reduced sulfur, nitrogen, or iron to produce energy for CO_2 fixation (in the case of chemolithoautotrophs).

This has now been modified, l. 98-> : Oxygen availability and pH play a significant role on sulphur and iron cycling as well. Sulphate and iron reduction typically require anoxic conditions, but if oxygen is available, thermodynamics favour oxidation reactions over reductive processes in groundwater. In acidic conditions, iron reducers have a significant advantage over sulphate reduction (Bethke et al., 2011).

Line 106: Please shortly introduce how microbial communities might be of interest for contaminant remediation. This is the first time it is named as an aim of the study.

We decided not to concentrate on the remediation, and thus, removed this from the manuscript.

Line 108: 'The region' or 'This region'

Rephrased, l. 115->: The study site is situated in the Southern Osthrobothnia region in western Finland, 70 kilometres from the Bothnian Sea shore (Fig. 1).

Methods

Line 128: Adding sub-headers to the Materials and Methods section would greatly improve clarity.

We have added sub-headers to the Materials and Methods section: 2.1 Field parameter measurements, geochemical and isotope sampling and analyses (l.147), 2.2 Isotope hydrogeochemistry analysis (l.169), 2.3 Residence time indicator sampling and analysis (l.192), 2.4 Microbiological sampling and analysis (l.226).

Line 137: Would be good if the weather conditions during this sampling were put into a broader context, e.g. did the precipitation differ significantly from the annual average, how variable are temperature and precipitation over a year?

According to statistics from the Finnish Meteorological Institute, the year 2021 was quite typical in terms of average temperature. The nationwide mean temperature was +2.8 degrees Celsius, which is only a tenth of a degree below the long-term average for 1991–2020. In most parts of the country, the annual precipitation was close to or slightly above normal. We decided not to incorporate this information to the manuscript to avoid extensive details, and as we only did one autumn sampling campaign, the seasonal variability remains to be studied.

Lines 224 to 225: Please provide additional information on visualization and statistical methods.

Tools are now mentioned in the text l. 266->: Tools used in RStudio included vegan, ggplot2, dplyr, ampvis2, psadd and cowplot.

Results

Line 235: If the chloride measurement is wrong, it can be excluded.

This has been corrected in the Table 2.

Line 239: “Level of system openness” sounds strange. Connection to surface, maybe? Please rephrase.

This has now been rephrased and moved to discussion (l. 450-458): The partial pressure of CO2 (pCO2) provides insight into the extent to which groundwater interacts with CO_2 produced in the soil (Clark 2015). As water infiltrates the subsurface, it first passes through the unsaturated soil zone, where it equilibrates with carbon dioxide produced by the decomposition of organic matter and root respiration. This results in pCO_2 levels that are higher than those in the atmosphere (Freeze and Cherry, 1979; Appelo and Postma, 2004). Groundwater with a log pCO2 over –2.0 indicates an open CO2 system (Clark 2015). In all water samples representative of unconsolidated aquifer groundwaters, pCO2 values higher than the atmospheric values suggest open-system conditions where groundwater is in equilibrium with the “soil zone” CO2. In contrast, the pCO2 value in bedrock groundwater suggests closed-system conditions, without CO2 input from the soil zone.

Line 240ff: This doesn't seem to belong to the results. Introduction?

This has now been modified and moved to discussion, see above.

Line 245f: Calculations can be explained in the methods section.

This has now been moved to methods section, l.155-156.

Line 282: Units are missing.

These have been added, l. 306.

Line 284: Units are missing.

D-excess has no unit.

Line 309: Section 3.4 is only one sentence. If this data is not relevant for the main storyline it can go to the supplement.

We had divided the residence time indicator results under sub-headings, thus there was only this one sentence under the main heading. We removed the subheadings here and all residence time indicator results are now under the 3.4 subheading, l. 327.

> Line 322: If there are exceptions, it's not "all samples."

This has been modified accordingly, see l. 341-357.

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> Line 232: What is that dissolved oxygen concentration?

This has been added to the text, l. 274: "...highest dissolved oxygen concentrations were measured at KUU19 and HÄJY30 (5,7 and 4,4 mg/l, respectively)". Please note that there was an error on two of the DO measurements, as we had mistakenly used the oxygen saturation percentage values for the two "supersaturated" readings of dissolved oxygen in Table2. The concentration (mg/l) values are 5,65 mg/l for KUU19 and 3,14 mg/l for HÄJY11. The confusion was from the fact that the readings from the YSI meter are displayed both as the concentration and as saturation percentage values for dissolved oxygen and from these two sites, the DO% was marked down to field notes.

> Lines 238 to 245: This information should be moved to methods.

We removed this information about pCO2 from here. We included some discussion about the partial pressure of CO2 in the discussion section(l. 516->).

> Line 344: It is unclear how the read numbers are distributed across the samples.

We decided that that we don't report the exact spread of the reads per sample in the text, but this information is available in the Supplementary Table 2.

> Line 345: Table 5 contains diversity indices not the microbial community data.

