Dear editor,

We would like to thank you and the two reviewers for the time you have taken commenting on our manuscript. We also would like to apologize for the delay in providing our responses to the reviewers' comments. Please find below our point-by-point reponses to the two reviewers' comments.

Reviewer 1 :

This is a very interesting and well written paper, dealing with the comparative effects of heat, humidity or pesticide applications on soil microbial community. Results are relevant since they show mostly effect of heat as compared to pesticides, and no legacy effects of previous applications. It would have been better to use at least two different soils, but I acknowledge the complexity of the experimental plan already for one soil.
It can be accepted after a minor revision of the points below.

A: Thank you very much for your kind words.

Introduction
In the first paragraph of the introduction most focus on soil microbial roles is on N cycle; please be broader
One of the main hypothesis of your work is to compare effects of climate change with pesticides application. You should mention in the objective section of the manuscript.
A : Agreed, we modified the corresponding paragraphs accordingly.

L. 44-47 : « *Soil microbial communities are major drivers of nutrient cycles that sustain plant growth and productivity. The nitrogen cycle (N-cycle), which regulates the availability of N in soil, is carried out by specific microbial guilds (Dominati et al., 2010; Aislabie and Deslippe, 2013; Whitman et al., 1998; Singh, 2015).”*

L.100-103 : *« Within this context, the objectives of the present work are, therefore, to compare effects of global change-related environmental disturbances and impacts of pesticidal active ingredients on soil microbial community structure and functioning and to evaluate their compounded effects.*"

Materials and methods
When you refer to 250 microcosms it is not clear if you refer to single replicates or treatments. Please specify, stating the number of replicates per treatment studied.

A: 250 microcosms refer to the total number of microcosms for our study. All treated and control microcosms were replicated 5 times as indicated in l. 144-145 and in Fig. 1.

L125. Is 25% total humidity or 25% of water holding capacity? I guess the first (but specify it). It is also important to indicate the %of WHC, since it is a pivotal parameter for soil microbial activity.

A : It is indeed the initial soil moisture, expressed as total humidity, and it corresponds to ~40% of WHC. This is modified in the revised manuscript.

L. 119-120 : *"The soil humidity after sieving was 26% which corresponds to ~ 40% of the WHC."*

Given the complexity of the experimental plan I suggest producing a figure summarizing it.

A : We provided a figure as Supp Fig 1 detailing the experimental set up. According to the reviewer's comment we have decided to include it in the main manuscript as Fig.1.

L207. It is not clear from what you write if you set 94% for OTU identification, which is weird (97% is usually applied)
A : We set the OTU picking threshold to 94% because we include in all our sequencing runs a mock bacterial community for which we know exactly the composition. Therefore, we use 94% because it is the threshold that allows us to retrieve the expected number of OTUs at the genus level.

Results
L300. Please provide info on the taxonomical affiliation of these 12 OTUs
A : This information is now provided in the text.

Discussion
You should discuss the limitation related to having studied a single soil. Conclusions should be taken with caution.

A : Agreed. We have added a sentence at the end of the discussion section pointing that out.

L. 413-416 : *« Future studies should be conducted in different soils with various physico-chemical properties to generalize our conclusions. Also, consideration of denitrifiers and N_2O emitters would"*

Reviewer 2 :

Title and abstract

I think the title is a good reflection of the article's content, but I find the term soil microbial endpoints a little vague. Given that a significant part of the article deals with the N-cycle, you might consider mention it in the title.

A : The reviewer is correct. The title has been revised accordingly.

The summary is clear on the context, methodology and results. I find, however, that the result part deserves a little more substance in comparison with the context and methodology.

Introduction

I find the structure of the introduction a little confusing. For instance: the introduction starts by describing the importance of the N cycle. Then comes a paragraph on environmental

pressures, a paragraph on pesticides, a paragraph on ecosystems and finally a paragraph on the context of your study. The introduction should be structured more from the general to the specific, and should be reworked. It's a formal remark, but I'd start with a section on microbial ecosystems, then talk about environmental and toxicological pressures, and finish with a section on how and why it's relevant to focus on the N cycle. For exemple, I think the paragraph on climatic disturbances (L55) and ecosystems (L88) should be combined.

A: We acknowledge that the introduction structure proposed by the reviewer would also be relevant however we think that our introduction structure is equally appropriate.

We first describe our studied ecosystem (soil) and function (N cycle), then we move to the effects of environmental disturbances on soil microbial communities, followed by pesticide impacts. Finally, we review the literature about compounded effects and what could be expected when combining disturbances of different nature before detailing our objectives and hypotheses.

The subject is well defined. Perhaps it lacks a few more elements on why focusing the N cycle is relevant. Why this one rather than another endpoint? The question should be answered beyond the fact that the study is also focusing on the effects of pesticides at agronomic concentrations (and that I understand that the study is part of a more global context of agriculture). More precise information about the climatic disturbances is also needed with regards of your study. You cited an exemple for heat but there is no information about humidity. This should be mentioned in your paragraph L55.

