

Biogeosciences Discuss., author comment AC1  
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## Reply on RC1

Laura Sereni et al.

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Author comment on "To what extent can soil moisture and soil Cu contamination stresses affect nitrous species emissions? Estimation through calibration of a nitrification–denitrification model" by Laura Sereni et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-265-AC1>, 2022

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***Does the paper address relevant scientific questions within the scope of BG?***

***R1 Yes, the topic is highly relevant to BG, encompassing physical, chemical, and biological processes as they impact nitrogen transformations in soil and subsequent emissions.***

**Does the paper present novel concepts, ideas, tools, or data?**

**R1 Yes, to my knowledge, the combined focus on impacts of copper on nitrogen transformations and integration of those impacts into an existing biogeochemical model is novel.**

Response: Thank you.

*Are substantial conclusions reached?*

**R1 Unsure. I am not entirely clear on what the authors' conclusions are, so I would request clarification of the conclusion section.**

Response: In accordance with this remark, we focused the conclusion with the main points raised in this study, that are the ability of the new DNDC-Cu version we performed to reproduce soil nitrates stocks for contaminated samples. Now the l.480-484 reads "). Based on a 3-day bioassay measuring soil NO<sub>3</sub>-N over time, we were able to adjust the DNDC model to take into account the Cu effect on soil N emission. The DNDC-Cu version we proposed was able to reproduce the observed Cu effect on soil nitrate stock with R<sup>2</sup>>0.99 and RMSE<10% for all treatments in the DNDC calibration range (>40% WHC)." .We also underlined that different decreases in N-species emissions when Cu concentration increases depending on the previous moisture. Now l.486-487 reads We showed that the effect of soil Cu contamination was different among moisture treatment and N species. And l.499-500 reads "as both contamination and rainfall patterns affect in a different way the soil NO<sub>x</sub>-N and N<sub>2</sub>O-N emissions."

*Are the scientific methods and assumptions valid and clearly outlined?*

**R1 The overall approach is valid. It is difficult to integrate experimental and modeling work, and this work does that well.**

Response: Thank you

**My most serious concern with the approach is that the bioassays investigating the effect of copper on nitrification were only conducted on soil that was not previously contaminated with copper. As microbial communities are quite adaptive, one could reasonably expect that there would be different results if the experiments were conducted on soil with a history of copper contamination. Indeed, there may already be studies on that topic; if so they would be relevant to incorporate. The question then becomes which situation is most relevant globally – short-term effects upon introduction of copper (such as the 3 days of exposure tested here), or longer-term consequences of contamination. Given the authors' focus on improving predictive capability of continental models under climate change, I'd expect longer-term effects would be more relevant.**

**It would also be helpful to provide more justification for the selected soil type and the copper concentrations used here. Consideration of bioavailable copper would also seem appropriate and might allow for conclusions that are more generalizable to other soils.**

Response: Indeed, you're right, and we agree that there would be different results if the experiments were conducted on soil with a history of copper contamination. The importance of chronic vs. abrupt contamination in ecotoxicological effect is actually debated. Indeed, some authors found that thresholds values for field contaminated soils are around 3 times largest than for spiked soils (Smolders et al., 2008) while others found no effect of aged contamination on bacterial structure, bacterial respiration or nitrification (Oorts et al., 2006; Brandt et al., 2010). These references and discussion upon history contamination have been added lines 83-86 to better justify our experimental setup ("It is not straightforward to assess that abrupt contamination can lead to distinct effects on microbial structure of functions than progressive contamination" ).

We actually focused on the scenario to follow the effect of two successive stresses of moisture and contamination on nitrification potential as an example of double (climatic and contamination) stress. Also, we hypothesized that first moisture stress will differentially select microbial communities involved in the nitrification/denitrification processes thus underlying the combined effects of these two stresses on soil functions. Furthermore, The DNDC model is also used to assess the short term effect of some cultural practices (Shah et al. 2020 <https://doi.org/10.1016/j.scitotenv.2020.136672> , Deng et al. 2016 <https://doi.org/10.1002/2015JG003239> ...) corresponding to our bioassay where no Cu ageing is involved ???. In this context of successive stresses, we needed a control not contaminated with copper. However, finding experimental sites where the only changing factor is copper contamination is very difficult. Moreover, copper contamination in the field is almost always associated to other contaminations. To avoid these discrepancies, we chose to contaminate ourselves the soil by spiking a non-contaminated soil. This is a classical method to study copper contamination effects through bioassays (Oorts et al., 2006; van Gestel, 2012).

Considering your remark about bioavailable copper, we actually made measurements of Cu contents in solution as a surrogate of the Cu available pool. But when writing the manuscript, we found that these values did not help us for additional arguments. However, you are right, it is legitimate to ask the question of the balance of copper in solution. Thus, according to your remark, we added a supplementary Table with these values that is now introduced lines 147-148 and reads ". Cu in solution values are provided in Suppl. Table 1.". Finally, our study took into account the fact that survey used for modelling are much often provided in terms of total Cu rather than of Cu in solution.

