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Hydrology and Earth System Sciences
Discussions

Interactive comment on "Environmental DNA simultaneously informs hydrological and biodiversity characterization of an Alpine catchment" *by* Elvira Mächler et al.

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Thank you for taking the time to read and comment on our manuscript, we appreciate your helpful and constructive feedback. Please find below a point by point response (in *italics*) to your comments. We feel confident that a clarification as response is sufficient to address the comments of the reviewers. These clarifications could be transferred to the manuscript in a more condensed manner, which we would be happy to implement.

Sincerely, the authors

Refree 2

This is a very "dense" paper where the authors propose DNA-based indicators that simultaneously include information about the hydrological and biological features of the stream network of Alpine systems. The approach comes from the consideration that in these systems the high variability of physio-chemical properties and flow paths frequently corresponds to that observed in biological habitats. In these habitats, highly specialised organismal communities tend to develop according to the trophic status, which in turn is related to the source of the water "type". For example the three aquatic environments (tributaries, springs, and the main channel) are unique habitats each with corresponding eukaryotic communities. Thus, the drift of biological organisms are expected to have the potential to trace connectivity of the stream network. As microorganisms leave traces of their DNA in the environment, this DNA (environmental DNA - eDNA) may be used as a tracer to derive flow patterns in a watershed using hydrologic models. In their paper, the authors evaluated the possibility of using eDNA in hydrologic assessments of an Alpine system and, contextually, to gain insights on where and when to sample eDNA in river networks for assessments of biological diversity. To do that, a very intensive monitoring campaign was set up in an Alpine catchment in Switzerland, where they monitored simultaneously eDNA, electrical conductivity, water temperature, stable isotope ratios of the water, as well as discharge at the catchment outlet and meteorological parameters at four stations distributed across the catchment at different a.s.l... The authors used so-called ZOTUs (clusters of very similar DNA sequences) as a rough proxy for a species present in different aquatic systems and thus indicating different water origins. At the same time, the authors also used the derivative of the discharge at the outlet, dq/dt, as a proxy for stream network recession and expansion. At the end, they discussed the relationships among the different indicators considered

General comments

The manuscript is very well structured. The introduction of the paper illustrates clearly the rationale and the objectives of the work. It provides a wide and exhaustive literature review about the approach used. The figures depict clearly the experimental data and, in general, the Materials and Methods are well explained. The number of techniques and methodological analyses used requires multidisciplinary skills to be correctly interpreted. I am not a biologist and the techniques to analyse the DNA should be revised by a reviewer with specific skills As for the approach and the interpretation of the results, based on my reading of the manuscript, I identified some strength and weakness points. The strengths mostly lie in the multidisciplinary approach on one side and, on the other side, in the number and quality of measurements the authors did in terms of eDNA, electrical conductivity, water temperature, stable isotope ratios of the water, discharge at the catchment outlet and meteorological parameters. Quite interesting is the use of the eDNA to identify (at least qualitatively) times of greater and lesser interconnection among water in different sites in the stream network, so that the main channel and tributaries resembled each other more (i.e., were more connected) on days with increased precipitation or snowmelt. The mechanism is quite clearly shown in the figure 7.

> Thank you for appreciation of our work and for highlighting the interdisciplinary approach of our study.

Weaknesses are mostly related to the interpretation of the measurements and the relationships between eDNA and "type" of water as related to its origin. For example, In the figure 5 I am not able to see a clear relationship between ZOTU richness and EC in the case of the main channel and tributaries, while it is a bit clearer for spring. I see a reversed situation in the relationship with dq/dt, even if, also in this case, a clear relationship does not exist even for main channels and tributaries.

> We agree that some of the relationships are not obvious when looking at the figure.

*It is important to note, though, that we are actually interpreting the data based on the statistical results presented in the Table 1, and not based on the figure itself. We tested for interaction (ZOTU richness = E.C. * water type), which allows us to identify if the intercept and slope of richness varies according to the water type, compared with an additive approach (ZOTU richness = E.C. + water type). An additive approach would only allow us to test whether the intercepts were different, while assuming the slopes were the same. To test for interaction was especially insightful in our case, because we identified different slopes (Figure 5a, dq/dt) for the each of the three water types (positive for main channel and tributary, but negative for spring). However, we only found a significant slope in terms of E.C. for the water types of main channel and the spring (Figure 5b). As you identified correctly, there is no significant interaction between E.C. and the water type of tributary (p-value of 0.203 in Table 1, i.e. the slope is not significant also indicated by the dashed line in the figure). In the case of the main channel, the interaction is significant, but perhaps adjusting the x-axis of E.C. for each water type would facilitate the interpretation, which we would be happy to do.*

In any case, most of the deductions the authors drew in the paper comes from a statistical analysis, which, at least in this specific case, can indicate something behind the observed behaviour but are not able "to see" the actual mechanisms inducing different DNA composition in the different water types in different times. In this sense, the deductions of the authors seems, to me, a bit speculative. Actually, the same authors stated: "Our analysis showed that the eDNA composition of the three water types was indeed different, but not to a level that made them entirely distinct. In fact, we always expect a portion of the eDNA signal that is non-informative on the water types, and this overlap can be explained by either shared species compositions due to ecological connectivity between sites and/or by transport of eDNA between hydrologically connected sites". Even the potentiality of using the eDNA to identify times of greater and lesser interconnection among water in different parts of the stream network seems mostly qualitative.

> We used a non-metric multidimensional scaling method, thus we would argue that our approach of the NMDS analysis is quantitative, rather than speculative. But as you have pointed out, the stress of the NMDS was reasonable but certainly not high enough to lead to an excellent discrimination. Although it is outside of the scope of our study, a future study might want to sample from more distinct, unrelated sources such as the glacier directly, pore-water, snow, rainfall, rock ice or any stagnant terrestrial water pools. Furthermore, we want to highlight that we are interpreting a biological response which perhaps is not as cut and crisp as a binary response of a purely physical process might be.

From the results analysis, it seems clear that the eDNA cannot replace the classical indicators (stable isotopes of water, water temperature, and E.C.) to discriminate among different origin of the water in the network. And yet, the eDNA analysis can still be used to support the observations with physio-chemical tracers, which are themselves not so simple to interpret.

> We agree that based on our results, eDNA cannot replace any of these classical indicators. However, our results do demonstrate that eDNA provides a huge amount of information that complements existing indicators. The metabarcoding approach in particular offers a thorough snapshot into the biological communities inhabiting this environment which will be useful for a wide variety of goals. In addition, we believe that in the future, eDNA will help discriminate hydrological processes in a more nuanced fashion than is currently possible with physical indicators.

Specific remarks
The first nine lines of the abstract should be moved to the Introduction section.

> The first nine lines of the abstract do in fact summarize our current introduction quite well, with each sentence or clause introducing one paragraph of the introduction. We

believe that it is valuable to have this summary of the major background, problem, and opportunity in the abstract as that is the most accessed and read part of a paper, especially online. However, if the editor supports the reviewers view, we would be happy to reconfigure the introduction by including these lines and thus shorten the abstract to focus on our study and its results.

In figure 4, the caption should indicate the meaning of NMDS1 and NMDS2.

> We will add 'NMDS' in parentheses after non-metric multidimensional scaling in the figure legend and clarify that they stand for the dimension 1 (NMDS1) and dimension 2 (NMDS2).

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