This document includes a detailed point-by-point response to the comments of the reviewers and editor. Our responses are written in blue.

REVIEWER 1

I think the authors have demonstrated a good understanding of processes across a large catchment. I was very interested in this work and the results. Overall, I think the paper is well written, with only a few sentences that need clarification. I think the majority of my comments are detail-oriented, but there are a few key things missing from the paper that I would recommend including in revisions:

- Discussion of the flow conditions during the sampling time period
- Statistics testing the downstream patterns in DOC, TDN, and fluorescence metrics
- More thorough incorporation of the ANOVA/Kruskal-Wallis (K-W) test results
- How did you define where the headwaters, transition zone, and floodplain are? How did you calculate the distance from the glacier?
- Please see my comments on salinity vs TDS vs conductivity – if all three of these are listed separately they should be derived from different measurements, rather than an estimate from a conductivity sensor.

We sincerely thank the reviewer for the positive and encouraging comments on our manuscript. A detailed point-by-point response to the reviewer's comments is provided below.

L43 paragraph – the RCC is an important foundation for this work, but in this paragraph I would make how the variables you measured relate to the RRC more clear.

We thank the reviewer for the comments. The paragraph has been modified to reflect the comment as follows: ¨The River Continuum Concept (RCC) offers a useful framework for interpreting longitudinal patterns in riverine biogeochemistry (Vannote et al., 1980). The RCC postulates that the structure and function of river ecosystems change predictably along the continuum, reflecting shifts in hydrology, energy sources, and biotic interactions (Ortiz Muñoz and Kominoski, 2025; Ruiz-González et al., 2015). Headwaters are typically dominated by allochthonous inputs of organic matter and strong hydrological control, whereas downstream reaches become progressively influenced by in-stream production and transformation processes. These changes may be reflected in the quantity and composition DOM, as terrestrial organic matter inputs are progressively transformed and supplemented by autochthonous production downstream, as well as in nutrient concentrations and availability through uptake, transformation, and remineralization (Graeber et al., 2012; Fovet et al., 2020). 625y, longitudinal changes in metal concentrations and mobility may result from differences in lithology, dilution, tributary inputs, redox conditions, and anthropogenic sources, while microbial diversity and community composition may respond to changes in organic matter quality, nutrient availability, and physicochemical conditions along the river network (Lu et al., 2018; Mayol et al., 2026). Although this conceptual model remains fundamental to limnology, increasing evidence suggests that many contemporary river systems deviate from this idealized continuum due to hydrological regulation, land-use change, and anthropogenic disturbance (Doretto et al., 2020; Roebuck et al., 2020). In catchments, watersheds, reservoirs, tributary inflows, agricultural runoff, anthropogenic activities, and urban pressure can introduce abrupt discontinuities in physicochemical conditions and material transport (Poole, 2002; Ellis and Jones 2013; Stanford and Ward, 1983; Ward and Stanford 2001). These discontinuities may alter DOM composition, nutrient cycling, metal mobility, and microbial

community dynamics, effectively reshaping downstream biogeochemical functioning. As a result, river systems may behave less as linear continua and more as mosaics of interconnected biogeochemical processing zones. Recent conceptual theories, including the river as a chemostat (Creed et al., 2015), the Pulse-Shunt Concept (Raymond et al., 2016), and the bending DOM framework (Casas-Ruiz et al., 2020), increasingly emphasize the importance of hydrological variability, residence time, and localized processing hotspots in controlling riverine carbon and nutrient dynamics along the fluvial gradient¨ (Lines 41-62).

- Ellis, L. E. and Jones, N. E.: Longitudinal trends in regulated rivers: a review and synthesis within the context of the serial discontinuity concept, Environ. Rev., 21, 136–148, https://doi.org/10.1139/er-2012-0064, 2013.
- Fovet, O., Cooper, D. M., Jones, D. L., Jones, T. G., and Evans, C. D.: Dynamics of dissolved organic matter in headwaters: comparison of headwater streams with contrasting DOM and nutrient composition, Aquat Sci, 82, 29, https://doi.org/10.1007/s00027-020-0704-6, 2020.
- Graeber, D., Gelbrecht, J., Pusch, M. T., Anlanger, C., and von Schiller, D.: Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams, Science of the Total Environment, 438, 435–446, https://doi.org/https://doi.org/10.1016/j.scitotenv.2012.08.087, 2012.
- Lu, H. and Yu, S.: Spatio-temporal variational characteristics analysis of heavy metals pollution in water of the typical northern rivers, China, Journal of Hydrology, 559, 787–793, https://doi.org/10.1016/j.jhydrol.2018.02.081, 2018.
- Mayol, E., Aguilar-Muñoz, P., Pérez, M., Pavlov, M. S., Rambaldi, G., Eissler, Y., Molina, V., Sola, I., Sáez, C. A., Cuny, P., Heimbürger-Boavida, L.-E., and Lavergne, C.: Microbial community's shifts in an Andean glacier-fed watershed under anthropogenic pressures, Journal of Environmental Management, 404, 129386, https://doi.org/10.1016/j.jenvman.2026.129386, 2026.
- Poole, G. C.: Fluvial landscape ecology: addressing uniqueness within the river discontinuum, Freshwater biology, 47, 641–660, 2002.
- Stanford and Ward: The serial discontinuity concept of lotic ecosystems, Ann Arbor Science Publishers, 470, 1983.
- Stanford and Ward: Revisiting the serial discontinuity concept, Regulated Rivers: Research & Management, 17, 303–310, https://doi.org/10.1002/rrr.659, 2001.

L52 – Cite some of the serial discontinuity research like Stanford C Ward 1983, 2001; Ward C Stanford; Ellis C Jones 2013; Poole 2002

We thank the reviewer for the literature suggestions. All these references were added: ¨In catchments, watersheds, reservoirs, tributary inflows, agricultural runoff, anthropogenic activities, and urban pressure can introduce abrupt discontinuities in physicochemical conditions and material transport (Poole, 2002; Ellis and Jones 2013; Stanford and Ward, 1983; Ward and Stanford 2001)¨ (Lines 54-57).

L84 – make sure you define a.s.l.

It has been defined accordingly as follows: ¨above sea level¨ (Line 92).

L89 – this isn't clear – is 310 km the entire length of flowpath? Or this distance between the

dam and the ocean?
To clarify, we restructured the sentence as: ¨The Rapel catchment is formed by the confluence of the Cachapoal and Tinguiririca rivers and drains westward into the Rapel Reservoir (artificial reservoir) and then via the Rapel River it reaches the Pacific Ocean at Navidad. The total fluvial length from the headwaters to the mouth is approximately 310 km¨ (Lines 95-97).

L95 – I'm assuming these average annual discharges are at the confluence of the two rivers? Please specify where if not.
Yes, it is.

L96 – reunited or just united?
We have changed it to ¨united¨ (Line 104).

L100 – I'm not sure that "at lower elevations" is specific enough to describe where these native vegetation communities are, as from my understanding, the agricultural areas are at lower elevation from these.
We thank the reviewer for this comment. Some details have been added to be more specific. Specifically, we wrote ¨Below 2,000 m a.s.l., native vegetation communities predominate along the Andean slopes and foothills, including forest and grazing lands (~25%)¨ (Lines 108-109).

L104 – Do you have a source describing the metal inputs form the mines?
To support our statement, we have added the following information and literature: ¨The catchment also hosts significant mining operations, particularly in the upper area of the Cachapoal River (>0.5%), where the largest underground copper mine is located, which contributes to metal inputs and potential contamination (Copaja et al., 2016; Lacassie and Ruiz del Solar, 2021)¨ (Lines 111-114).

- Copaja, S. V., Nuñez, V. R., Muñoz, G. S., González, G. L., Vila, I., and Véliz, D.: Heavy metal concentrations in water and sediments from affluents and effluents of mediterranean chilean reservoirs, Journal of the Chilean Chemical Society, 61, 2797–2804, https://doi.org/10.4067/S0717-97072016000100011, 2016.
- Lacassie, J. P. and Ruiz-Del-Solar, J.: Integrated mineralogical and geochemical study of the Rapel fluvial system, central Chile: An application of multidimensional analysis to river sedimentation, Journal of South American Earth Sciences, https://doi.org/10.1016/j.jsames.2021.103289, 2021.

L111 – Are sies R-R1 and R-R2 reservoir samples?
Yes, R-R1, R-R2, and R-R3 are the three samples from the reservoir, as it is shown in Fig. 1.

Fig 1 – This map is clear visually, and I like that it shows the elevation, since this is a key part of how you define the different zones within the catchment. However, I do think it may be helpful to try to include the land use data in the main text (e.g. SI Fig 1). I recognize this may not be possible to incorporate into the Fig. 1 map without negatively impacting the legibility. However, it may be helpful to include high level annotations of the cities, the Rapel reservoir, and maybe even outlines of the land use zones. Ultimately I think this edit is a matter of personal opinion, but at the very least I would define the headwater, transition zone, floodplain

on the map.