*Are the results sufficient to support the interpretations and conclusions?*

**(See comment on conclusions above)**

**The experimental results seem not to be considered as leading to any conclusions. For the sentences in the conclusion about N<sub>2</sub>O, and the last sentence of the abstract, I do not find sufficient support in this paper. N<sub>2</sub>O was not measured here, and DNDC has difficulty predicting the proportion of N<sub>2</sub>O from denitrification (e.g. <https://doi.org/10.1016/j.scitotenv.2018.07.364>), so it seems quite speculative to make these conclusions based only on results from a model that was only calibrated and validated with respect to nitrate. I have similar concerns about conclusions about the abundance of denitrifying bacteria, particularly since they are typically facultative and thus may not directly correspond to the concentration of nitrate. Modeling is not my core expertise, as is probably evident from my comments. However, to conclude that a new model performs better, I would typically want to see it compared to calibrated versions of existing models.**

Response: Thanks for your remark. We acknowledge that the measurement of NO<sub>3</sub> only is a limitation of our study, this is why we noticed at the previous lines 410-412 that this study did not allow us to determine if the double stress rather affect nitrification or denitrification ("However, the experiments performed here did not allow us to determine if the soil Cu contamination rather affects nitrifying bacteria (e.g. decrease in nitrifying activity and in NO<sub>3</sub>-N production) or denitrifying bacteria (e.g. increase in denitrifying activities and NO<sub>3</sub>-Nconsumption)").

Following your remark, we now underlined this difficulty in DNDC to reproduce N<sub>2</sub>O emissions in lines 414-417 that now reads: "Also, our modeling approach of N<sub>2</sub>O-N, N<sub>2</sub>-N and NO<sub>x</sub>-N production in the contaminated context could have been more constrained with measurement of denitrification rate to assess the effect of Cu on proportion of production and consumption of NO<sub>3</sub>".

Also, the 4.3 paragraph (previously 4.2) deals with the difficulty in upscaling for modelisation of bio-physical processes. However, the calibration of the model for an absolute estimation of N<sub>2</sub>O/NO<sub>x</sub> was not the purpose of our work as we aimed at estimating the relative effect of the double stress on N<sub>2</sub>O/NO<sub>x</sub> emissions. Indeed, all the Figures showed emissions rates in function of Cu concentration. Nevertheless, to underline these aspects, we decided to include in the new version of the manuscript, the Supplementary Fig. 1 (now introduced lines 313-314) showing the difference between modeled and measured NO<sub>3</sub> concentrations for the initial DNDC version and for our DNDC-Cu version, for each Cu concentrations.

*Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)?*

**I have several questions about how the experiments were conducted. No replication is mentioned in 2.2, but data from replicates is given in Table 1. In addition to having these be consistent, I'm wondering if the samples were from replicate microcosms or from replicate samples taken from the same microcosm. How was sampling conducted? In particular, were microcosm soils mixed before sampling for measurements and bioassays? I'm not clear about how the drying was done, or how long it took for microcosms to reach the drought/dry state. What temperature was used for the microcosm incubation? How long was the microcosm incubation? Section 2.2 says 45 days, but other places refer to one month and five weeks. This also relates to another question – were the drought and dry-rewetting treatments at a dry or wet state for their final week of incubation before the bioassays? When was  $\text{NH}_4^+$  added to the bioassays, and at what concentration? I'm not clear on how much liquid was in the bioassays, not sure how to interpret soil solution 1:12. Does this mean that the approximately 5 g of soil was mixed with 60 mL of liquid? Did you account for the moisture differences, or simply take 5 g wet weight from the relevant microcosm? Line 124 and 130 contradict. Line 285 gives yet a third description. On the modeling side, I have an even greater level of confusion. In the abstract it states that DNDC was used, with modifications to account for soil copper effects. Yet in the methods it states both DNDC and a model by Zaehle and Friend ... which are two different models ... and later it states that the model was written in R, which suggests that DNDC was not used at all. Given this fundamental confusion, I have not done a detailed review of section 2.3. It would be helpful to have more clarity on which equations are taken directly from previous models and which have been modified in this work.**

