Peer Community In
Ecotoxicology & Environmental Chemistry

Impact of environmental disturbances and pesticides on soil microbial communities involved in the Nitrogen cycling

Abdulsamie Hanano *and* ***Christian Mougin*** *based on peer reviews by* ***Edoardo Puglisi***, ***Vincent Laderriere*** *and 1 anonymous reviewer*

Camilla Drocco, Anja Coors, Marion Devers-Lamrani, Fabrice Martin-Laurent, Nadine Rouard, Aymé Spor (2024) Evaluating the Effects of Environmental Disturbances and Pesticide Mixtures on N-cycle related Soil Microbial Endpoints. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. https://doi.org/10.1101/2024.01.22.576671

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Soil microbial communities play a crucial role in maintaining ecosystem health, driving key processes such as nutrient cycling, organic matter decomposition, and soil fertility. However, these microbial populations are highly sensitive to environmental changes and chemical stressors, including pesticides. The preprint "Evaluating the effects of environmental disturbances and pesticide mixtures on soil microbial endpoints," provides valuable insights into how soil microbial communities respond to environmental fluctuations and pesticide exposure (Drocco et al., 2025). By integrating experimental soil microcosms with targeted microbial assessments, the study offers a comprehensive view of the resilience and vulnerability of soil microbiota under multiple stress conditions.

The study aimed to assess how temperature and humidity fluctuations, along with pesticide exposure, impact soil microbial communities. A total of 250 soil microcosms were subjected to three different environmental conditions: heat disturbance, high humidity simulating heavy rain, or a control with no disturbance. Following a three-day recovery period, the microcosms were exposed to different pesticide active ingredients—clopyralid (herbicide), cypermethrin (insecticide), and pyraclostrobin (fungicide)—either individually or in combination at standard (1x) and elevated (10x) agronomic doses.

By evaluating microbial endpoints related to diversity and community structure, the researchers were able to determine how environmental disturbances and chemical exposure influence soil microbial functions

(Bacmaga et al., 2015). Of particular interest was the focus on microbial guilds involved in nitrification, a critical process for soil nitrogen cycling and agricultural productivity (Dominati et al., 2010).

The study's findings reveal a complex interplay between environmental stressors and pesticide exposure on microbial communities. Some key observations showed that heat and high humidity significantly altered microbial diversity and composition before pesticide application. This suggests that climate-driven disturbances can precondition microbial communities, potentially influencing their subsequent responses to chemical exposure. Moreover, the pesticide effects depend on dose and combination, while individual pesticides had measurable impacts on microbial endpoints, their effects were amplified when applied in mixtures or at elevated doses. This underscores the importance of considering real-world pesticide applications, where mixtures are commonly used. Furthermore, the results indicate that the microbial guilds involved in nitrification appeared to be disproportionately affected by pesticide exposure, raising concerns about long-term soil fertility and nitrogen availability in treated soils.

These findings have significant implications for sustainable agriculture and soil health management. Understanding how soil microbiota respond to environmental and chemical stressors can inform strategies to mitigate negative impacts, such as adopting precision agriculture techniques, improving pesticide formulations, and implementing soil conservation practices.

Despite its valuable contributions, the study has some limitations. The controlled microcosm approach, while useful for isolating specific variables, may not fully capture the complexity of field conditions. Long-term effects of pesticide exposure were also not assessed, leaving questions about microbial recovery and ecosystem stability over extended periods. Future research should focus on field-based experiments and long-term monitoring to validate and expand on these findings.

In conclusion, the current study highlights the intricate interactions between environmental stressors and pesticide exposure on soil microbial communities. By leveraging a robust experimental design and providing open-access data and statistical scripts, the research enhances our understanding of soil microbial dynamics and their implications for agricultural sustainability. As climate change and intensive pesticide use continue to shape soil ecosystems, such studies are essential for developing resilient and sustainable soil management practices.