We appreciate all of the reviewer's suggestions and have addressed most of them. Specifically, we have added the locations of the main cities, identified both reservoirs, and delineated the headwaters, transition zone, and floodplain areas on the map. However, we could not incorporate land-use information, as doing so would negatively affect the readability of the map.

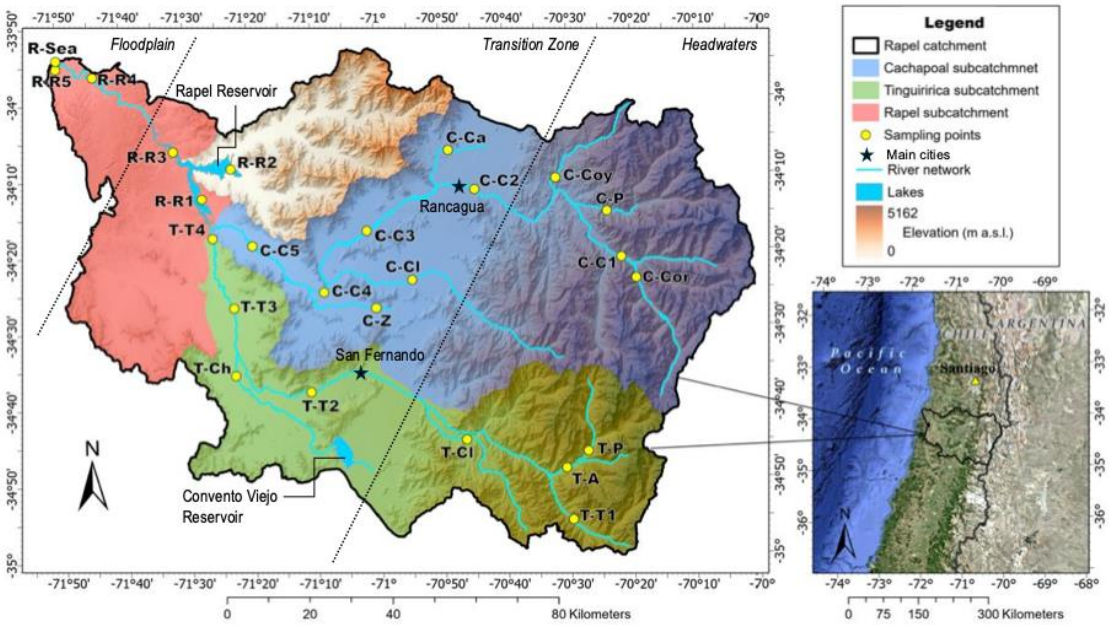


Fig 1 – What is this other lake upstream of T-Ch? On the map it looks to be about the same size as the Rapel Reservoir by surface area.

The other lake upstream of Chimbarong Creek is another artificial Reservoir (Convento Viejo Reservoir, see Fig. 1). Convento Viejo is an artificial reservoir primarily used for irrigation, with a storage capacity of approximately 237 million m^3, which is considerably smaller than the Rapel Reservoir (approximately 695 Mm^3, line 104).

L 123 – What were the flow conditions during your period of sampling? Did you do anything to make sure flow was consistent across days? How do you think that sampling during snowmelt influenced your results?

The upper-catchment sites were sampled over two consecutive days, after which we followed the planned sampling sequence from the transition zone to the lower catchment and floodplain. Thus, the sampling was conducted over a relatively short period and following a consistent spatial schedule. Although snowmelt likely influenced flow conditions in the upper catchment, its influence was progressively reduced downstream, particularly below the Rapel Reservoir, where flow is regulated. We therefore considered the sampling period representative of the natural spatial gradient during spring. Snowmelt may have influenced our results by modifying the transport and dilution of dissolved and particulate constituents, as well as by mobilizing and introducing new material into the upper reaches. However, assessing the specific influence of snowmelt would require sampling under different hydrological conditions throughout the year. Therefore, while we acknowledge its potential influence, our study was not designed to isolate this effect.

L124- Based on your variation in temperature in the results, I would recommend reporting your conductivity values as specific conductance.

We thank the reviewer for the suggestion. The reported conductivity values correspond to specific conductance (i.e., conductivity normalized to 25 °C). We have clarified this terminology as follows: ¨Physico-chemical parameters (temperature, pH, dissolved oxygen (DO), and electrical conductivity normalized to 25 °C (EC) were measured in situ at each site using a Hanna HI 98194 multiparametric probe (Hanna Instruments, Italy)¨ (Lines 141-143).

L144- Did you do an inner filter effect correction? If so, make sure it's listed here.

We thank the reviewer for raising this issue. To correct the EEMS for Inner Filter Effect we considered the threshold value of absorbance of 0.042 (in a 1 cm cuvette) as recommended by Kothawala et al. (2013). None of our samples exceeded this value; therefore, no Inner Filter correction was made.

- Kothawala, D. N., Murphy, K. R., Stedmon, C. A., Weyhenmeyer, G. A., and Tranvik, L. J.: Inner filter correction of dissolved organic matter fluorescence, Limnology and Oceanography: Methods, 11, 616–630, https://doi.org/10.4319/lom.2013.11.616, 2013.

L204 – Unless I missed it, I didn't see salinity mentioned in your methods? Is this in parts per thousand?

The reviewer is right. This information was missing. However, as suggested in the following comments, we decided to remove the salinity and TDS values because they were indirectly derived from conductivity measurements.

L210 – Is the conductivity measurement coming from the same sensor as the salinity measurement? Same with the TDS measurement. I am not familiar with the Hanna probe, but I know with some instruments conductivity, salinity, and TDS all come from the same conductivity sensor. If so, I would report only one.

We thank the reviewer for the comment. Indeed, conductivity, salinity, and TDS measurements were obtained using the same conductivity sensor of the Hanna probe, with salinity and TDS being calculated from the conductivity measurement. Therefore, following the reviewer's suggestion, we decided to retain only the conductivity values and remove the salinity and TDS values from the manuscript (Sections 2.2 and 3.1 and Table S2).

L212 – elevated compared to what?

For clarification, we have added the text ¨as relatively elevated values (e.g., >500 µS/cm)¨ (Line 239).

L213 – Did you test this EC pattern with the ANOVA/K W?

The EC pattern we observed was tested by the Kruskal-Wallis test since the data did not follow a normal distribution. We then performed a Wilcoxon rank-sum exact test for pairwise comparison, confirming the observed EC pattern. The manuscript was modified in the Methods and Results sections as follows:

In Materials and Methods:

¨Significant differences of physicochemical parameters among subcatchments were assessed

using Kruskal Wallis test due to non-normal distribution of the data, followed by a Wilcoxon rank sum exact test for pairwise comparison using the Holm method for p-value adjustment. Significant trends along the watercatchment were determined using the spearman correlation between the distance from the glacier base and the tested parameter; note that spearman correlation was chosen after a multivariate normality test (mvn function, 'MVN' package; Korkmaz et al., 2014). A PERMANOVA was used to identify the significant difference of the metal and metalloids studied among the different subcatchments. The dispersion within groups was tested using the betadisper function ('vegan' package; Oksanen et al., 2007) and pairwise multilevel comparison were assessed using the pairwise.adonis function ('pairwiseAdonis' package; Arbizu, 2020) with the Bonferroni p-value correction¨ (Lines 199-205).

- Korkmaz S, Goksuluk D, Zararsiz G. MVN: An R Package for Assessing Multivariate Normality. The R Journal. 2014 6(2):151-162.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. A. S. S. (2007). The vegan package. Community ecology package, 10(631-637), 719.
- Arbizu, M. (2020). pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version.

In Results:
¨The Tinguiririca subcatchment significantly displayed the lowest EC values (Pairwise comparisons, $P < 0.05$)¨ (Line 240).

L214 – What do you mean here by continental stations?
This part of the text has been removed, as it referred to the TDS values.

Section 3.1. and 3.2. – Here you have mostly qualitative descriptions of the data, it would be good to incorporate the ANOVA/K-W in Section 3.1. and apply stats to say if something was actually significantly increasing or decreasing along the flowpath in Section 3.2.
We agree with the comment. When testable (i.e., according to the number of samples per category) and significant, the statistical analysis has been added to the main text. We also added significant trends along the flowpath using the distance from the glacier base as a proxy. The statistical procedure has been added to the "Material and Method" section (see previous comment) and the results of the tests have been added in sections 3.1 and 3.2 as follows: ¨The Tinguiririca subcatchment significantly displayed the lowest EC values (Pairwise comparisons, $P < 0.05$)¨ and ¨The lowest DOC concentrations were observed in samples collected in the Andes Mountains, with concentrations significantly increasing downstream (Fig. 2a; Spearman correlation with the distance from the glacier: $r = 0.79$, $P < 0.05$ Fig S3)¨ (Lines 240, 245-247).