A: A sentence about 'the importance of the role of soil microbial communities on nutrient cycles that sustain plant growth and productivity' has been added in lines 45 and 46.

About pesticides, more information about the concentrations used on a crop cycle should be adressed. This would allow the reader to better situate your concentrations in a more global context other than 1x or 10x and before reading the methodology section.

A : the annual dose of application of each a.i. has been added. The dose applied in the soil microcosms has been calculated by considering the annual dose of application, 10 cm depth and mean soil density of 1.3 t per m3.

Finally L107, key microbial community members for ? On N cycle I supposed, worth mentioning it.

A : Yes, this is now mentioned at the end of the introduction.

Materials

Methods and analyses are sufficiently detailed to allow replication by other researchers, and statistical methods and analyses are appropriate and well described. The experimental design is ambitious, a schema would be useful for the reader. But I understand that it's not an easy task, given the design.

A: According to this and a similar comment from reviewer 1, we have decided to move Supp Fig 1, describing the experimental design, to the main manuscript.

I would like to see more informations about your sampling site. pH, physico-chemical characterization, temperature and humidity during the sampling season? This would make it easier to justify your temperature and humidity parameters later,

A : More informations about the sampling site is now provided in the text.

For exemple, why a 42-degree heat wave? Is it because such a heat wave happened on your study site (L124)? L125, was humidity monitored during heat waves?

A : 42 degree was chosen because it is a realistic maximal temperature at the sampling site, but also because it has been referenced in the literature as a heat-stress temperature in other studies (Calderon et al., 2018). Humidity was monitored over the entire experiment and was adjusted after the disturbance cycles to 22% total humidity.

L131, I am working on metals so maybe my remark is not relevant, but why these 3 pesticides? Are they commonly used and are they representative? The point should be addressed. L142, no homogenization, but I imagine the volume of the solution was sufficient to distribute the pesticide dose?

A: These specific pesticides (herbicide: clopyralid, fungicide: pyraclostrobin and insecticide: cypermethrin) were chosen because they can all be applied during the corn cropping cycle and are quite commonly used to protect corn in Europe. The reviewer is correct, all a.i were applied with 1mL of water which is sufficient to ensure an homogenous distribution of the solution in the microcosms.

L144-146: "*These three a.i are all constituents of commercial formulated products commonly used in agriculture, and can be all used in corn cropping during a growth cycle."*

Results

First, i have a general remark on graphics, the tables are huge. I understand the difficulty to make some clear figures regarding the experimental design (e.g. number of conditions, sampling dates, number of endpoints, etc) but because all your results are not significant, a figure focusing on your endpoints regarding the heat condition would be welcomed.

A: See below for a detailed response to this comment.

The rest can be left in a table. If not, at least highlight the significant differences (more than just an asterisk, maybe with colors?).

A : Significant differences have now been highlighted in bold in all tables.

It will really help the reader to directly see that the major effect in your study is the heat wave but not necessarily for all your endpoints and above all, in comparison to your rainfall conditions. Your standard deviations are quiet low so I imagine that boxplots are not the best choice but maybe histograms? Because all the endpoints do not show significant differences (e.g. alpha diversity), these parameters may not be included in order to simplify the

representation. For pesticides, a table is fine because even you have some tendencies, few significant differences are observed.

A: We wanted to focus our message on the global picture rather than on specific variables. This is why we preferred to keep in the main manuscript figures that were based on multivariate data. Also, the length and the direction of the arrows on the PCA figure directly inform about the size of the effects for each individual endpoint. Therefore, adding boxplots or histograms would be redundant to the data already reported in the manuscript.

Another point, why pcoa (L259) after all your endpoints ? Because some of your endpoints are specific to some groups, why not describe the pcoa and so, the general bacterial compositional changes first and second, more deeply with your N cycle endpoints?

A : The logic behind this choice is to put more emphasis on data that are more meaningful in terms of biological interpretation. We wanted to answer first whether the treatments impacted the functioning of the community, and then whether it would translate into compositional changes.

L286, I found the sentence confusing. You say considering all individual endpoints so are you talking about the table 2 ? In your table 2, I don't find statistical differences on AoA, ComaA, ComaB etc. Are you refering of table 3 ? Please precise, I think I am confused by the term individual endpoint in your sentence.

A: Sorry for the misunderstanding. The reviewer is correct, there is no significant difference in pairwise comparisons for AoA, ComaA and ComaB when compared to the untreated control microcoms, however a significant amount of variance was attributed to the Pesticide_Dose and/or Pesticide_Dose-by-Time effects in the ANOVA model for those specific variables. This indicates that there might be significant differences between different pesticide treatments but this was not the focus of our study.

L295, again, why presenting the results of pcoq after your targeted endpoints ? It seems more logical to me to present first the overall community and then describe more precisely the changes observed. I also wonder why but you don't put more emphasis on the repartition on your abundance data. At least one sentence should be added to describe the distribution of your 3 groups with regard to the different pressures exerted in comparison to the control. This is relevant information for microbial ecosystem in general but also for studies interested in other endpoints and functions.

