**Reviewer 1: Thank you for your helpful comments and the time you put into reviewing the paper. Below, our responses are provided in bolded text following each reviewer comment. The responses are primarily in the form of our plan to address each comment in a revised manuscript, if afforded that opportunity by the editor. We look forward to your further evaluation.**

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 (ERWC) 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 ERWC ranging from basic physicochemical measures to high-resolution DOM data.

**Thank you for the encouraging remarks.**

Laan et al. hypothesized that ERWC values increase from upstream to downstream (approximated by drainage area) and conclude from their results that rather local factors are driving ERWC 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 ERWC 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 ERWC values (i.e. rates slow down) from upstream to downstream. However, based on Fig. 3b, I see a decreasing trend of ERWC (i.e.rates fasten) from upstream to downstream. I suspect that this discrepancy is due to strong effects of other variables on ERWC, which also strongly correlate with drainage area (Fig. S4). I suggest to try partial regression plots to illustrate the net effects of predictors on ERWC. For Fig. 3b this could mean that you show how ERWC 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.

**In a revised manuscript we plan to address the high-level point of collinearity influences by adding more acknowledgement of the collinearity among explanatory variables. We will emphasize relationships between LASSO-identified variables and ion concentrations**

**that seem to indicate influences of agricultural inputs. We plan to retain our approach of emphasizing N, rather than TDN or $NO_3$, as to avoid assumptions about which of those variables is most important. We will keep our approach of refraining from placing an order of importance to the variables and maintain a focus on each as explaining $ER_{wc}$ across the Yakima River basin.**

**To address the more specific point about total drainage area, we made the following five observations and plan to take actions summarized in each bullet.**

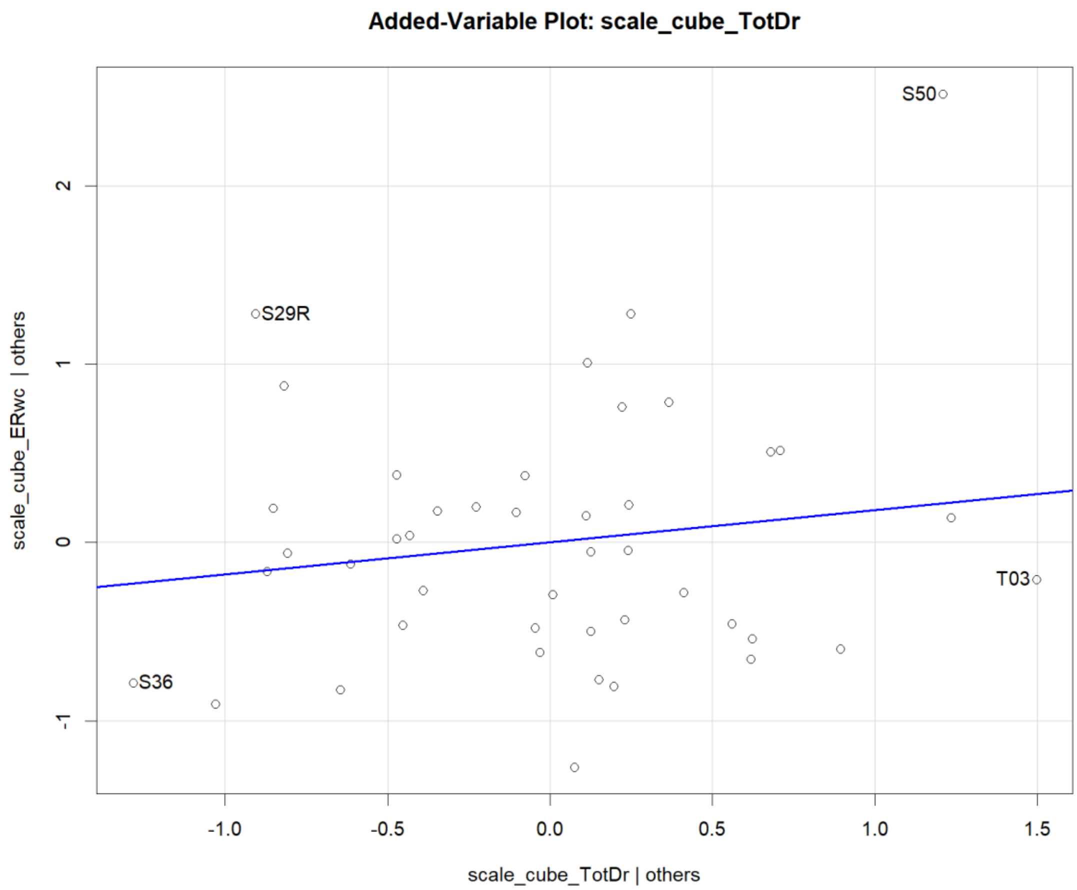
1) **To help minimize spurious outcomes that could arise due to collinearity across parameters, we used LASSO regressions across 100 random seeds, averaging the model coefficients. This reduced the likelihood of arbitrarily selecting one variable over another, which could happen with high collinearity. This revealed relatively small standard deviations of β coefficients compared to mean β coefficient values, indicating that the most important variables are consistent across seeds. In a revised manuscript, we plan to add a small amount of additional text to help make these points clearer.**