Figure 2 – How did you define distance from glacier base and the locations of the transition zones? You don't discuss glaciers in your site description, where are they? What glacier are you using for the downstream sites (e.g. Rapel), where presumably there are multiple glaciers in the headwaters? Are the tributaries plotted based on their confluence with the main stem? These same questions go for Figure 3.
We understand that it can be confusing. The glacier's base locations were defined by looking at satellite images and have been used as a reference starting point for the two Tinguiririca

and Cachapoal, which originate from the corresponding glaciers. We chose this reference method instead of using, for example, coordinates for better readability of the figures and to highlight the short distance between the headwaters and the ocean and the short distance between sampling locations. The Rapel Reservoir main inputs are the Cachapoal and Tinguiririca rivers, and the distance between the two "glacier base" and the Rapel Reservoir is approximately the same (140 - 145 Km); we therefore considered it to be 145 Km downstream from both glaciers, following the river continuum.
The tributaries' locations are plotted based on their confluence in the main rivers.
These details have been added in the main text as follows: ¨To represent the spatial dynamics of the measured parameters in a reader-friendly representation, a reference site was chosen as the spring of the Tinguiririca and Cachapoal rivers according to the lowest reach of the two glaciers that originate them (located from satellite images), in the results, some data are therefore presented as a function of the distance from the glacier base (Km). The main tributaries are plotted in correspondence with their confluence with the main river. The confluence of the two rivers into the Rapel Reservoir is approximately at the same distance from the two glaciers (140 - 145 Km), it was therefore decided to consider it to be 145 Km distant from both glaciers¨ (Lines 127-132).

L264 – again, I'm looking for some sort of statistical trend analysis here. I agree with your qualitative descriptions of these patterns, but it would be good to look at the significance of the downstream trends.
We performed the statistical analyses to test the significance of the trend, and no significant correlation was found in this particular case. For this reason, we maintained the text as it was. A correlation matrix has been added as a Supplementary figure (Fig. S3).

Fig 3 – I think there's a discrepancy between your units in Figure 3 (x102 R.U. uM-1) vs in the text (R.U. uM-1).
We thank the reviewer for catching this oversight. We have corrected all the units and added a sentence to explain the unit used. It was changes as follows: ¨To improve figure readability and sample's fluorescence data easily comparable, the values of the normalized fluorescence was multiplied by 10^2 and therefore reported as 10^{-2} R.U. μM^{-1}¨ (Lines 289-291).

L297 – I don't think that you have evidence here to "confirm" that the copper concentrations are due to the mining; perhaps you can say "potentially related"
As suggested by the reviewer, we have changed it to: "Within the Cachapoal subcatchment, C-Coy was characterized by elevated concentrations of Cu (4.710 ppm), potentially related to the upstream copper mining activities in this tributary¨ (Lines 325-326).

L300 – What do you mean by "lower" and "intermediate" metal concentrations? Did you use the ANOVA/K-W test here to compare the subcatchments?
In order to clarify and support our statements, a PERMANOVA was performed associated with a pairwise multilevel comparison. The results were added in a supplementary table (Tables S3 & 4) and cited in the main text in section 3.4 (methods are detailed in the Materials and Methods section as reported above).

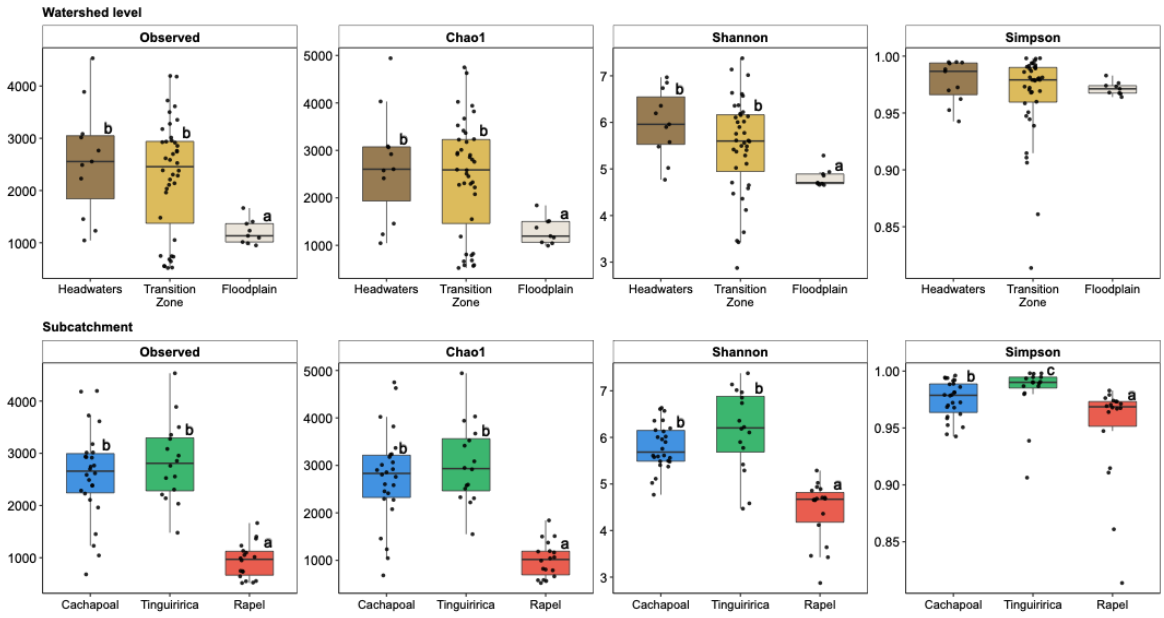
L320 – By "lower zone" do you mean the Rapel subcatchment? I would make sure you're referring to these zones consistently throughout. I think how you break downs the site groups

in Figure 4 is clear, and can be a guiding example throughout the text. I think what's missing in the map is the Headwaters/Transition Zone/Floodplain.

We thank the reviewer for this relevant comment. For consistency, we have decided not only to add the Headwaters/Transition Zone/Floodplain zonation to Figure 1, as already shown in Figures 2, 3, and 4, but also to specify this zonation in the text.

Figure 5 – Can you visually add the results here from your ANOVA/K-W test? E.g. labeling the significant groups a/b/c (this website contains examples: https://statdoe.com/one-way-anova-and-box-plot-in-r/)

We thank the reviewer for this very helpful suggestion. The significant groups have been labelled accordingly.

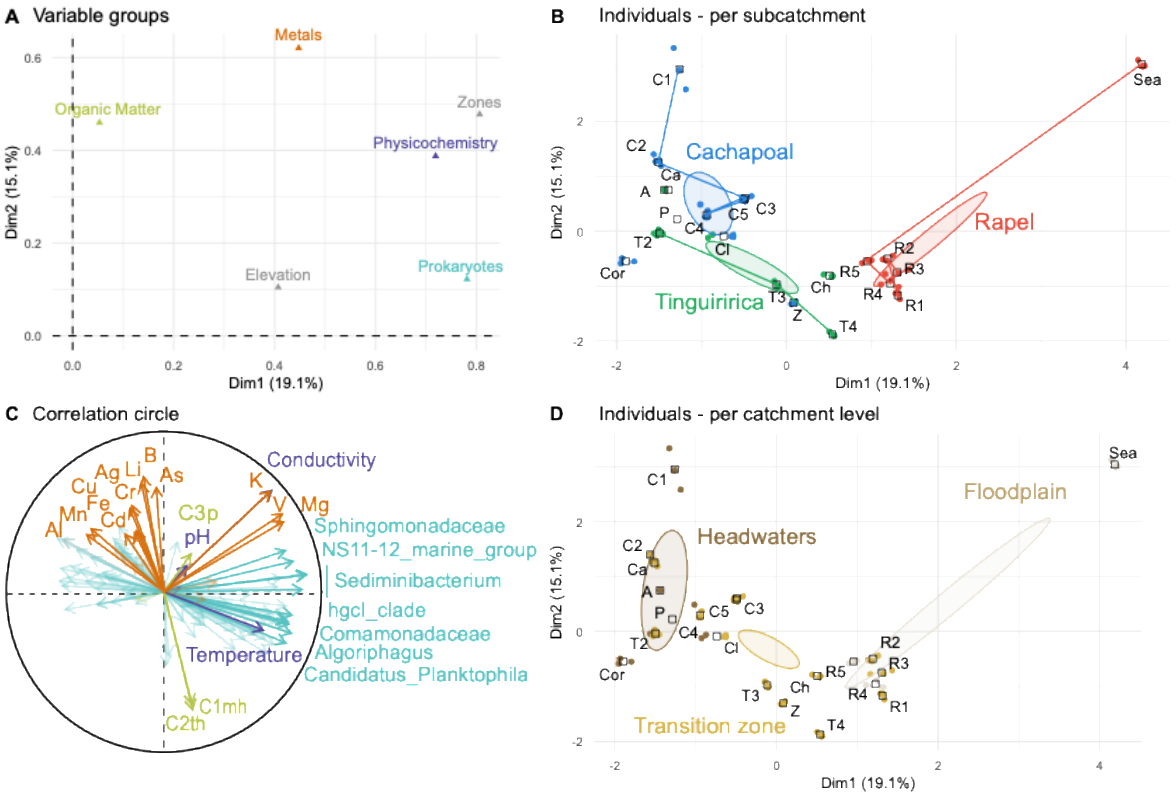


L356-358 – I think this relationship is a really interesting finding.

