

Rebuttals of Manuscript PCIMicrobiol #49 entitled: “Long-term sulphide mitigation through molybdate at shrimp pond bottoms”

The manuscript specified above has been revised. The remarks of the reviewers have been incorporated. We appreciate the effort of the reviewers and their suggestions have allowed us to improve the quality of the paper. Please find our replies to the comments of the reviewers in the section below. Some minor changes to enhance grammar and overall readability are not marked.

Legend to the layout of this document:

Comments of the reviewers (all, complete) are shown in a box in italics.

The replies of the authors to the comments of the reviewers are shown in plain text. Changes in the revised manuscript are referred to with the page number and starting line. ~~Deletions are crossed out~~ and **additions are shown in bold.**

Reviewer #2:

Reviewer #2 asked whether the "ANOVA was preceded by a study of the underlying distribution of the data to determine if it was indeed normally distributed." From the manuscript and from your reply it is not entirely clear what exactly you modelled in this case. From your reply, it seems that you compared the relative abundance of each OTU across all the biological replicates of a given treatment to conclude if they differ. If this is the case, the procedure is termed "differential abundance testing" and is non-trivial. There is a vast literature on the subject and many methods. Briefly, however, ANOVA is not suitable for such data primarily because it is compositional in nature and not absolute (see 10.3389/fmicb.2017.02224).

ALDEX2 (10.1371/journal.pone.0067019) and ANCOM-BC2 (10.1038/s41467-020-17041-7) are two of the most popular methods for running such tests these days.

However, I would argue that there is no real reason to test the difference between the biological replicates. Instead, you should run a differential abundance test between your treatments or days to see which phyla changed (ANCOM-BC2 is particularly suitable for running tests on higher taxonomic levels). This is optional since I believe that the manuscript can be recommended even without such a test. However, the ANOVA test should be omitted.

Answer: We agree with this comment from Reviewer #2, and we have removed the repeated measures ANOVA test from the manuscript (1)-(2).

- (1) Page 11 Line 230: ~~A repeated measures analysis of variance (ANOVA, aov function) was used to validate that the biological replicates showed no significant ($P < 0.05$) differences in bacterial community composition. Next, Absolute singletons were removed, and the different samples were rescaled via the "common-scale" approach (McMurdie and Holmes, 2014) through which the proportions of all OTUs were taken, multiplied with the minimum sample size, and rounded to the nearest integer.~~
- (2) Page 14 Line 304: ~~Repeated measures ANOVA revealed no significant differences ($P < 0.0001$) between the biological replicates.~~