2) **In preliminary analyses, not currently shown in the paper, we tested the sensitivity of our LASSO results to collinearity by removing highly correlated explanatory variables. In this case, we had to select between co-correlated variables and include one of them in the LASSO. We selected the variable to keep based on it having the higher (univariate) Pearson correlation coefficient with $ER_{wc}$. This did not change identities of the most important explanatory variables, as identified via LASSO. This consistency suggests that collinearity did not meaningfully alter the key findings from the LASSO modeling. Including these analyses in the revised manuscript does not feel necessary, but we can include these analyses in the supplementary material if the editor deems it necessary; we will await guidance on this point.**

3) **The variables identified as the most important via LASSO also had the largest Pearson correlation coefficients with $ER_{wc}$. This consistency between multivariate and univariate methods provides additional confidence in the robustness of the LASSO outcomes. This is already discussed in the paper and we plan to retain that text.**

4) **A partial regression based on total drainage area, controlling for all the variables that had non-zero β coefficients in the LASSO model, is shown below. The relationship between total drainage area and $ER_{wc}$ remains weak in this partial regression plot. Additionally, the direction of the relationship is opposite from the univariate correlation; $ER_{wc}$ was faster moving downstream in the univariate analysis but slower moving downstream in the LASSO model. We do not plan to add the partial regression plot to the manuscript, but will add more discussion of**

how the relationship between total drainage area and other explanatory variables may relate to $ER_{wc}$ as described below.




5) The highest Pearson correlation of total drainage area was r = 0.63 with temperature, followed by an r = 0.58 correlation with TDN. Temperature and TDN are indicated by the LASSO model as being important for explaining variation in $ER_{wc}$. Clear mechanistic connections between $ER_{wc}$ and both temperature and TDN suggest that any relationship between drainage area and $ER_{wc}$ is not causal. Instead, we infer that the relationship between total drainage area and $ER_{wc}$ is simply because of correlations among the explanatory variables, and not a mechanistic connection. This means that drainage area itself is not the key factor, but instead may act as a proxy for influences of temperature and TDN. Further, TDN is strongly correlated with other explanatory variables, including $NO_3$, Cl, and $SO_4$.. The covariation among these variables likely reflects increasing influences of agricultural inputs that, in turn, lead to faster $ER_{wc}$ by supporting microbial metabolism. We collectively infer that increasing temperature and nutrients, potentially from agricultural inputs, are the most likely drivers of $ER_{wc}$ rates in the YRB. In a revised manuscript, we plan to add more discussion of these points.

2a. The model does not account for spatial autocorrelation, which could further lead to an overseen stronger shift of ERWC 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 ERWC by shaping the spatial TDN pattern across the entire river network.

**We plan to add discussion on the possibility of regional watershed processes influencing key explanatory variables such as total dissolved nitrogen, as suggested by the reviewer. We find this very helpful and we certainly agree that the chemistry observed at a given site is the outcome of processes occurring throughout the upstream drainage area (watershed).**

2b. Further, I suggest to change negative ERWC 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 “ERWC increases”, because in these cases I was often not sure if the authors mean whether ERWC rates increase (i.e. rates fasten) or ERWC 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.

**We appreciate the suggestion to express all values as positive to more closely match literature representations and improve ambiguity. To clarify interpretation in the text, we will replace the occurrences of “rates increase” with revised language in lines 28, 71, and 74 to explicitly state that we are referring to rate magnitudes (i.e., faster or slower). However, we plan to retain the sign of $ER_{wc}$ values to reduce bias that would be introduced by setting small positive values, likely associated with instrument noise, to zero. As described in lines 245 - 252 and 291 - 294, these small positive values represent measurement uncertainty overwhelming the rate of oxygen consumption, which means the rate of oxygen consumption must have been very slow. Retaining the sign allows us to preserve this information across the full data set, where more negative values indicate faster oxygen consumption.**

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.

**We will improve clarity by presenting results in the Abstract as something similar to "...rates ranged from effectively zero to -7.38..."**

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 ERWC rates to reach-scale ERtot rates across the Yakima River basin are likely highly variable", because a comparison of a set of ERWC values with another set of ERtot 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 ERWC and ERtot within classes.