We also believe that this finding is very interesting, and this has been revealed by our innovative use of the MFA analysis. For the same reason, we went deeper in the discussion section into this finding.

Figure 6 – are the replicates analytical replicates? This wasn't discussed previously. Also, are the tributaries included in here? Since you made a distinction with the tributaries in figures 2 and 3 you may also consider doing it here, where you can trace site by site.

In the MFA analysis, we used field replicates. We added the information in the Material and Method section as follows: ¨A multiple factor analysis (MFA) was performed (MFA function, 'FactomineR' package; (Husson et al., 2013)) to explore the relationship between microbial composition, metals and metalloids, environmental variables, and components of DOM; field triplicates were used as observations (n = 60)¨ (Lines 215-217). Additionally, the analysis included the tributaries, and according to the suggestion of the reviewer, we added the path traced site by site as shown in Figures 2 and 3 in Figure 6.



L380-382 What do you mean by "different y-axis scales between the protein components and the humic ones"?

We thank the reviewer for pointing out this sentence. We wanted to highlight the marked difference in the range of fluorescence of the protein-like components compared to the humic ones. The sentence was modified as follows: ¨more than 10 times higher fluorescence intensities of the protein-like components compared to the humic ones¨ (Lines 413-414).

L393 – why would the volcanic environment lead to more protein-like DOM?

We thank the reviewer for raising this question, as we failed to discuss it properly in the manuscript. The discussion was expanded as follows: ¨These previous studies attribute the high protein-like fluorescence to biological production (i.e., chemosynthesis) or abiotic factors that are promoted by the elevated temperature. The humic-like compounds might instead be more easily thermally degraded (Tedetti et al., 2026). Higher values of the BIX fluorescence index, indicating a higher contribution of in-situ produced DOM, were also found in a volcanic site compared to a reference area (Retelletti Brogi et al., 2019). Abiotic production of DOM at increasing temperature was also demonstrated in laboratory-controlled experiments (Lin et al., 2017)¨ (Line 428-433).

- Lin, Y.-S., Koch, B. P., Feseker, T., Ziervogel, K., Goldhammer, T., Schmidt, F., Witt, M., Kellermann, M. Y., Zabel, M., Teske, A., and Hinrichs, K.-U.: Near-surface Heating of Young Rift Sediment Causes Mass Production and Discharge of Reactive Dissolved Organic Matter, Sci Rep, 7, 44864, https://doi.org/10.1038/srep44864, 2017.

- Retelletti Brogi, S., Kim, J.-H., Ryu, J.-S., Jin, Y. K., Lee, Y. K., and Hur, J.: Exploring sediment porewater dissolved organic matter (DOM) in a mud volcano: Clues of a thermogenic DOM source from fluorescence spectroscopy, Marine Chemistry,

https://doi.org/10.1016/J.MARCHEM.2019.03.009, 2019.

- Tedetti, M., Guigue, C., Mahieu, L., Martinot, P. L., Benavides, M., Dupouy, C., Nunige, S., Pulido-Villena, E., Dimier, C., Tilliette, C., Bonnet, S., Guieu, C., and Lefèvre, D.: Dissolved organic matter (DOC, CDOM, FDOM) in the western Tropical South Pacific: Depth- and subregion-resolved variability, and hydrothermal influence, Progress in Oceanography, 242, 103664, https://doi.org/10.1016/j.pocean.2025.103664, 2026.

L397 – what are the common characteristics specifically?

To address any remaining doubts, we have added the following information: ¨commonly associated with relatively undisturbed Andean headwaters, including limited anthropogenic influence and strong controls exerted by lithology, cryospheric processes, and mountain hydrology on water chemistry and microbial organization, as also observed in other high-mountain regions(e.g., Alpine and Tibetan headwaters) (Brighenti et al., 2026; Garcia et al., 2018; Hotaling et al., 2017; Maurischat et al., 2022)¨ (Lines 437-442).

- Brighenti, S., Delpero, M., Bearzot, F., Bertolotti, G., Tolotti, M., Bruno, M. C., Fischer, A., Winkler, G., Voto, G., Aguzzoni, A., Tirler, W., and Comiti, F.: Cryosphere and lithology influence the hydrological gradients of high elevation Alpine catchments, CATENA, 263, 109676, https://doi.org/10.1016/j.catena.2025.109676, 2026.

-Garcia, R. D., Diéguez, M. del C., Gerea, M., Garcia, P. E., and Reissig, M.: Characterisation and reactivity continuum of dissolved organic matter in forested headwater catchments of Andean Patagonia, Freshwater Biology, 63, 1049–1062, https://doi.org/doi:10.1111/fwb.13114, 2018.

- Hotaling, S., Hood, E., and Hamilton, T. L.: Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections and implications of a warming climate, Environmental Microbiology, 19, 2935–2948, https://doi.org/10.1111/1462-2920.13766, 2017.

- Maurischat, P., Lehnert, L., Zerres, V. H. D., Tran, T. V., Kalbitz, K., Rinnan, Å., Li, X. G., Dorji, T., and Guggenberger, G.: The glacial–terrestrial–fluvial pathway: A multiparametrical analysis of spatiotemporal dissolved organic matter variation in three catchments of Lake Nam Co, Tibetan Plateau, Science of The Total Environment, 838, 156542, https://doi.org/10.1016/j.scitotenv.2022.156542, 2022.

L407-408 – If this were a pristine catchment, would you still expect terrestrial OM to accumulate along the continuum?

We thank the reviewer for the comment. Yes, even in a relatively pristine catchment, terrestrial OM can naturally accumulate in aquatic systems, including in headwaters. However, Lines 407-408 refer specifically to the Transition Zone, which is a distinct section of the subcatchment. Therefore, the discussion of terrestrial OM in the Transition Zone does not refer to the pristine/headwater zone.

L419-423 – Can you link some of these differences between the reservoir to other studies comparing reservoirs vs streams? I would expect them to operate pretty differently.

We thank the reviewer for pointing out this issue. We reviewed the literature for studies addressing the influence of dams and reservoirs, but found that most focus exclusively on the reservoir itself rather than comparing conditions upstream and downstream. Many of these studies also focus on drinking-water production or sediment dynamics within the reservoir,

making direct comparisons with our results difficult. Nevertheless, we identified a few published studies reporting an increase in DOC from the upstream river to the reservoir, as well as changes in CDOM. We have therefore added a sentence to the text referring to these studies. To reflect the comment, we added the following information: ¨This dynamic has been observed in other regulated rivers where the presence of dams resulted in an increase in DOC concentration and/or a higher relative abundance of in-situ produced DOM such as protein-like fluorescence (Acharya et al., 2023; He et al., 2025; Zhuang et al., 2023)¨ (Lines 467-469).

- Acharya, S., Holland, A., Rees, G., Brooks, A., Coleman, D., Hepplewhite, C., Mika, S., Bond, N., and Silvester, E.: Relevance of tributary inflows for driving molecular composition of dissolved organic matter (DOM) in a regulated river system, Water Research, 237, 119975, https://doi.org/10.1016/j.watres.2023.119975, 2023.

- He, D., Wang, W., Yi, Y., Zhong, J., Mostofa, K. M. G., Hu, X., Shi, W., He, D., and Li, S.-L.: Cascading river damming amplifies photosynthetic organic matter production and DOC transport, Water Research, 284, 124036, https://doi.org/10.1016/j.watres.2025.124036, 2025.

- Zhuang, W.-E., Chen, W., and Yang, L.: Coupled effects of dam, hydrology, and estuarine filtering on dissolved organic carbon and optical properties in the reservoir-river-estuary continuum, Journal of Hydrology, 617, 128893, 2023.

L427 – Reference indicating these taxa are reflective of these conditions.

We thank the reviewer for this suggestion, and we added three references to support our statement (Line 475):

- Cabello-Yeves, P. J., Picazo, A., Camacho, A., Callieri, C., Rosselli, R., Roda-Garcia, J. J., Coutinho, F. H., and Rodriguez-Valera, F.: Ecological and genomic features of two widespread freshwater picocyanobacteria, Environmental Microbiology, 20, 3757–3771, 2018.

- Pitt, A., Lienbacher, S., Schmidt, J., Neumann-Schaal, M., Wolf, J., and Hahn, M. W.: Description of a new freshwater bacterium Aquirufa regiilacus sp. nov., classification of the genera Aquirufa, Arundinibacter, Sandaracinomonas, and Tellurirhabdus to the family Spirosomataceae, classification of the genus Chryseotalea to the family Fulvivirgaceae and Litoribacter to the family Cyclobacteriaceae, as well as classification of Litoribacter alkaliphilus as a later heterotypic synonym of Litoribacter ruber, Archives of Microbiology, 206, 79, 2024.