Response: Thank you for pointing out the inaccuracies in the experiment's description. The initial incubation procedure is described in the first paragraph in 2.2 (now l.111 to 121 starting with "For the 5 weeks' initial incubation, five microcosms were » while the second paragraph now (l. 122 to 149 " At the end of the initial incubation period, we performed a nitrification bioassay") describes the bio-assay. In line 122 we now mention that the bio-assay was made in triplicate, using 3 replicates taken from one microcosm per initial incubation condition. For clarity, the number of dry and wet periods have been clarified l.116-121 and reads ". One, thereafter called "Drought" (or DO), started with one week at 60% WHC and then the soil was left for 3 weeks without added water to mimic a dry period until 10% of the WHC before rewetting at the initial 60% WHC. The other, thereafter called "Dry-rewetting" (or DR) encountered 2 cycles of one-week near-saturation period (90% WHC) followed by one-week dry period (10% of the WHC) ending by one week near saturation period. Drying was performed by natural evaporation (gentle air-drying at the laboratory temperature, i.e. 20°C). and controlled by weighting. ". The 5 weeks initial incubation period the DR treatment ended by a near-saturation period. Also, we added more information about the amount of water used for bioassay, the soil solution ratio and the sub-sampling of the different incubated microcosms (now l. 123-127 that's reads "Bio-assay consisted in nitrate production measurement over a short-term aerobic incubation in soil slurries (ratio soil:solution 1:10) with ammonium in excess and in the presence of gradients of Cu. Briefly, 3.5 g of fresh soil (approximately 3 g of soil equivalent dry weight), were mixed in a 50 mL Falcon® tubes with 29mL of a 10 mM HEPES buffer solution (hydroxyethyl piperazineethanesulfonic acid, Sigma-Aldrich, France) to maintain a constant pH"). The 5g mentioned refers to the fresh weight at the moment of sampling while for the bio-assay we took only 3.5 g (corresponding to 3g in a dry-

weight basis) of each moisture treatment then added water. This is also added in the new version of the manuscript at line 124-125. Considering the  $\text{NH}_4^+$  for the bioassays it was added at the beginning of the bioassay in a large excess (3mM). This precision has been added l. 128 and reads "containing the substrate  $(\text{NH}_4)_2\text{SO}_4$  (3 mM) (Sigma-Aldrich, France).".

Considering the model, we used a simplified version of the DNDC of Changsheng Li et al. (1992) that have been adapted by Zaehle & Friend (2010b). This simplified version was written in Fortran and to facilitate the manipulation, we rewrote it in R. Also, equations came directly from the DNDC model with two modifications concerning the k value and the Cu effects through the equations numbered in the text Eqs.13, 28-31 that have been added. We hope that now l.225 is clearer with the specification introduced ("We wrote the model adapted from Zaehle & Friend, (2010) in R...").

*Do the authors give proper credit to related work and clearly indicate their own new/original contribution?*

**There is a wide body of literature on the effects of copper on soil microbial communities, which is not really incorporated here. Also many studies looking at the effect of moisture on N cycling in soil. These omissions make it difficult to make a clear argument for the novel contribution here.**

Response: This is now done. We added 8 references ((Butterbach-Bahl et al., 2013; Galloway et al., 2008, Baath et al. 1989, Schimel, 2018; Stark and Firestone, 1995, Borken and Matzner, 2009; Fierer et al., 2003; Guo et al., 2014), 2 being review articles, to better assess the effects of copper on the soil microbial communities, and of moisture on the N cycling respectively at l.45, 46, 50, 56. Also, considering your previous remark on Cu ageing, we added references at lines 82-84 that provide controversial results about the importance It is not straightforward to assess that abrupt contamination leads to distinct effects on bacterial structure of functions than progressive contamination (Brandt et al., 2010; Oorts et al., 2006; Smolders et al., 2009)."

*Does the title clearly reflect the contents of the paper?*

**In my opinion, the title is not representative of the paper, promising much more than is delivered. It examines copper, not a wide range of soil contaminants. Similarly, the focus is on N<sub>2</sub>O, not the full array of greenhouse gases. There may be similar issues with the modeling description, but that is harder to evaluate given my confusion with the methods.**

Response: Done. Considering that the title might indeed be too promising we proposed a new title: "To what extent can soil moisture and soil Cu contamination stresses affect nitrous species emissions? Estimation through calibration of a nitrification/denitrification model" to better highlight the focus on Cu contamination and on N GHG emissions.

*Does the abstract provide a concise and complete summary?*

**The abstract is well-organized and covers the expected content. Clarity could be improved, in particular around where the double stress came into the experiments. (see also language comments below)**

Response: Done. Also, considering your following remarks, we rephrased both the abstract and the material and methods parts in some points to better clarify what corresponds to the initial incubation and what was the bio-assay. The abstract can now reads ("For that, initial incubations of soils were performed at different soil moistures in order to mimic expected rainfall patterns during the next decades and in particular drought and excess of water. Then, a bio-assay was used in the absence or presence of Cu to asses the effect of the single (humidity) or double stress (humidity and Cu) on soil nitrate production " l. 25-27 while the material and methods part is clearly separated in two paragraph starting respectively by "For the 5 weeks' initial incubations" L.110 and by "At the end of the initial incubation period," l.121

