The line numbers that I reference in my response to reviewers are those of the marked-up version of the manuscript.

**Reviewer #1**

**Hardly ever does a reviewer read a manuscript where he finds himself muttering "told you so" and "just as I always said" again and again. For me this is such a manuscript. Dr. Schloss makes a fine case why the Emperor's new ASVs are no actual improvement over the shoddy OTUs microbial ecologists have been stitching together from partial 16S rRNA gene sequences during the last decade.**

I appreciate the positive feedback from Reviewer 1. The manuscript is improved because of their feedback. It is comforting that they have confirmed much of my analysis and reading of the literature with their own experience.

**One important aspect that has not been mentioned are errors introduced by PCR amplification and sequencing. I realize that this is not the topic of the study, but maybe one should mention that here you are looking at an artificial " best-case scenario", i.e., one assumes that all variability in the 16S rDNA data set is actually "real".**

I have added text that addresses the comment made by the reviewer (L111-115). One benefit of using distance-based thresholds is that this approach merges very similar sequences that are sequencing errors. In the real-world application, ASVs on noisy data would exacerbate the problems described in this study.

**Minor points:**

**l.45-47: This sentence sounds like the authors of the cited references were "dismissive of concerns" about inter-operon variability, which they are not. Reformulate.**

I am grateful that the noticed our misattribution of the citation in this sentence. I have restructured the surrounding text (L44-52) to make it clear that the listed references call attention to intragenomic variation and that these concerns have been minimized by proponents of ASVs (L49). I have replaced “dismissive of concerns” with “minimize” to soften the language.

**l.65: it is somewhat odd that the first figure cited in the manuscript is from the supplementary material.**

Unfortunately, the Observation format is limited to two figures. As much as I would like to have Figure S1 in the main body of the paper, I felt that the other two figures were more important to include as primary figures. Figure S1 is more descriptive of the dataset and the overall problem of total intragenomic variation in the dataset. Because it helps lay the foundation for the problem, which I also feel is kind of obvious, and there is limited space for figures, I elected to make it a supplemental figure.

**L.84-92: This test does not seem to be equally meaningful than the test for splitting genomes. Its outcome very much depends on the phylogenetic composition of the tested set of species, i.e., how many closely related species there are. It is also somewhat trivial, because the whole reason for using ASVs is their higher resolution compared to distance-based OTUs.**

I agree with the reviewer that the test of lumping is not as meaningful as splitting. At the same time, the risk of using a broader definition is lumping species that people consider different based on phenotypes. I felt it was important to include this analysis to counter any critiques that I was overly critical of ASVs without addressing the limitations of OTUs. I have replaced the concluding sentence from this paragraph to state the point the reviewer is making (L96-101).

**L. 92: This statement is somewhat arbitrary and reflects more a personal opinion than a conclusion derived from the conducted analysis or literature research.**

I have removed this sentence and replaced it with a different concluding sentence to the paragraph that addresses the previous point from the reviewer. The same sentiment was already in the manuscript (L106) with supporting citations.

**l.99: If you talk to microbial taxonomists, the first thing they tell you is that taxonomy is pragmatic and, like any classification, serves a specific purpose (what you call operational).**

I appreciate this confirmation!

**L.103: Why "at best"?**

I have removed “at best” and added “not necessarily” to the sentence at L110-111. I was trying to communicate the point that OTU definitions don’t really seek out a biological foundation. Rather, they are a pragmatic/operational solution.

**L.108: I wholeheartedly agree with this statement.**

I am grateful for the reviewer supporting this assertion.

**Fig. 2, y-axis legend: "theshold"**

I am grateful to the reviewer for detecting this typo and it has been corrected in the revised version of the manuscript

**Reviewer #2**

**This "Observation" manuscript on the provocative topic of ASVs vs OTUs is well written, to the point, and informative. Essentially, we need to accept that bacterial taxonomy is a mixture of phenotype + genomic information and its simply not possible to get to species level with 16S rRNA gene sequence. Many of us have long ago accepted this, but as pointed out, this does not stop people from trying. The paper quantifies the risk of artificially splitting genomes into separate clusters using ASVs vs clustering different species into the same OTUs at various cutoffs. The contribution is of high impact, as the dataset and scripts are available via GitHub and the conception and analysis is on YouTube. It will be a superb teaching tool in the lab, in addition to the value of the findings presented in the manuscript. My only comment for improvement is that there could be more emphasis on why the risk of splitting genomes using ASV is of greater concern than clustering multiple species into the same OTU.**

I am grateful to Reviewer 2 for their positive comments regarding the manuscript and the process of developing and writing the analysis.

**1. Accepting that genomes are artificially split by ASVs and this results in inflating "cluster" numbers and diversity, how does this impact downstream measures and interpretations? The data supporting this risk is robust; but what could be better emphasized / communicated is WHY this makes zero ecological sense and how it can impact interpretations. From the devil's advocate perspective (and ignoring biology), does it matter if diversity estimates are inflated if everything is relative?**

I have added text to the concluding paragraph to emphasize the problems with inflated cluster numbers in the context of making relative comparisons (L119-123).