- Qiu, Y.-L., Tourlousse, D. M., Matsuura, N., Ohashi, A., and Sekiguchi, Y.: Draft genome sequence of terrimicrobium sacchariphilum nm-5t, a facultative anaerobic soil bacterium of the class spartobacteria, Genome Announcements, 5, 10–1128, 2017.

Paragraph at Line 401 – Can you break this up into shorter paragraphs with some overview sentences? Currently it is hard to digest in one long paragraph. Maybe have one paragraph describing what is going on in each subcatchment/zone?

As suggested by the reviewer, we have decided to restructure this paragraph to make it easier to read and understand. The changes are as follows: ¨Moving downstream, the transition zones of both subcatchments showed the clearest influence of anthropogenic land use on the river's biogeochemistry. These sectors were characterized by the accumulation of nutrients, dissolved ions, metals, and organic matter, together with shifts in microbial community composition. Elevated NO_3^- concentrations coincided with increases in DOC, TDN, EC, and dissolved metal concentrations, particularly downstream of tributaries influenced by agricultural activities (Fig. S1). For example, the Cadenas tributary (C-Ca) exhibited elevated phosphorus

concentrations (Table S2), likely associated with agricultural runoff and diffuse inputs from surrounding cultivated areas. These chemically distinct tributaries disrupted simple longitudinal gradients and generated localized biogeochemical “hotspots” within the river network. In parallel, the transition sectors were characterized by a progressive increase in the C2th* humic-like component (Fig. 3b), reflecting the accumulation of terrestrial organic matter along the river continuum.

The influence of these transition zones was also evident in the microbial communities, although the response differed between the two subcatchments. The simultaneous enrichment in nutrients, dissolved ions, and DOC in these impacted sectors was accompanied by clear shifts in microbial community composition relative to the upstream headwaters. The Cachapoal subcatchment, for instance, was characterized by the occurrence of the Betaproteobacteria family Neisseriaceae within the Cadenas tributary (C-Ca), a bacterial group that can be found in freshwater environments but which also includes genera associated with wastewater and anthropogenic contamination (de Santana et al., 2025). In the present case, its occurrence may be linked to effluent discharges from the wastewater treatment plant located a few kilometers upstream. In contrast, the Tinguiririca subcatchment showed a more pronounced shift in microbial organization, characterized by the increased abundance of hgcI_clade and Terrimicrobium. Terrimicrobium has frequently been associated with surrounding agricultural soils and may reflect terrestrial inputs into the aquatic system, whereas hgcI_clade represents one of the most abundant and ubiquitous groups of free-living planktonic bacteria in inland waters worldwide (Wang et al., 2024). Its high relative abundance in these sectors may also reflect the changing nutrient conditions along the transition zone (Ghylin et al., 2014).

Further downstream, the Rapel Reservoir represented a major biogeochemical transition zone within the catchment, where hydrological retention and in-reservoir processing altered the composition and distribution of organic and inorganic constituents. DOC reached its highest concentration within the reservoir, whereas humic-like fluorescence decreased relative to upstream sectors, indicating substantial in-reservoir transformation of DOM, with removal of the terrestrial humic-like fraction through processes such as photochemical degradation and microbial remineralization. This dynamic has been observed in other regulated rivers where the presence of dams resulted in an increase in DOC concentration and/or a higher relative abundance of in-situ produced DOM, such as protein-like fluorescence (Acharya et al., 2023; He et al., 2025). Simultaneously, microbial community composition shifted toward the dominance of hgcI_clade and an increased presence of lacustrine-associated taxa such as CL500-29_marine_group (Wang et al., 2024). The microbial composition within the reservoir was similar to that of the Tinguiririca transition zone, suggesting a strong microbial and biogeochemical influence from the Tinguiririca inflow. The local enrichment of taxa such as Terrimicrobium, Sandaracinomonas, and Cyanobium within the transition sectors likely reflects the increasing influence of nutrient-enriched conditions, total dissolved solids, longer water residence times, and flow regulation associated with agricultural landscapes. In parallel, the accumulation of major ions and several dissolved elements at R-R2 (Table S2) suggests that hydrological retention and internal biogeochemical cycling contribute to solute enrichment within the lacustrine system, potentially due to external inputs from the Alhué stream. This is a small stream originating outside the Rapel catchment that flows into the Rapel Reservoir next to the R-R2 sampling site.

Downstream of the reservoir, the floodplain and estuarine sectors reflected the continuing influence of these major hydrological and biogeochemical transitions, while also showing

distinct downstream characteristics. The floodplain samples generally reflected the reservoir pattern for most biogeochemical variables. By contrast, the estuarine interface displayed a unique fingerprint associated with seawater input (Anderson and Harvey, 2022; Zhang et al., 2025). Together, these patterns indicate that the reservoir and its upstream transition zones act as areas of intensified ecological and biogeochemical transformation within the aquatic continuum, decoupling local biogeochemical dynamics from simple upstream–downstream transport¨ (Lines 442-486).

L431 – have there been any studies at the Alhue stream showing it has high concentrations and can be a source? Is this stream the orange/beige part of the map?
We thank the reviewer for this comment. To our knowledge, there are no specific studies demonstrating that the Alhue stream is a source of high concentrations of these compounds. We have therefore modified the text to refer to the Alhue stream as a "potential" source rather than a confirmed source. The Alhue stream belongs to the broader Rapel catchment but corresponds to a separate subcatchment and was not included in the river network investigated in this study. Therefore, it is not represented on the map.

L461- Be more specific here than "upper Andean sectors" – do you mean you observed more variability in these upper areas?
To address the reviewer's comment, we have revised the sentence as follows: "Our results from the entire Rapel catchment partially support these expectations, particularly in the upper Andean headwaters, but beyond these upper reaches, the system departed markedly from the progressive and predictable longitudinal organization proposed by the RCC¨ (Lines 510-513).

L475 – Even though this type of headwater to ocean study is uncommon, there must be studies looking at how dams impact the downstream properties of organic matter. Are your results typical?
We agree with the reviewer that these specific dynamics can be discussed and compared with other studies, even though we feel that this comparison is better fitted in paragraph 4.1. The following part was added: ¨This dynamic has been observed in other regulated rivers where the presence of dams resulted in an increase in DOC concentration and/or a higher relative abundance of in-situ produced DOM such as protein-like fluorescence (Acharya et al., 2023; He et al., 2025; Zhuang et al., 2023)¨ (Lines 467- 469).

L493-495 and L 511-516– Do you have references for these statements? I don't think that these statements (particularly regarding changes to climate/hydrology) are clearly supported by your results, which measured only one time period.
We thank the reviewer for the comment. We have revised the text to distinguish our results from broader climate and hydrological trends, which are now supported by relevant references, and clarified that these longer-term changes cannot be assessed from our dataset. The changes are as follows: ¨Finally, the observed dynamics within the Rapel catchment highlight the potential sensitivity of Mediterranean-Andean rivers to the combined influence of land-use and hydrological pressures, and suggest how these systems respond to increasing environmental pressures. Within our study area, biogeochemical patterns varied along the catchment in association with differences in land use, with some sites showing distinct characteristics in areas where specific anthropogenic activities occur (e.g., Coya and Cadenas Creeks, C-Coy and C-Ca). These observations are consistent with previous studies

highlighting the importance of land use as a key factor shaping riverine biogeochemistry (Williams et al., 2010; Maurischat et al., 2023). Beyond these local pressures, regional changes in climate and hydrology may further modify the functioning of such systems. Declining snow cover and glacier retreat have been documented in the Central Andes, together with changes in river discharge and snowmelt contributions (Ohlanders et al., 2013; Ragetti et al., 2016; Masiokas et al., 2020). Although these longer-term climatic and hydrological changes cannot be assessed directly from our dataset, they provide an important context for considering how future changes in water availability may interact with existing land-use pressures and reservoir regulation in Mediterranean-Andean rivers¨ (Lines 543-554).

- Masiokas, M. H., Rabatel, A., Rivera, A., Ruiz, L., Pitte, P., Ceballos, J. L., Barcaza, G., Soruco, A., Bown, F., Berthier, E., Dussaillant, I., and MacDonell, S.: A Review of the Current State and Recent Changes of the Andean Cryosphere, Frontiers in Earth Science, 8, 2020.

- Maurischat, P., Seidel, M., Dittmar, T., and Guggenberger, G.: Complex dissolved organic matter (DOM) on the roof of the world – Tibetan DOM molecular characteristics indicate sources, land use effects, and processing along the fluvial–limnic continuum, Biogeosciences, 20, 3011–3026, https://doi.org/10.5194/bg-20-3011-2023, 2023.

- Ohlanders, N., Rodriguez, M., and McPhee, J.: Stable water isotope variation in a Central Andean watershed dominated by glacier and snowmelt, Hydrology and Earth System Sciences, 17, 1035–1050, https://doi.org/10.5194/hess-17-1035-2013, 2013.