**To clarify, we plan to revise the results/discussion text to emphasize that this comparison is exploratory and intended to assess the potential overlap between our measured $ER_{wc}$ values and $ER_{tot}$ reported in the literature. While both datasets span a range of stream conditions, we acknowledge that differences in environmental context limits direct comparability. Nevertheless, the overlap between $ER_{wc}$ and $ER_{tot}$ values suggests that $ER_{wc}$ could contribute a variable portion of $ER_{tot}$, with some instances indicating the potential for substantial contributions. Substantial contributions of $ER_{wc}$ to $ER_{tot}$ are not guaranteed, however, which we will emphasize in the revised manuscript. The results fail to reject the hypothesis that $ER_{wc}$ can be a significant portion of $ER_{tot}$. If we had observed consistently very slow $ER_{wc}$ rates across the Yakima River basin, we would see effectively no overlap with $ER_{tot}$ values. This would have clearly rejected the hypothesis that $ER_{wc}$ can contribute substantially to $ER_{tot}$.**

**We also agree that a more robust analysis would involve applying consistent stream classifications across datasets and comparing $ER_{wc}$ and $ER_{tot}$ within those classes. We will revise the manuscript to more clearly state the limitations of this initial comparison, while still noting that it provides useful context for understanding the range of $ER_{wc}$ values observed in the YRB.**

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

**We plan to change this sentence to: We were able to explain 40% of ERwc variability via a combination of temperature, dissolved organic carbon, total dissolved nitrogen, and total suspended solids.**

71: I miss an explanation why you expect absolute ERWC 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 ERWC values will increase from upstream to downstream, because ERtot 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.).

**We plan to add additional material to the introduction to provide more rationale for the hypothesis that $ER_{wc}$ increases moving downstream. For example, we plan to highlight the increase in gross primary production (GPP) (Marzolf and Ardon, 2021; Segatto et al., 2021) moving down the stream network. Increases in GPP are known to influence ecosystem respiration, such that we would expect an increase in $ER_{wc}$ moving downstream. Additionally, increases in nitrogen processing in the water column (Wang et al., 2022), may also indicate that $ER_{wc}$ would increase with stream order.**

**Marzolf, N.S., and Ardón, M. (2021). Ecosystem metabolism in tropical streams and rivers: a review and synthesis. *Limnology and Oceanography, 66* (5), 1627 - 1638.**

**Segatto, P.L., Battin, T.J., and 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.**

**Wang, J., Xia, X., Liu, S., Zhang, S., Zhang, L., Jiang, C., Zhang, Z., Xin, Y., Chen, X., Huang, J., Bao, J., McDowell, W.H., Michalski, G., Yang, Z., and Xia, J. (2022). The Dominant Role of the Water Column in Nitrogen Removal and N2O Emissions in Large Rivers. *Geophysical Research Letters, 49* (12).**

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

**We plan to add “dissolved oxygen” at line 148.**

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

**We plan to add the following information to the methods section describing this calculation for identifying outliers. Pairwise differences between NPOC, TDN, and DIC measurements from all replicates were calculated. The sample that had the largest difference from the other samples was removed if the coefficient of variation was greater than 30%. This coefficient of variation threshold for sample removal is based on inspecting histograms of these data types, and determining the point at which sites likely contain anomalous outlier values.**

255: perform instead of performs

**We plan to change this.**

292: I wonder, whether keeping positive ERWC 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 ERWC values and subsequently use the same approach. I definitely do not suggest to erase cases with positive ERWC values below 0.5 from the results but I find it more intuitive to change positive ERWC 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.

**To the best of our knowledge, other published studies of $ER_{wc}$ do not discuss positive rates, so there is no precedent to base our decision off of. We are left with needing to set the precedent. We make the assumption that variation among near-zero positive $ER_{wc}$ rates does carry some real information such that keeping those rates carries that information into the statistical analyses. If we change all near-zero positive $ER_{wc}$ rates to zero, we lose that information. In turn, we prefer to retain the near-zero $ER_{wc}$ rates and in the revised manuscript we plan to further emphasize the assumption we're making.**

**Regarding the influence of this decision on our comparison to literature values of $ER_{wc}$, in our view the main take away from our study is that within a single basin the range of $ER_{wc}$ is broader than all literature values combined. This outcome won't be altered by changing near-zero positive $ER_{wc}$ rates to zero. This further motivates us to retain the near-zero positive $ER_{wc}$ rates in a revised manuscript.**

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

**We plan to soften the language at this point in the manuscript to acknowledge this point. We will edit the text to something like "...is inconsistent with our hypothesis and below we use a multivariate analysis for further evaluation."**

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

**We plan to edit this sentence to clarify that we expected $ER_{wc}$ to be fastest in the highest order rivers, not mid-order rivers, by changing the text to something like "... as opposed to our hypothesis of $ER_{wc}$ being fastest in the highest stream orders."**

364: Change to "Regression analyses showed ..."

**We will change this in the revised manuscript.**

Technical corrections
88: Remove space previous to dot.
**We will change this in the revised manuscript.**

216: The reference is missing at the end of the sentence.
**We will change this in the revised manuscript.**