A: we cannot figure out what was the point made here so we did not answer to this comment.

Discussion

L327, commonly used. This information is not present before.
A : This information has been added in the Material & Methods section.
L144-146: "*These three a.i are all constituents of commercial formulated products commonly used in agriculture, and can be all used in corn cropping during a growth cycle."*

L334, does the cited study analysed other parameters like microbial abundance ? Yes it's a shift in the community however your table 1 show also a decrease in microbial abundance especially with a factor 2 for ITS and 18S but not so much for 16S. Do you have an hypothesis ?

A : In Calderon et al., the authors did not investigate the impact of heat disturbance on ITS and 18S abundances. An hypothesis for the absence of recovery for these groups is the longer generation time of these groups and the relative short duration of the experiment (60 days). We could hypothesize a possible recovery for these groups at a longer term.

L342, you observe higher AoB abundance in the heat disturbed samples compared to the control, I found the term sensitivity non-ideal to highlight the augmentation in the AoB abundance in your heat disturbed condition. It can be a direct or an indirect effect, you just have abundances to support your hypothesis but the ComaA and B seems to decrease. AoB communities could just take advantage of the lower abundance of species more sensitive to pressures explaining the lower functional redundancy.

A: The reviewer is correct. We decided to replace 'sensitivity' by 'tolerance' in the manuscript.

L351, as mentioned previously, the discussion regarding the effect of pesticides should put into perspective with the concentrations used in your study. Yes you have not significant effects but is this in agreement with the literature and from what thresholds have other studies seen effects ? Are there mechanisms for the degradation of these molecules that can take place over days, particularly in conditions of high heat or high humidity? No informations about this point are present in the article. Maybe the remark isn't relevant, but in my case, I work more with inorganic contaminants. More details would be interesting for this type of audience.

A: As mentioned earlier we have chosen to apply to soil microcosm the annual agronomical dose of each a.i. (x1) and the worst-case scenario (x10) corresponding to misuse (error in the dilution of formulated pesticide on farm) or to the accumulation of the a.i. in the soil following repeated application a.i. DT50 of these three a.i. are in the same range (clopyralid dt50=40 days; cypermethrin dt50=91j and pyraclostrobin dt50=60 days). Even if the evolution of a.i. was not measured, we can hypothesize that it was fully removed at the end of the incubation. Here we report no effects of a.i. in our conditions of exposure as compared to a control treatment (not exposed). To our best knowledge, the evaluation of the ecotoxicological impact of a.i. on microbial endpoints is done by comparison to a control not exposed to the a.i. For a given endpoint there are no threshold set up to estimate the importance of a possible effect as the normal operating range of microbial endpoints are not known. From the regulation point of view, up to 30% decrease of a given endpoint can be accepted if this effect does not last more than one year.

L367, at which concentration so ? Is the gap important regarding the concentrations used ?

A : The cited studies were not conducted with the same active ingredients, but were designed with the same logic (1X, 10X or 100X the agronomical dose, which directly depends on the studied pesticide).

L 368, This might be due to the large variability observed between biological replicates? Please precise your idea. If I look your tables, I found your standard deviations on your mean endpoints below the 10%, not a huge variation to me. Need more contextualisation.

A : Here we mentioned bacterial community composition, for which biological variation between replicates is stronger than the variation induced by the pesticide treatments (see Figure 4, no clustering by color).

L373, I don't understand the sentence without more information on the cited study. Toxic metals deriving from pesticides so the study tested the toxicity of pesticides containing metals in the formula or pesticides and metals in mix? It's unclear to me why you cited this, you should add a sentence to specify what you are really meaning.

A : The reviewer is correct, the sentence was very poorly worded. The cited study actually mentioned that the relative abundance of acidobacteria in soils can be an indicator of soil landuse changes because the abundance of this group was significantly lower in farmland subjected to metal contamination, that might be linked to the use of metal containing pesticides, compared to forests.

L 383-385 : Previous studies described this phyla to be a good biological indicator of land-use change from forest to farmland because of its sensitivity to various toxic metals, potentially deriving from metal containing pesticides (Kim et al., 2021).

L400, there is too little discussion about the impact of your results on a large scale? I understand that ultimately showing few effects of pesticides on your parameters is surprising and not what was expected but the effect of heat is clear, what are the repercussions of your research? Why is this innovative and important?

A: See below for an answer to this and to the next comment.

I would have liked more contextualization of your results with regard to the general context. What are the implications for the environment and agriculture or the issues described in your introduction? L409, your perspective is interesting but what would allow us, apart from the fact of a better understanding, what impact? and in relation to other functional soil processes? Especially in the context of global change.

A: The heat effect, *per se*, is not surprising since several studies, cited in our manuscript, already demonstrated that heat disturbance can induce strong changes in soil microbial communities. On the other hand, an absence of effect of pesticides at agronomical doses, is also not very surprising. What is novel and important here, is the combination of an environmental disturbance and pesticides treatments.