Author’s response to reviews

Title: Vinasse Fertirrigation Alters Soil Resistome Dynamics: An Analysis Based on Metagenomic Profiles

Authors:
Lucas Braga (lppbraga@cena.usp.br)
Rafael Alves (rferrazalves@gmail.com)
Marina Dellias (madellias@gmail.com)
Acácio Navarrete (navarrete@cena.usp.br)
Thiago Basso (thiagobasso@usp.br)
Siu Tsai (tsai@cena.usp.br)

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Author’s response to reviews:

Reviewer #1: The authors have revised their manuscript to address my comments. Especially, the use of rigorous statistical tests has improved the quality of this manuscript. However, there is a serious inconsistency problem in results.

I compared Table 1 in the original manuscript and Figure 1 in the revised one, and found that (i) 24 out of 51 proteins (47%) in Figure 1 are not found in Table 1, and (ii) among 27 proteins observed both in Figure 1 and Table 1, 14 (52%) proteins have different relative abundance in V+ and V-. For example, RF0008 was more abundant in V- than V+ in Table 1, but more abundant in V+ than V- in Figure 1.

I don't understand how this is possible after just changing the way of testing. One of the analyses in the original manuscript and in the revised one may be wrong? I think this needs to be clearly explained before the manuscript is further reviewed.

Another drawback of Figure 1 is the lose of abundance change in terms of elapsed days, which was observed in Table 1 in the original manuscript. That may be one of important features showing the dynamics of resistome.

Authors comment:

>>> The first question provided by Dr. Kim in his previous analysis of the manuscript led us to dig further in order to bring out more details regarding the metagenomic datasets that were used:
“Reviewer 1#:

1. The authors used 18 datasets from total 57 in the MG-RAST server. It would be better if readers can find more information of those 18 datasets.”

>>> When we started to revisit/review the metagenomic datasets for further details regarding this specific question, we concluded that the dataset used as control treatment (V-) was not ideal. We noticed that the V+ dataset used received also basic fertilizations of nitrogen. Therefore, we thought that it would be appropriate to use as control (V-) the dataset which received also nitrogen but not vinasse, instead of the control utilized in the first analysis (presented in Table 1, in the original manuscript) that was not supplied with nitrogen. In this way we would effectively detect solely the effect of vinasse amendments, which is the central question of our manuscript. We have informed this point in the revised version of the manuscript by (i) providing more details about the datasets that were used and by (ii) correcting the topic “Availability of data and material” to inform the right collection of datasets:

>>>Revised manuscript (L71-L76):

“In the referred study, a greenhouse experiment was performed in which vinasse was repeatedly applied to sugarcane-cultivated soils in plastic pots (100 L) filled with 90 kg of soil (n = 3) treated with urea (450 g N kg⁻¹) at a rate of 60 kg N ha⁻¹ and mineral fertilizers (150 kg ha⁻¹ of P2O5 and 80 kg ha⁻¹ of KCl) following the basic recommendations for sugarcane field production [9].”

>>>Revised manuscript (L196-L199):

“The datasets supporting the conclusions of this article are available in the MG-RAST server repository, in http://metagenomics.anl.gov/linkin.cgi?project=10854. Files V9T7, V10T7, V11T7, V9T157, V10T157, V11T157, V9T217, V10T157 and V11T217 were obtained form the pots without vinasse.”

>>> So the dataset generated from pots V9, V10 and V11 are more appropriate to be contrasted with V+ instead of the datasets from V1, V2 and V3 as performed in the original version. Thus, the revised version of the manuscript is based on a more appropriate setup in which not only more rigorous statistical tests were used, but also more appropriate metagenomic datasets were chosen. Although we have informed the change in the revised version (L196-L199) we should have made it clear in our resubmission. To reinforce that, we are attaching a second version of the revised manuscript in which we rewrite the paragraph referring to treatments used in our analysis (Revised manuscript version 2: L71-L79). We do apologize for that and we are grateful to the reviewer earlier question’s, which helped us to improve and provide a more accurate analysis. We will be happy to provide any other explanation needed and hope that now our work can be considered for further review and possible publication.