- Ragettli, S., Immerzeel, W. W., and Pellicciotti, F.: Contrasting climate change impact on river flows from high-altitude catchments in the Himalayan and Andes Mountains, Proceedings of the National Academy of Sciences, 113, 9222–9227, https://doi.org/10.1073/pnas.1606526113, 2016.

- Williams, C. J., Yamashita, Y., Wilson, H. F., Jaffé, R., and Xenopoulos, M. A.: Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems, Limnology and Oceanography, 55, 1159–1171, https://doi.org/10.4319/lo.2010.55.3.1159, 2010.

General questions for the discussion -

How does the rain/snow regime influence the differences you might see between the upland headwaters and further downstream, particularly since you sampled during a time period with snow runoff?

We thank the reviewer for these general and relevant questions. The snow runoff may contribute to the biogeochemical differences observed between the upper Andean headwaters and the downstream transition and floodplain zones by regulating the timing and magnitude of water and material inputs. Importantly, this sampling was conducted shortly after the onset of snowmelt according to DGA hydrological records (The General Water Directorate, https://mapas2.mop.gob.cl/). The distinct biogeochemical characteristics observed in the upper headwater zone compared with the transition and floodplain sectors may therefore partly reflect the influence of early-season snowmelt and the associated hydrological connectivity between cryosphere-influenced headwaters and downstream reaches. However, because we did not sample before and throughout the snowmelt period, we cannot directly quantify its contribution to the patterns observed. Downstream characteristics likely also reflect the cumulative influence of tributary inputs, land use, and reservoir regulation. Thus, our results should be interpreted as a snapshot of catchment biogeochemistry under early snowmelt conditions rather than as a direct assessment of seasonal snowmelt dynamics.

We should add that the project that allowed us to carry out this sampling has a bigger goal and

that the same sampling is being carried out 4 times per year for three consecutive years. This will possibly allow us, in the end, to make some assessment of seasonal effects on the rivers' biogeochemistry, including snowmelt. This has been added as future perspectives at the end of the manuscript as follows: ¨To make a step further in studying the river continuum and to try to relate riverine biogeochemistry to climate, it is paramount to assess both the spatial and the temporal dynamics. This study represents indeed the first step of a larger project which will investigate the spatial dynamics across all seasons and several years¨ (Lines 605-607).

What would you expect to see if you compared first order streams in the upper Andean zone with low order streams at a lower elevation?

The influence of stream order and topography is an interesting aspect to consider. Indeed, a comparison between first-order streams in the upper Andean zone and low-order streams at lower elevations would likely reveal differences in both the sources and processing of organic and inorganic matter and, more broadly, in riverine biogeochemistry (Maurischat et al., 2023). According to the River Continuum Concept, stream order represents a longitudinal gradient in which physical conditions, organic matter inputs, and biological communities change progressively from small headwater streams to larger downstream reaches (Vannote et al., 1980). In this framework, organic matter is progressively processed along the river network, so downstream communities are expected to capitalize on organic matter remaining after upstream processing, with DOM becoming progressively transformed along increasing stream order (Raymond et al., 2016; Mosher et al., 2015). However, in the Rapel catchment, stream order is associated with strong environmental gradients. Upper Andean first-order streams are more strongly influenced by cryosphere-derived water and local lithology (Ohlanders et al., 2013), whereas lower-elevation streams, even at similarly low stream order, are more likely to integrate inputs from surrounding soils, vegetation, tributaries, and human activities. In addition, water travel time may provide an important integrative control on the progressive transformation of organic matter and microbial community turnover along the river network (Demeter et al., 2025). Thus, in the Rapel catchment, differences between upper and lower elevation streams should be interpreted as the combined result of stream position, elevation, hydrological connectivity, and local environmental conditions rather than as a simple effect of stream order.

- Demeter, K., Savio, D., Kirschner, A. K. T., Reischer, G. H., Kolarevic, S., Parajka, J., Derx, J., Jakwerth, S., Wurzbacher, C., Blaschke, A. P., Mach, R. L., Blöschl, G., Farnleitner, A. H., and Eiler, A.: Hydrological regime of a continental river system predicts bacterial macroecological patterns, ISME J, 19, wrag013, https://doi.org/10.1093/ismejo/wrag013, 2025.

- Mosher, J. J., Kaplan, L. A., Podgorski, D. C., McKenna, A. M., and Marshall, A. G.: Longitudinal shifts in dissolved organic matter chemogeography and chemodiversity within headwater streams: a river continuum reprise, Biogeochemistry, 124, 371–385, https://doi.org/10.1007/s10533-015-0103-6, 2015.

- Ohlanders, N., Rodriguez, M., and McPhee, J.: Stable water isotope variation in a Central Andean watershed dominated by glacier and snowmelt, Hydrology and Earth System Sciences, 17, 1035–1050, https://doi.org/10.5194/hess-17-1035-2013, 2013.

- Raymond, P. A., Saiers, J. E., and Sobczak, W. V.: Hydrological and biogeochemical controls on watershed dissolved organic matter transport: Pulse- shunt concept, Ecology, 97, 5–16, https://doi.org/10.1890/14-1684.1, 2016.

How does the strong altitudinal gradient influence the transferability of the results from this study?

The strong altitudinal gradient is an important consideration when transferring the results of this study to other river systems. The biogeochemical patterns observed in the Rapel catchment emerge from the interaction between elevation, cryosphere influence, hydrological connectivity, land use, and downstream regulation, rather than from a single longitudinal gradient. Consequently, the specific magnitude and direction of the observed changes may not be directly transferable to rivers with different climatic, geomorphological, or hydrological settings. Nevertheless, the broader finding that longitudinal biogeochemical patterns can be strongly modified by environmental transitions, tributary inputs, land use, and flow regulation is likely applicable beyond the Rapel catchment, particularly in other Mediterranean mountain river systems.

In order to incorporate these critical general questions, section 4.3 has been revised and modified as follows: ¨More broadly, the strong spatial heterogeneity observed across the Rapel catchment indicates that longitudinal biogeochemical organization cannot be explained by downstream transport alone. The upper Andean headwaters were distinct from the transition and floodplain sectors, while tributary confluences, land-use transitions, the reservoir, and the estuarine interface represented additional zones of biogeochemical change. This reinforces the idea that river corridors are active components of regional carbon and nutrient cycling rather than passive conduits linking terrestrial and marine ecosystems. In this context, elevation, stream position, hydrological connectivity, and local environmental conditions interact to shape the sources, transformation, and transport of organic and inorganic matter along the catchment.

The timing of sampling also provides an important context for interpreting these spatial patterns. The November campaign was conducted shortly after the onset of the snowmelt period according to DGA (The General Water Directorate) hydrological records (https://mapas2.mop.gob.cl/). Snowmelt may therefore have contributed to the hydrological conditions observed in the upper Andean zone by increasing connectivity between cryosphere-influenced headwaters and downstream reaches. However, because this study covers a single annual cycle and does not include sampling before and throughout the snowmelt period, its specific contribution to the observed biogeochemical patterns cannot be directly quantified. The differences between the upper headwaters and downstream sectors should therefore be interpreted as reflecting the combined influence of seasonal hydrological conditions, elevation, catchment position, tributary inputs, land use, and reservoir regulation rather than as a direct signature of snowmelt alone.

Finally, the results of this study provide a spatial perspective on biogeochemical organization within a strongly heterogeneous Mediterranean-Andean catchment and highlight the potential sensitivity of these systems to future environmental change. Glacier retreat, reduced snowpack, and changes in runoff seasonality may modify hydrological connectivity, residence time, and DOM sources, while existing land-use pressures and reservoir regulation may further influence these processes. These changes may alter microbial processing pathways, carbon turnover, nutrient dynamics, and contaminant transport across the aquatic continuum. However, these longer-term changes cannot be assessed directly from our dataset, which covers only a single sampling season. Predicting the future functioning of Andean river systems will therefore require integrated approaches capable of linking hydrology, microbial ecology, organic matter dynamics, and inorganic geochemistry across the entire catchment, together with multi-year and seasonally resolved observations¨ (Lines 564-588).

REVIEWER 2

General comments:

The manuscript *Riverine biogeochemistry from the Andes to the ocean: longitudinal patterns along a Mediterranean Andean catchment in central Chile* by Derrien et al. presents important and timely findings on riverine biogeochemical dynamics in a Mediterranean Andean system. The authors provide a comprehensive longitudinal assessment across the Rapel catchment,integrating dissolved organic matter (DOM) optical properties, nutrient chemistry, dissolved metals, and microbial community composition. The dataset is extensive, the spatial coverage is strong, and the multi-compartment biogeochemical approach represents a major strength of the study. The work is highly relevant for understanding hydrological regulation and land-use pressures in Andean watersheds.

We sincerely thank the reviewer for the positive and encouraging comments on our manuscript.