References:

Bacmaga, M., et al., 2015. Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican + mesosulfuron-methyl + iodosulfuron-methyl-sodium. Environ Sci Pollut Res Int. 22: 643-56, doi: 10.1007/s11356-014-3395-5

Dominati, E., et al., 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecological Economics. 69: 1858-1868, doi: 10.1016/j.ecolecon.2010.05.002

Drocco, C., Coors, A., Devers-Lamrani, M., Martin-Laurent, F., Rouard, N., Spor A. 2025. Evaluating the Effects of Environmental Disturbances and Pesticide Mixtures on N-cycle related Soil Microbial Endpoints. ver.3 peer-reviewed and recommended by PCI Ecotoxicology and Environmental Chemistry doi: 10.1101/2024.01.22.576671

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2024.01.22.576671
Version of the preprint: 2

Authors' reply, 06 March 2025

Dear Recommender,

We corrected the last minor spelling errors throughout our manuscript. A new revised version of the manuscript has been uploaded to biorXiv.

Thanks again for your time and consideration,

Best,

Aymé

Decision by Abdulsamie Hanano and Christian Mougin, posted 24 February 2025, validated 03 March 2025

Dear colleague

Thanks for condisering the last comments of the reviewer and submitting an updated version of the preprint.

Best regards

Christian

Reviewed by Vincent Laderriere, 17 February 2025

I am okay with the authors' justifications and choices, it can be accepted. Ambitious experimental design and a nice paper!

On your answer on my last comment: 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.

Sorry for the misunderstanding, my comment was not clear. I just wanted to point out that an additional sentence that recontextualizes your findings in the context of the lack of data on the effects of pesticides in cocktails would be great. A bit like you did at the end of paragraph L398 with the following sentence: "Hence, we can conclude that the present experiment confirms the findings of Cedergreen (2014) as no exceptionally stronger effect was found in the mixture treatments (pointing at a potential synergistic interaction) compared to the single a.i. treatments." The fact is that you had significant effects on your endpoints at agronomical dose, as highlighted L375. I was thinking of confounding factors such as the physico-chemical characteristics of the soil or the presence of contaminants other than pesticides, parameters which vary in the case of a field study. A sentence with a reference emphasizing his point would have been relevant, in my opinion. But on second reading, I think it's clear and you added a sentence L413-416 regarding the limitations so it works for me. Results are well presented and commented.

Just few additional remarks,

L163 colorimetry?

L178 and 182 the thermal cycling conditions

L185 then is misspelled

L276 did not induce

L376 abovementioned without space

In addition, I don't know if the number of keywords is limited, but maybe heat stress can be added? This would allow better referencing of your study, which shows a strong impact of heat. Also, you're interested in the N cycle as a soil function, perhaps a keyword with the idea of soil functionning would be nice and may be more precise than ecotoxicology. Up to you

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2024.01.22.576671
Version of the preprint: 1

Authors' reply, 09 January 2025

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Decision by Abdulsamie Hanano and Christian Mougin, posted 12 June 2024, validated 12 June 2024

Request for a revised version of your preprint

Dear Dr Spor

Thank you again for submitting your manuscript to PCI EcotoxEnvChem. We have now received two reviews of your manuscript, and are sorry for the delay. We would be pleased to receive a revised version of your manuscript, including a point-by-point reply to comment and suggestion made by the reviewers.

Best regards,
Christian Mougin

Reviewed by Edoardo Puglisi, 03 June 2024

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.

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.

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.
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.
Given the complexity of the experimental plan I suggest producing a figure summarizing it.
L207. It is not clear from what you write if you set 94% for OTU identification, which is weird (97% is usually applied)

Results
L300. Please provide info on the taxonomical affiliation of these 12 OTUs

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

Reviewed by anonymous reviewer 1, 12 June 2024

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.

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.

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. 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.

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

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

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? 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. 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.DiscussionL327, commonly used. This information is not present before.

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 ?

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.

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.

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

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.

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.

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?Conclusion

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.