

Response to Topic Editor

Topic editors' comments:

Topic editor: As indicated by the reviewer, the authors have successfully addressed the comments and suggestions raised previously. There is still a pending issue related to the linear mixed modelling introduced in this last revised version and a couple of minor edits that need to be addressed before accepting the manuscript.

A: Thanks for the feedback. We have taken into consideration all the critical points raised by the topic editor. Detailed responses are provided below.

1. Regarding the Linear mixed modelling, in agreement with the reviewer, there is something that is not clear: The use of random variables in the modelling can help address the limitations of the experimental design to a certain degree: There are no statistics that will remove the pseudoreplication that is derived from the experimental design, since you have only 1 plot per type (crop × fertilization rate) and 3 sampling spots within the plot. In fact, the sampling spots might be less than 5 meters apart? So that is effectively a pseudoreplication.

However, experimental sites like this have their value, and we have to take them as they are. Regardless of this issue, using random variables can improve the robustness of the analyses. You indicate that sampling date was used as a random variable to account for repeated measurements, which is ok. But also that different fertilization rate was used to account for the spatial structure. The problem is that you cannot use the same variable as fixed and random effect. If you are testing for the effect of fertilization rate, how can you include it as a random variable too? I wonder if what you used as a random variable was actually the plot ($n=12$), so that the 3 sampling spots are grouped. This way the model would account for the spatial structure (12 plots, 3 spots per plot). You would need to clarify and correct this issue if needed.

It would also be adequate that the lack of proper replication, and thus the limitation of the study, is briefly acknowledged in the discussion.

A: We have revised the manuscript and have now used only temporal (sampling dates) effect as a random effect in the linear mixed-effects model. We used it to test statistical differences between N_2O emissions of different fertilization rates in plots with different crop types. The experiment was organized into 12 plots in a systematic block design (Figure 1 in the manuscript) with three sampling spots per plot. We must take into consideration that replicates of sampling might not be statistically independent since the experiment had 1 plot per type (crop type and fertilization rate) and three samples were taken within the same plot.

We have revised the Discussion by adding the limitations of the study to the text as follows:

“However, it is important to consider that the sample replicates may not be fully statistically independent, as the experiment involved only one plot per treatment combination (crop type × fertilization rate), with three samples taken within the same plot.”

Response to Anonymous Referee #3

Reviewers' comments:

Anonymous Referee #3: In this version of the manuscript “Interactions of fertilisation and crop productivity on soil nitrogen cycle microbiome and gas emissions”, the authors have addressed most of the critical comments that were raised in the review of the previous version, thus improving its quality.

A: Thank you for the valuable feedback.

1. Anyway, there is one aspect, not mentioned in the preceding version, on which I would kindly ask for clarification. In fact, in contrast with the stated objectives of this work, and with the way results are presented, in this version of the manuscript it is specified that, for statistical analyses, “For N₂O emissions and gene parameters, spatial (different fertilisation rate) and temporal (sampling dates) effects were used as random effects. For N₂ emissions, spatial effect (different fertilisation rate) was used as a random effect” (L.s 214-216). I don't understand why these factors would be considered as random effects and, for the way results were presented, I'm given to understand that probably they weren't. Therefore, I think there might be a possibility that this could be a mistake in the text. If so, I think it should be addressed. If this is not just a mistake in the text, in my opinion, there would be a contrast between statistical analyses and results. Therefore, I would kindly ask the authors for further explanation on this point.

A: We have excluded spatial (different fertilisation rate) effect as random effect from the linear mixed-effects model. Now, only the temporal effect (sampling dates) is used as a random effect in the linear mixed-effects model.

2. L. 292 “expect” I think that this is one is a typo.

A: Done! We have corrected this error.

3. L. 311 “genetic parameters” I don't think that this phrase is fitting in this context to describe the abundance of microbial groups.

A: Thank you for the comment! We have changed the subtitle as follows:

“3.4 Relationships between environmental parameters, gene abundances and ratios, and N emissions”