

Bouilloud et al. have put in a significant amount of time and effort into revising this manuscript and it really shows. The overall story is much easier to understand, the writing is much clearer, and the ideas presented are more fully developed. This new iteration was enjoyable to read.

The authors have addressed most of my previous concerns, though I'm still concerned about the use of an older taxonomic classifier. But as long as an appropriate conformational check to show it's not affecting the results is provided it should be ok. Please see my comment below for more details. The only other concern I have is the lack of references provided. There are quite a few sentences where ideas, information, and previous studies are talked about without a proper reference provided. The rest of my comments are mostly minor and whose purpose is to help improve the manuscript just a little bit more.

Major comments

Lines 222 – 224: I agree that it is not always possible to use the most up to date version of such resources as they are updated routinely. However, SILVA SSU 132 was released in December 2017, presumably before you conducted your bioinformatics analyses in 2018. Also, version 138 was released in December 2019 (effectively 2020), more than 2 years before this paper was first submitted to PCI, providing plenty of time to incorporate it into your study. I agree that proper reporting of your methods is important and helps to interpret your results, but using such outdated information can cause misleading or inaccurate results. Especially since there have been significant increase in the number of bacterial species described and cataloged, as well as taxonomic classifications being reworked. It is the same reason why the greengenes reference database is not typically used anymore as it has not been updated in nearly 10 years. SILVA SSU 128 is only a couple years newer than greengenes. I understand that redoing all your analyses following OTU clustering is a major task that you are hesitant to do. I would be as well. Using a more updated reference database ultimately may not change the results of your study, especially your diversity analyses unless it is able to provide taxonomic classification for a larger number of OTUs that remained unclassified at the family with SILVA 128. What I am most concerned about is that it could change your Firmicutes / Bacteroidetes ratio estimates and lead to very different results for your differential abundance analyses (DESEQ2). I highly recommend you at least provide some form of confirmation that this is not impacting your results. For example, showing that the Firmicutes / Bacteroidetes ratio is similar for both the 128 and 138 releases.

Minor comments

General comment on the use of commas: Generally speaking, in scientific writing it is better to avoid the use of commas for stylistic purposes and instead use them only for grammatical reasons. The overuse of commas for stylistic purposes can lead to the tendency of creating unnecessarily long and complex sentences that requires the reader to read multiple times to really understand it. It makes the reader tired, and is more difficult and time consuming to read through the manuscript. Please check their use throughout the manuscript as it was common throughout. Just to point out a few, there should be no comma after “fitness” (Line 77), “bacteriota” (Line 82), or “weight loss” (Line 105).

Also, please check the proper use of the singular / plural form of nouns.

Lines 102-104: Similar to the use of transition words at the beginning of a paragraph, “may thus” implies cause and consequence. However, what you are referring to when using “thus” is information provided within the previous paragraph. I recommend rephrasing this as something along the lines of, “Helminth – gut bacteriota interactions may be positive or negative with potentially both local and systemic physiological changes affecting host health”.

Line 106-108: References?

Lines 116 to 124: Each of these sentences requires references.

Lines 119 – 120: No “...” in the parentheses. The same on line 123.

Lines 131 – 132: References?

Lines 132 – 133: References?

Line 154: Do you mean “health status of the host”? In this particular sentence it is unclear if this is referring to the “health status” of the host or the gut microbiota.

Line 196: “lower segments” not “segment”

Line 198: How was the lumen removed? Washed off with a solution or scrapped off with a tool?

Line 219-220: I agree with your argument outlined in your response letter that OTUs have received a bad reputation recently, but that they are not obsolete and that sticking with OTUs for this manuscript is appropriate. That being said, the way this is currently phrased, it sounds like the purpose of this method for OTU clustering is to produce something similar to ASVs which begs the question, “why not just use ASVs?”. Also, there is no clustering of ASVs like OTUs, so they are inherently different which makes this statement a bit confusing. Do you mean it is of similar quality or reliability?

Line 234 - 235: “Number of reads per OTU” not “OTUs number of reads”?

Line 269: Referencing the references in another paper is not appropriate. Please provide the references you are referring to here.

Line 422-431: this should be one paragraph.

Line 587: The same as my comment regarding the reference on line 269. Referencing the references in another paper is not appropriate. Please provide the actual references you are referring to here.

Line 596: This is a bit misleading as you are reporting a correlation between *H. mixtum* and *Lactobacillaceae* abundance whereas Reynolds et al. 2014 showed a direct relationship through experimental inoculation. I think it is really interesting that you found a similar correlation with *H. mixtum*, but I recommend rephrasing the last part of this sentence, “as observed in our study” to better reflect the limitations of your own data.

Lines 606 - 607: “of this later”? This may have been accidentally left in from a previous version of this sentence.

Lines 607 – 610: This is more appropriate for the results section. I recommend deleting this entire sentence and making the sentence on line 605 – 607 the start of paragraph beginning on line 611.