While the scientific foundation of the manuscript is solid, several issues require attention before final publication. These include editorial inconsistencies, missing values, formatting errors, and areas where methodological or interpretive clarity could be improved. Addressing these points will enhance readability, coherence, and overall professionalism. In its current form, the manuscript warrants moderate revision.

A detailed point-by-point response to the reviewer's comments is provided below.

Specific comments:

- Line 65 and 83: The Rapel catchment area should be consistent. You have got two different numbers/significant figures.

We thank the reviewer for highlighting this inconsistency. We have revised the Introduction accordingly to ensure consistency with the information provided in the Materials and Methods: ¨In this study, we investigated the longitudinal biogeochemical dynamics of the Rapel catchment in central Chile, a large Andean-to-coastal Mediterranean ecosystem covering 13700 km²¨ (Lines 68-69).

- Line 108-114: Clarify the naming conventions, they are not very clear especially for Tinguiririca and Cachapoal subcatchment. They were described in results, but given the number of samples, a short description in the methods would be helpful for the flow.

We thank the reviewer for this comment. We have revised the text to clarify the station code structure as follows: ¨To examine the complete river continuum, 25 sampling sites were selected along the catchment, from the Andes to its mouth in Navidad (Fig. 1). Of these, 11 sites were located within the Cachapoal subcatchment, 8 within the Tinguiririca subcatchment, and 6 within the Rapel subcatchment. Station names follow a standardized nomenclature in which the first letter indicates the subcatchment (C = Cachapoal, T = Tinguiririca, and R = Rapel), while the following letter(s) identify the river within the corresponding subcatchment, followed by the station number, when applicable. For example, C-C1 refers to sampling point 1 on the Cachapoal River within the Cachapoal subcatchment, whereas C-P refers to the sampling point on the Portillo River within the Cachapoal subcatchment. Thus, the station code provides information on both the subcatchment and the river where the sampling site is located¨ (Lines 117-124).

- Line 133: Maybe specify the maximum storage duration before extraction in this sentence “Sterivex filters were frozen at -20℃.”

We thank the reviewer for the suggestion. The following information has been added: ¨Sterivex filters were stored frozen at −20 °C for a few weeks to a maximum of 4 months before extraction¨ (Lines 151-152).

- Line 134: Sample Analysis? “Samples collection” Section title appears twice (2.2 and 2.3).

We thank the reviewer for this comment. The section title has been changed to " 2.3 Analytical measurements¨ (Line 153).

- Line 169: “retained XX% of the raw reads.” Place the missing value here.

We thank the reviewer for this comment. The missing value has been added ¨This process resulted in an average of 155,640 ± 37,086 reads per sample and retained 83.9 ± 3.6% of the raw reads¨ (Line 189).

- Line 191: “C1mh, C2th, C3p, C4trp” Component naming inconsistent across manuscript Please Standardize them. Also, the component names could also be included in section 2.3 to avoid confusion.

We thank the reviewer for catching this. The naming of the components has been reviewed and homogenized across the manuscript. Component names have also been added in section 2.3. ¨A 4-component model was validated (98.9% explained variance, Fig. S2) and the components were compared with similar components reported in the literature using the OpenFluor database (Murphy et al., 2014) and attributed to specific groups of fluorophores (Table S1; C1mh: Marine Humic-like, C2th: Terrestrial Humic-like; C3p: Protein-like, C4p: Protein-like)¨ (Lines 165-168).

- Line 196: catchment levels?

Indeed, it has been corrected as follows: ¨Similarly, for representation purposes, the three subcatchments (i.e., Cachapoal, Tinguiririca, Rapel) and the three catchment levels (i.e., headwaters, transition zone, floodplain) were added as supplementary qualitative variables¨ (Lines 224-225).

- Line 220: a better way might be to reference figures as- (Fig. 2a) instead of (Fig. 2 (a)) here and in the rest of the manuscript.

This way of referencing this figure has been corrected across the manuscript.

- Figure 2: revise the following sentence “Dots and lines represent the main rivers; single empty dots represent the tributaries.” with something like “line and scatter plots (with filled symbols) represent the main rivers while void symbols represent the tributaries.”

We thank the reviewer for the suggestion. The caption of Figure 2 has been revised as follows: ¨Figure 2: Dissolved Organic Carbon (DOC) concentration (a) and Total Dissolved Nitrogen (b) in the three subcatchments. Line and scatter plots (with filled symbols) represent the main rivers while open symbols represent the tributaries. In the Rapel subcatchment, the reservoir sample is shown in a lighter shade. The code names of the sampling sites are shown next to the corresponding dots. Note that the y-axis of DOC concentration has a break between 140 and 200 µM due to the marked difference between the concentration in C-Ca and R-R2 and the rest of the sites, helping in highlighting the concentration dynamics in the lower

concentration sites¨ (Lines 264-269).

Also, Given the importance of Rapel reservoir in this study, highlighting it using a different color or symbol in the figures would improve readability.

We thank the reviewer for the suggestion. The comment has been reflected in Fig. 2 and Fig. 3 using a lighter shade for the reservoir samples.

- Figure 3: revise the caption following the comment for Figure 2.

We thank the reviewer for the suggestion. The caption of Figure 3 has been modified as follows: ¨Figure 3: Concentration normalized fluorescence of the 4 identified components: microbial humic-likeC1mh* (a), terrestrial humic-like C2th* (b), protein-like C3p* (c), and tryptophan-like C4p* (d) in the three subcatchments. Line and scatter plots (with filled symbols) represent the main rivers while open symbols represent the tributaries. In the Rapel subcatchment, the reservoir sample is shown in a lighter shade. The code names of the sampling sites mentioned within the text are shown next to the corresponding dots¨ (Lines 311-315) .

- Line 244-252: Consider adding specific excitation/emission maxima at their first appearance and check for consistency in the terms used to represent the components (i.e., C4trp vs C4p) throughout the manuscript and figures.

We thank the reviewer for catching the mistake in naming the components; it has been reviewed and homogenized across the manuscript. Components' excitation/emission maxima have been added as follows: ¨The PARAFAC analysis of the EEMs and comparison of the results within the Openfluor database (Table S2) resulted in the identification of two humic-like and two protein-like components. C1mh (λEx/λEm max: 315/409 nm) has characteristics that are usually attributed to humic-like compounds that have been reprocessed by the microbial community. C2th (λEx/λEm max: 260(365)/469 nm) has the typical excitation and emission maxima attributed to terrestrial humic-like compounds. C3p (λEx/λEm max: 300/348 nm) has fluorescence maxima that resemble those of protein-like compounds in water, i.e., tryptophan, but its excitation maximum occurs at longer wavelengths than the typical protein-like components of DOM. This suggests that its fluorescence may be influenced by factors such as being bonded within bigger molecules or complexed with metals (Aiken, 2014). Interestingly this component was found to be more than 98% similar (Openfluor comparison) to components identified in other regions affected by the presence of ice (Chen et al., 2018; Maurischat et al., 2022). C4p (λEx/λEm max: 275/338 nm) fluorescence maxima are those usually related to protein-like fluorescence¨ (Lines 272-281).

- Acknowledgements: If Financial Supports section is a requirement, consider removing the same information from Acknowledgements.

As suggested by the reviewer, the financial support information has been removed from the Acknowledgements (Lines 625-630).

EDITOR

Two reviewers acknowledged the novelty and significance of your exploration of longitudinal variations in biogeochemical characteristics and microbial community composition across the Rapel catchment in the Chilean Andes. While the overall evaluation is positive, the reviewers have identified several areas for improvement. I agree with both reviewers that your study offers rare insights into the unique biogeochemical patterns in this understudied Andean region, within the wider context of examining local patterns through the lens of the river continuum concept (RCC). However, I also see that further refinements are required to improve your presentation and interpretations of the comprehensive dataset, which is nonetheless based on a single field campaign.

We thank the Editor for his careful review and comments. A detailed point-by-point response to the Editor's comments is provided below.

Based on these reviews and my own evaluation, I recommend "Reconsider after major revisions." Please revise your manuscript by incorporating both the reviewers' comments and my suggestions as follows:

- Hypotheses: To explain the observed longitudinal patterns, you emphasize the complex interplay between natural geomorphological and anthropogenic drivers throughout the manuscript, as illustrated by some selected sentences below. However, these general explanations need to be based on specific hypotheses. Please consider articulating specific hypotheses as to why and how the expected biogeochemical patterns would deviate from the traditional RCC and its recent modifications. From your major findings, such as higher protein-like fluorescence in alpine headwater streams, I thought it would be meaningful to hypothesize the limited validity of the conventional RCC model (developed for temperate systems) in the Chilean Andes. It would also be helpful if you could specify key anthropogenic drivers unique to each subcatchment in both the Introduction and Discussion sections.

L 371-374 "the combined influence of natural geomorphological gradients and contrasting land-use pressures across the Andes-to-ocean continuum. Along the river network, the changes in these biogeochemical variables were not independent but rather covaried spatially, revealing coupled processes operating at the catchment scale, as shown by the MFA (Fig. 6)."