*Is the overall presentation well structured and clear?*

**Organization of the intro and methods was straightforward. I found the results very difficult to follow. It would be helpful to me if the experimental measurements were presented before the explanation of how they were incorporated into the model. Or, if those are not considered part of your results, then frame the whole paper as a modeling paper rather than an experimental and modeling paper. Similarly with the discussion –there is currently very little, if any, discussion of the experimental results.**

Response: Done. Following your remarks, we re-organized the results and discussion section to better separate the ecological implications in terms of  $\text{NO}_3/\text{NH}_4$  ratio and on N species emissions (lines 331-362). The description in terms of relative variations was also enlighten with deletion of some sentences (previously lines 317-321, 322-323 and 338-341). Furthermore, we modified the order of the paragraphs in the discussion section (4.2 and 4.3, actual lines 406-459) to follow the same order in the results and in the discussion.

*Is the language fluent and precise?*

**The language is at times not fluent or precise, but the intent is generally clear. Highlighting some early examples:**

**Title Extend should be extent**

**Abstract: prospect I think should be predict**

**Abstract: nitrate production modulation**

**Intro lines 70, 75**

**Another area of confusion for me is the word preincubation. From the figure I understand that this is referring to the initial incubation at different moisture levels, but in the abstract this period was referred to as an incubation, and in section 2.2 as an incubation and as microcosms. It would be helpful to be consistent about how you name this part of the experiment.**

Response: Thank you for identifying the mistakes and inappropriate words that have been corrected. As mentioned above we proposed a new title: "To what extent can soil moisture and soil Cu contamination stresses affect nitrous species emissions? Estimation through calibration of a nitrification/denitrification model" to better highlight the focus on Cu contamination and on N GHG emissions.

We also emphasized that the preincubation refers to the 5 weeks under various moisture treatments while the bio-assays refers to the 3 days with Cu gradient in the materials section (lines 109-111).

To be more precise, we used your words and changed the word preincubation by "initial incubation" throughout the entire manuscript

*Are mathematical formulae, symbols, abbreviations, and units correctly defined and used?*

**No major concerns here. In my experience, the "N" would follow the compound name, e.g.**

**N<sub>2</sub>O-N.**

Response: We are more used to the abbreviation with "N" at the beginning of the words, but considering your remark it seems that both are employed. Also, for convenience we modified to write with "N" at the end of the word throughout the entire manuscript.

*Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated?*

**I found the discussion difficult to follow. You might consider organizing it around your conclusions. Please use different symbols/line types as well as different colors in the figures. This is a fairly easy change that will make them more accessible for color blind readers and for those who still print in black and white.**

Response: According to your remarks, we combined line type to color type for Figures. However, we choose to keep the current color in the supplementary, especially colors that are helpful to represent error associated with model.

Concerning your remark about discussion, we reordered the paragraph to follow the conclusions. As mentioned above we re-organized the results and discussion section to better separate the ecological implications in terms of NO<sub>3</sub>/NH<sub>4</sub> ratio and on N species emissions (lines 331-362). The description in terms of relative variations was also

enlighten with deletion of some sentences (previously lines 317-321, 322-323 and 338-341). Furthermore, we modified the order of the paragraphs in the discussion section (4.2 and 4.3, actual lines 406-459) to follow the same order in the results and in the discussion. Also, we rephrased some parts, like the description of the initial incubation effect on PNA sensitivity to Cu stress (l.392-402 that's now reads "More complex dose response functions have been used in (Sereni et al., 2022) to assess thresholds and loss of functions after such a double stress. These results are in relatively good agreement with those presented here using the quadratic fit, especially for the highest half of [Cu].") or the limitations of DNDC in N<sub>2</sub>O estimations (l 492-495 that now reads This result points out two main difficulties in biogeochemical modelling: i) the difficulty to take into account hydrological dynamics (produced NO<sub>3</sub>-N and NH<sub>4</sub>-N could be expected to leach) and soil structures at different spatial scale (denitrification is estimated based on rough estimation on anaerobic soil volume which also controlled emissions rates through diffusion processes).

*Are the number and quality of references appropriate?*

**The paper is generally well-supported by references. Some specific comments on references: I would have liked to see more recent references included in the first paragraph (lines 44 and 50). Although it is an excellent work I don't find Jones et al 2014 a good support for the sentence in lines 46-47. The reference list is inconsistent in format and several references are lacking information such as page number or doi. See also comment on related work above.**

Response: We agree, and following your comment, we changed the Jones reference for Butterbach-Bahl et al. 2013 and Galloway et al. 2008, references and added several references as mentioned for the commentary above. Moreover, reference list has been completed with page numbering and doi.

*Is the amount and quality of supplementary material appropriate?*

**Yes. I appreciate that the authors have posted so much data.**

Response: Thank you.