Review of "Water Column Respiration in the Yakima River Basin is Explained by Temperature, Nutrients and Suspended Solids" by Laan et al.

General comments:

Laan et al. submitted a well-written manuscript with a clear structure. The key strengths of this work are that Laan et al. could reliably measure ecosystem respiration in the water column (ER_{WC}) at a broad spatial scale in environmentally diverse rivers, which are located at different positions in the Yakima River network and that they measured a comprehensive set of explanatory variables for ER_{WC} ranging from basic physicochemical measures to high-resolution DOM data.

Laan et al. hypothesized that ER_{WC} values increase from upstream to downstream (approximated by drainage area) and conclude from their results that rather local factors are driving ER_{WC} in river networks. Although I, in general, agree with the conclusion, I do not think that the data analysis is robust enough to really rule out the effect of drainage area (and regional processes in general) on ER_{WC} because of two potential problems:

1. Laan et al. use LASSO regressions for variable selection and regularization. LASSO analysis does not provide p-values per se, however, one could argue that only important variables are selected in the model. Total drainage area was selected in the final model with a low positive mean beta coefficient suggesting a slight increase of ER_{WC} values (i.e. rates slow down) from upstream to downstream. However, based on Fig. 3b, I see a decreasing trend of ER_{WC} (i.e. rates fasten) from upstream to downstream. I suspect that this discrepancy is due to strong effects of other variables on ER_{WC}, which also strongly correlate with drainage area (Fig. S4). I suggest to try partial regression plots to illustrate the net effects of predictors on ER_{WC}. For Fig. 3b this could mean that you show how ER_{WC} changed as a function of the drainage area after statistically holding the effect of other important predictors (e.g. TDN) constant. This strong collinearity among predictors (Fig. S4), however, may have even more influence on the analysis: If there are highly correlated predictors, LASSO's variable selection might only select one of them. I suggest to provide an explanation why you do not think that this is an issue for your analysis or a comparison of your current method with methods using elastic net penalties (combination of penalties of the LASSO and ridge method) which may deal better with multicollinearity.
2. The model does not account for spatial autocorrelation, which could further lead to an overseen stronger shift of ER_{WC} from upstream to downstream. Either you directly use spatially explicit methods for your analysis, such as spatial stream network models (*e.g. Peterson, E. E. & Ver Hoef, J. M. A mixed-model moving-average approach to geostatistical modeling in stream networks. Ecology **91**, 644–651 (2010)*) or you discuss this issue more thoroughly. For example, when looking at TDN along the drainage area (Fig. S4), I assume that TDN concentrations increase from upstream to downstream along two major branches of the network, which finally merge into the most downstream sites, suggesting a strong spatial autocorrelation of TDN

concentrations. Hence, regional processes (transport of water chemical from upstream to downstream) might have an indirect effect on ER_{WC} by shaping the spatial TDN pattern across the entire river network.

Further, I suggest to change negative ER_{WC} values to positive values (consumption of O2), as I think it makes the interpretation of positive and negative beta coefficients in the LASSO analysis easier and it is more common in the literature. This would also solve ambiguity when writing "ER_{WC} increases", because in these cases I was often not sure if the authors mean whether ER_{WC} rates increase (i.e. rates fasten) or ER_{WC} values increase (i.e. rates slow down).

Overall, considering that my major concerns and specific comments will be addressed in a major revision, this study warrants publication in Biogeosciences.

Specific comments:

26: Reading about positive ER values in the Abstract is very confusing. After reading the method section I understand that you kept positive values below 0.5 because they are difficult to distinguish from zero. Still, I wonder if you could avoid stating positive ER values in the Abstract to avoid confusion by the readers early on. But also see my specific comment to line 292.

26: Although you clearly state that you "did not test this directly", I think it is still too much to say "that the contribution of ER_{WC} rates to reach-scale ER_{tot} rates across the Yakima River basin are likely highly variable", because a comparison of a set of ER_{WC} values with another set of ER_{tot} values, which both derive from a wide range of streams differing in size, stream order, environment, etc. does not give you that information. You might come closer to such a result if you apply the same classification to the streams from the literature as you have done to your study streams and then compare ER_{WC} and ER_{tot} within classes.

28: I suggest to keep the statement that you "did not observe a clear **increase** in ER_{WC}" more neutral. Because at that point of the manuscript, it is not clear why you expect an increase in absolute ER_{WC} values from upstream to downstream. But see also my next specific comment.

71: I miss an explanation why you expect **absolute** ER_{WC} values to increase. At the beginning of this paragraph you state that water column processes are expected to become increasingly important from upstream to downstream due to a shift from benthic-dominated processes to water column dominated processes. This statement is about the **relative** contribution of water column processes. However, I do not think that this easily translates to your hypothesis that **absolute** ER_{WC} values will increase from upstream to downstream, because ER_{tot} is expected to decrease (rates slow down) from upstream to downstream (e.g. *Segatto, P.L., Battin, T.J. & Bertuzzo, E. (2021). The Metabolic Regimes at the Scale of an Entire Stream Network Unveiled Through Sensor Data and Machine Learning. Ecosystems, 24, 1792–1809.*).

148: Please write out the abbreviation "DO" first time you mention it.

200: Can you please clarify how calculating “the distance between each of the replicate samples” helps to identify the outlier.

255: perform instead of performs

292: I wonder, whether keeping positive ER_{WC} values below 0.5 in your data biases your results (Fig. 2) towards low respiration rates. Maybe this is the reason why you find on average lower respiration rates compared to studies from the literature. Since you are comparing your data to the literature, it might be reasonable to investigate how studies from literature were dealing with positive ER_{WC} values and subsequently use the same approach. I definitely do not suggest to erase cases with positive ER_{WC} values below 0.5 from the results but I find it more intuitive to change positive ER_{WC} values below 0.5 to zeros, as you rightfully stated that positive values are biologically unrealistic and are not distinguishable from zero. However, if studies from the literature research also keep positive values, I would suggest to keep the current approach to make results comparable.

342: I understood that you use LASSO analyses to investigate which variables are selected for your final model. Hence, a rejection of your hypothesis at this point of the manuscript based on the correlation in Fig. 3b seems premature. See also my general comment to this topic

347: This part of the sentence is not clear: “… as opposed to a higher order river“, please clarify.

364: Change to “Regression analyses show**ed** …”

Technical corrections

88: Remove space previous to dot.

216: The reference is missing at the end of the sentence.