L 433-434: "reservoir and its upstream transition zones act as areas of intensified ecological and biogeochemical transformation within the aquatic continuum, decoupling local biogeochemical dynamics from simple upstream–downstream transport"

L 461-464: "Our results from the entire Rapel catchment partially support these expectations, particularly in the upper Andean sectors. Beyond these upper sectors, the Rapel catchment departed markedly from the progressive and predictable longitudinal organization proposed by the RCC. Instead of a smooth downstream transition, DOM, dissolved metals, nutrients, and microbial communities were strongly reorganized by tributary inputs, land use, and hydrological regulation"

We thank the Editor for this comment and the helpful suggestions. The Rapel catchment is considered as a temperate system but we *a priori* identified various factors that allowed us to hypothesize a deviation from the classic RCC model in this system. Hence, new hypotheses have been formulated so it makes the expectations of our work clearer as follows: ¨Because several discontinuities were identified in the Rapel catchment, we hypothesized that the conventional RCC model may have limited validity in this system. Specifically, we hypothesized that, rather than showing smooth gradients from headwaters to the estuary, the catchment would exhibit pronounced shifts driven by hydrological conditions and

anthropogenic pressures¨ (Lines 79-82).
We also added the main anthropogenic activities per subcatchment in the Introduction and the Discussion for a better understanding of the study system as follows: ¨The basin encompasses strong altitudinal and hydrological gradients, contrasting land uses, intense agricultural (mainly in the Tinguiririca subcatchment) and mining activities (strongly influencing the Cachapoal subcatchment, Lacassie and Ruiz-Del-Solar (2021)), and the presence of one of the largest reservoirs in central Chile (Rapel subcatchment, Bravo-Linares et al. (2024))¨ (Lines 69-72).
And ¨Mining-affected tributaries (mainly affecting the Cachapoal river, Lacassie et al. (2021)) introduced localized enrichments in Cu, Mn, Zn, sulfate, and NO_3^-, while agricultural sectors contributed elevated nutrient inputs¨ (Lines 514-516).

- Mismatches between DOM optical characteristics and microbial communities: Although you emphasized synchronous patterns (e.g., L 440-443), the intriguing dominance of protein-like fluorescence across the catchment appears to deviate from the spatial patterns of DOC and microbial community composition. Please compare this observed decreasing trend (even in the lower reaches downstream of the reservoir and urban centers) with findings from other recent studies. I also wonder if other widely used optical indices, such as Humification Index and Fluorescence Index, would reveal similar or complimentary patterns.
L 440-443: Rather than behaving as isolated components, DOM, dissolved inorganic compounds, and microbial assemblages responded synchronously to shifts in environmental conditions and land use, from oligotrophic Andean headwaters to agriculturally and mining-influenced transition zones, reservoir processing environments, and estuarine interface.
L 500-504: "The predominance of protein-like fluorescence across most stations contrasts with the classical view of progressively humified riverine DOM along the downstream continuum. Instead, our observations suggest that a fraction of DOM is produced in situ throughout the catchment, including in high-altitude sectors. These findings suggest that Andean headwaters and cryosphere-influenced environments may represent important sources of organic matter to downstream ecosystems, potentially sustaining microbial activity and carbon processing along the aquatic continuum."
We thank the editor for this comment. It was indeed surprising also for us to find such high protein-like fluorescence. This part of the discussion was highly integrated, including comparison with recent literature, by following your comments as well as the comments from the other two reviewers. The paragraph now reads: ¨FDOM instead showed unexpectedly high fluorescence of the two protein-like components, C3p and C4p, across most upstream sampling points (Fig. 3; e.g., more than 10 times higher fluorescence intensities of the protein-like components compared to the humic ones). This very high fluorescence suggests that DOM was dominated by in situ-produced and possibly labile organic matter in these environments. This is in agreement with the dominance of protein-like fluorescence in small Chinese rivers during spring (Shi et al. 2024), where one of the driving factors of water quality was nitrates concentration. Protein-like fluorescence in high mountains can also be derived from glaciers and snowmelt (Drapeau et al., 2025; Dubnick et al., 2022). Indeed, there is strong evidence supporting the in situ production of organic matter, particularly protein-like compounds, by photoautotrophic microorganisms within glaciers (Anesio et al., 2009; Maurischat et al., 2022) or also sea ice (Retelletti Brogi et al., 2018; Stedmon et al., 2011). On one side, and as also reported from field and experimental studies, the highest C3p* and C4p* values coincided with the greatest abundance of cyanobacteria, including Cyanobium PCC-

6307 (Li et al., 2025; Tedetti et al., 2026; Yin et al., 2025). Most of the cyanobacteria retrieved were likely not free-living but instead associated with Nostoc cyanobacterial mats observed on stream rocks (Hentschke et al., 2025). Cyanobacterial mats act as a dynamic environment where both organic matter produced and microorganisms can be retrieved in the water and play an important role that may be considered in future research (Scott and Marcarelli, 2012). On the other side, the extremely high C3p* and C4p* values observed in the Azufre tributary (T-A, Fig. 3c and d) may be also likely related to its distinctive origin within volcanic and hydrothermal-influenced environments associated with the Tinguiririca volcanic complex, as observed also in the marine environment (Tedetti et al., 2026 and references therein). These previous studies attribute the high protein-like fluorescence to biological production (i.e. chemiosithesis) or abiotic factors which are promoted by the elevated temperature. The humic-like compounds might instead be more easily thermally degraded (Tedetti et al., 2026). Higher values of the BIX fluorescence index, indicating a higher contribution of in-situ produced DOM was also found in a volcanic site compared to a reference area (Retelletti Brogi et al., 2019). Abiotic production of DOM at increasing temperature was also demonstrated in laboratory controlled experiments (Lin et al., 2017). Finally we cannot exclude the anthropogenic contribution to the protein-like FDOM pool, in agreement with Liu et al. (2026) which recently reported the dominance of protein-like DOM (average approx. 70%) along a small river impacted by mining¨(Lines 412-435).
Regarding the suggested optical indices, we acknowledge that indices such as FI and HIX are commonly used to gather complementary information. However, we decided not to include them in the present study because their interpretation and commonly used thresholds are strongly context-dependent and calculation-dependend as suggested in two recent reviews on optical indices (D'Andrilli et al., 2022; Korak and McKay, 2024). Indeed, the HIX values obtained in our study fall below the traditional interpretation thresholds, making direct application of these thresholds potentially misleading for our system. We therefore considered that the relative patterns of the fluorescence components provide a more robust and internally consistent approach for our dataset.

- Limitation of one-time sampling: This is a clear limitation given the substantial temporal variability typical of DOM characteristics. Please discuss how potential seasonal variations would reveal unnoticed patterns, thereby expanding your views on the RCC framework.
We agree with this comment. It was also raised by one of the reviewers. To address this point, we have highlighted some of the limitations associated with our one-time sampling approach in Section 4.3 of the Discussion, as follows: " Finally, the results of this study provide a spatial perspective on biogeochemical organization within a strongly heterogeneous Mediterranean-Andean catchment and highlight the potential sensitivity of these systems to future environmental change. Glacier retreat, reduced snowpack, and changes in runoff seasonality may modify hydrological connectivity, residence time, and DOM sources, while existing land-use pressures and reservoir regulation may further influence these processes. These changes may alter microbial processing pathways, carbon turnover, nutrient dynamics, and contaminant transport across the aquatic continuum. However, these longer-term changes cannot be assessed directly from our dataset, which covers only a single sampling season. Predicting the future functioning of Andean river systems will therefore require integrated approaches capable of linking hydrology, microbial ecology, organic matter dynamics, and inorganic geochemistry across the entire catchment, together with multi-year and seasonally resolved observations¨(Lines 580-588).

This issue was also addressed in the conclusion of the manuscript where we highlighted the need for longer temporal scale in such kind of studies and that indeed the project within which this study was carried out will include intra and interannual variability: ¨To make a step further in studying the river continuum and to try to relate riverine biogeochemistry to climate, it is paramount to assess both the spatial and the temporal dynamics. This study represents indeed the first step of a larger project which will investigate the spatial dynamics across all seasons and several years¨(Lines 605-607).

- Terms: Please use these terms consistently: catchment (vs. watershed), main stem (vs. main river). Headwater is usually referred to low-order streams. Please define it at its first use and use the term consistently throughout the manuscript.

We thank the Editor for this comment. All the manuscript has been revised for consistence. We have added this information as follows: "Headwaters (i.e., low-order streams) are typically dominated by allochthonous inputs of organic matter and strong hydrological control, whereas downstream reaches become progressively influenced by in-stream production and transformation processes" (Line 44).

- Headwater-transitional zone-floodplain: As one reviewer suggested, this critical classification requires a clear definition in the text, as well as a more efficient visualization in the study map (together with major anthropogenic sources).

These points have been addressed in the manuscript as suggested by one of the reviewers. Figure 1 has also been modified in response to the same reviewer' comments.