This has been changed to reference the correct supplementary material.

> Line 386: This is surprisingly little and it might be good to revisit the bioinformatics pipeline to determine why so little was retained. Chimera removal can lead to relatively large losses for fungal sequences, which might have happened here but this should be clarified.

Thanks for this comment. We went back to the dada2 pipeline and determined that our stringent QC on filter and trimming stage discarded many sequences. A lot of these were reverse reads with poorer quality, but we decided to stick with using the paired-end method and merging the remaining reads, as it is the standard procedure and the most accurate way of working with ASVs. We added an explanation to the manuscript on the issue, l. 410-411)

Discussion

The discussion of fungal and archaeal taxa beyond the biodiversity patterns is missing. A short discussion of their potential role in the studied system would be important.

We have added some discussion on specific archaeal and fungal groups and their potential functionality in relation to geochemistry data to the discussion 4.2 (l. 610->): Archaeal communities were dominated by Woesearchaeales. These have been previously detected from groundwater environments and are believed to thrive in anoxic conditions performing fermentation-based metabolism (Castelle et al. 2015, Liu et al 2018). Similarly, other dominant group Bathyarchaeia, have been associated with complex organic matter degradation and fermentative lifestyle (Hou et al. 2025). In R56 and HARJA10, a significant fraction of the archaeal community affiliated with freshwater anaerobic methane oxidizing archaea Methanoperedenaceae (Haroon et al. 2013), while in MIHP15, archaeal community was clearly dominated by hydrogenotrophic methanogens (Methanoregula, Methanobacterium). The reducing conditions in these samples likely create ecological niches that support the persistence of anaerobic archaea. Despite the limited understanding of fungal metabolic capabilities in groundwater systems (Retter et al. 2024), most taxa identified here belong to saprotrophic lineages that are typically adapted to oligotrophic conditions. It is likely that the fungal communities in Kurikka sites are originating from surface environments, as many taxa are associated with soil, plant litter or phyllosphere inputs (Retter et al. 2024) and are distinct from the deeper bedrock groundwater communities such as those described in Sohlberg et al. (2015) and Purkamo et al. (2018).

Line 422: Please adjust typo: "...have groundwaters also have high TDS..."

This has been fixed, l. 445.

Line 429: This is an interesting connection to above ground activity, and it would be helpful to have better contextualization (potentially in the introduction together with the bioremediation of aquifer contaminants).

We decided to omit the bioremediation aspect from the introduction as well as from here, to keep the focus and to avoid too long discussion.

Lines 432 to 436: This would benefit from better integration into the other data and seems detached from the rest of the discussion.

We have omitted this part of the discussion.

Lines 459 and 460: Please define the "microbial process".

Microbial processes fractionating sulphur isotopes are explained in the following sentence (l.503): Due to the microbial reduction of sulphate, the lighter ^{32}S isotope is removed from the solution (Kaplan and Rittenberg 1964).

> Lines 460 to 466: This would be an interesting place for an introduction of which microbes in the microbial community could be performing sulphate reduction.

We have discussed the sulphate-reducing community detected in the samples below in the section 4.2.1, but added a note here (l. 510).

> Lines 507 to 509: Please expand on the reasons for low Sulfate reducers in the high sulfur well. Also please clearly define which taxa are considered sulfate reducing. This is also true for the iron and nitrogen cycling microbes.

This section has been improved according to these suggestions: The lowest abundance of sulphate reducing bacteria was seen in the water sample from NOPPA15, explaining the low $\delta^{34}S$ values (Fig. 6, 7). Although sulphate is available at NOPPA15, the growth of sulphate-reducing microbes is hindered by the oxic environment. The known sulfate reducers are explained on l. 555 and on Figure 7.

The discussion on iron cycling microbes is also improved and modified, see.l. 570->.

> Line 519ff: If dissolved oxygen is present at all sites, aerobes should be able to grow.

This section has been rewritten, see above.

> Line 546: Interesting, please clarify and cite literature.

Literature is cited here (Bärenstrauch et al., 2022; Zhang et al., 2021), l. 594.

> Lines 565 to 567: Would be an interesting place to expand on the role of nitrogen cycling microbes in contaminant remediation.

We decided to omit the bioremediation aspect from the manuscript to keep the scope clear.

> Line 570: What about a correlation analysis between microbial species and environmental parameters?

We have now mostly rewritten this part of the discussion l. 651->.

For example NMDS and Pearson correlation between microbial community structure and geochemistry were calculated but these did not provide significant clarification to the system characterisation, so we have not incorporated these to the manuscript.

Line 615: In what way are the results relevant for groundwater extraction endeavors? This could be discussed a bit more.

Conclusions part has been rewritten as well, l. 681-> : For example, strontium isotope signatures provide an important reference for identifying mixing of groundwaters in the Kurikka buried valley aquifer system as pumping tests continue and monitoring baseline for the large-scale extraction.

Specific microbial taxa, such as Hydrogenophaga, show promise as indicators of deep bedrock-originating groundwater.