The manuscript “Barium stable isotopes as a fingerprint of biological cycling in the Amazon River Basin” by Charbonnier et al. describes the use of stable Ba isotopes as proxy for estimating the role of the biota on Ba fluxes in large catchment areas. Based on the approach of using stable isotope proxies (e.g., Li) to calculate river mass budgets, Charbonnier et al. found that part of the Ba dissolved from the bedrocks in the catchment is missing. Following several lines of evidence, based on (isotope) mass balance calculations, correlations with ecosystem dynamics and river mass budget, they concluded that biological cycling in the critical zone is responsible for the missing Ba component. Further implications of their findings are that earlier calculations of the CO_2 consumption of the catchment, based on river cation loads, might be considerably underestimated.

General comments:

The manuscript is well written and organized and a reader can follow the authors’ argumentation. The English of the second version of the manuscript benefited greatly from proof reading after the access reviews. I encourage the authors to also check the supplement for English grammar and style. I recommend publication of the manuscript in *Biogeosciences* after moderate revisions. My comments, which I hope the authors will find useful and constructive, are listed below.

Specific comments:

The main conclusion of the study is that a considerable amount of Ba dissolved from the bedrock and transported by the rivers is taken up and stored by biota. I was wondering how exactly plants and/or (micro)organisms utilize Ba. To my knowledge, Ba is not considered an important nutrient. The authors even state that Ba could be a limiting factor for biota growth (page 19, line 424). This has to be further discussed.

Did the authors propagate uncertainties of single parameters in their models? Most figures do not have error bars and it is thus difficult to assess whether apparent trends are real or not within uncertainty. Furthermore, I am missing estimations on the uncertainties of, for instance, the 20% underestimated CO_2 consumption, the main impact this study might have.

For the isotope mass balance in section 4.2 the authors assume congruent dissolution of the bedrock, i.e., no Ba isotope fractionation. Assuming this assumption is wrong, how large would be the impact of isotope fractionation during rock dissolution on the model output? Would it be negligible?

In section 4.4 the authors describe an apparent trend in biological Ba cycling with ecosystem dynamics (Fig. 7c,d). As the figure is now, I fail to see the trend. The only obvious is that the Madeira tributaries have lower GPP and TER values than the rest. However, there is a discrepancy between GPP data in Fig. 7a and 7c. Also, error bars are missing.

In section 4.7, I do not agree with the authors’ interpretation that $R_{(sil+bio)/sil}$ increase with very low W/D, based on Fig. 12. The argumentation is apparently based on one data point. Also, this figure lacks error bars.

Appendix B: The authors estimated the Ba isotope fractionation between dissolved Ba and Ba taken up by biota, admitting that it is poorly constrained. Yet, they state the fractionation with a fairly high precision of ±0.05 ‰. How reliable is the estimated fractionation?

The authors made a great effort in computing and quantifying data and parameters. However, not all derivations of equations can be followed easily. For instance, I failed to understand how equations C5, D5 and D6 are derived given the provided information.

Page 3, line 59: ^{130}Ba is a primordial nuclide and can be considered stable under geochemical aspects.

Page 5, line 134: What are plutonic rocks in this case? What is their lithology?

Page 10, line 214: Please define * in the main text, not only in the figure caption and supplement.

Page 19, line 433: Why is the residence time of water longer along steeper slopes?

Page 29, line 654: I could not find any data/figure supporting the argument that mainly K weathering flues are influenced by biological cycling. If they are to be found, e.g., in the supplement, please refer to it. Otherwise data have to be provided.

Page 30, line 669: Please quantify this significant uncertainty!

Page 31, line 672: […] to be source <u>mainly</u> from silicate rocks […]

Page 33, line 754: Charbonnier et al. (2018) is a review paper. When literature data are used, please cite the original publications (also later in that appendix).

<u>Technical comments:</u>

Fig. 7: GPP data are different in panel a) and c)!

Fig. S2: Are the error bars correct? They show approximately ±0.15 ‰ on $\delta^{138/134}$Ba. Long-term precision for BaBe27 and JB-2 is however given as ±0.08 ‰.