

Response to reviewer

Anonymous Referee #3: This work presents a relevant topic, that is soil N₂O emissions management, to understand N fertilization and crop type impact, along with a possible involvement of soil microbiome. Therefore, in my opinion, it is relevant to SOIL aims and scope. Introduction is well constructed, and it highlights the relevance of the study in a broader context. Some modifications in text structure are required. Hypotheses and objectives are clearly stated and coherent with the methodology used, even though some of them might require improvements.

A: Thank you for your valuable feedback. We have carefully reviewed your comments and addressed all the critical points raised. Detailed responses are provided below.

1. The biggest problem of this paper is a major lack in appropriately describing the experimental design. In fact, it is necessary to specify which crops immediately preceded the ones tested in the current experiment and which fertilization treatments they received. The reason why it is so important is that this experimental design would not be valid if this wasn't a long-term experiment, as there aren't multiple separate and randomized plots. In fact, I think that what you refer to as "replicates" are only multiple sampling points of one unique plot per treatment. If this lack in methodological information will not be addressed appropriately, I fear that it might seriously undermine the reproducibility of this work. In addition, some information about one of the treatments is lacking (manure amendment).

A: Thank you for pointing this out. We have revised the manuscript and added additional information about the experimental design according to reviewers' suggestions.

Study is made on long-term three-field crop rotation experiment established in 1989. All fertilizer treatments have applied continuously from first harvest year in 1990. Manure treatment is amended with solid farmyard manure (ca 40 t ha⁻¹) in every third year before sorghum/potato. Last year of manure amendment was in year 2022. The farmyard manure is cattle dung with straw bedding, freely fermented before use 6-8 months in heap. In the Material and Methods section, we already had the following information about the preceded crops: "Initially, the crop rotation was potato–spring wheat–spring barley (Astover *et al.*, 2016). In 2019, potato was replaced with sorghum-sudangras hybrid."

We will include the use of linear mixed-effects models in the revised version of the manuscript. We use it to test statistical differences between N emissions of different fertilisation rates in plots with different crop types. We use spatial (different fertilisation rate) and temporal (sampling dates) effects as random effects. This model will help account for both fixed and random effects inside the experimental design, which provides better analysis of data.

2. Results have been described quite clearly, although sometimes too much detail is given about findings that don't have a wide importance. In some graphs I think there are some mistakes in results presentation. In the discussion section there are some problems related to the flowing of the text. In fact, often the description of the same topic is divided into multiple, short paragraphs, thus creating some confusion for the reader. Moreover, some of

the speculations are too strong based on the presented results. Overall, the manuscript has a great potential to be improved, but only if the issue with the experimental design is correctly and extensively addressed, as it is the most serious problem of this work.

A: Thank you for the constructive feedback. We have revised the manuscript according to reviewer's feedback. In Discussion section, we have removed the repetitive parts and improved flowing of the text. We have also consolidated paragraphs, where discussion of the same topic was previously divided into multiple paragraphs. We have also revised the figures and the Discussion section. In addition, we smoothed the text where needed.

3. L. 12: N₂O I think the term emission is missing here.

A: Done!

4. L.s 16-17 You mean higher compared to the application of mineral fertilizers?

A: Yes, we mean in comparison to mineral fertilisation. We have clarified it in the manuscript.

5. L. 19 **Microbial analyses** Could you be more specific here?

A: Thank you for pointing this out. We have revised the sentence followingly: "Quantification of nitrogen cycle functional genes also showed the potential role of denitrification, comammox and DNRA processes as a source of N₂O."

6. L. 23 **sorghum** It is not necessary to repeat the term sorghum here again.

A: Done!

7. L. 33 **maise** There is a spelling mistake here.

A: Done! We have corrected this error throughout the entire manuscript.

8. L. 34 **in present agricultural regions due to climate system changes** I am sorry, but this is not very clear to me. Could you please rephrase it.

A: We have removed this sentence from Introduction section to avoid too verbose text and redundancies.

9. L. 38 This sentence does not fit well in this paragraph. I think it is better to move it to the following one, when you introduce the problem of N₂O emissions.

A: Done!

10. L.s 51-62 I think it's better to unite these two paragraphs in one. Otherwise, it results confusing since the topic discussed continues from the first to the second.

A: Done!

11. L. 63 **contributes** Probably some words are missing here.

A: Done!

12. L. 63 for **biological production as a N fertiliser** This seems confusing to me. Could you rephrase it?

A: We have clarified the sentence. The revised sentence is “Dissimilatory nitrate reduction to ammonium (DNRA) supplies NH_4^+ to the soil, conserves bioavailable N and prevents the leaching of NO_3^- (Bai *et al.*, 2020; Pandey *et al.*, 2020).

13. L. 65 **both requiring NO_3^-** I don't think it is necessary to repeat this here again.

A: Done!

14. L. 68 **clad** There is a spelling mistake here.

A: Done!

15. L. 71 **the Hatch-Slack pathway** This phrase requires to be included in commas.

A: Done!

16. L. 71 **maise** This is a spelling mistake.

A: Done! We have corrected this error throughout the entire manuscript.

17. L.s 83-84 Could you rephrase this part? It sounds confusing to me.

A: We have revised the sentence followingly: “The general objectives of the study were to evaluate temporal patterns of gaseous N loss, link N-cycle processes with abundances of functional N cycle genes in arable mineral soil, and evaluate the performance of different crops (including novel crop in Northern Europe) in terms of biomass production and N_2O emissions under mineral and organic fertilisation.”

18. L. 86 **in arable mineral soil** What do you mean by "mineral soil"?

A: Mineral soil is typically defined to have less than 12% organic carbon in topsoil. Soil in our experiment is definitely classified as mineral soil. We have now removed the word “mineral” from the sentence to avoid confusion. Also, in methods we have mentioned the soil type, which indicates that it is a mineral soil.

19. L. 88 **decreases** This term here is not fitting.

A: Thank you for the comment! We have made changes, and the updated sentence fragment is “(3) in arable mineral soil, low soil moisture results in reduced N_2O losses.”

20. L. 88 **affects the soil microbial community** This hypothesis seems too general. Could you be more specific?

A: Yes, we agree with reviewer's comment. We have specified the hypothesis: “(4) amendment of manure fertiliser increases soil N_2O emissions and affects the abundances of functional N cycle genes”

21. L. 89-90 This hypothesis does not sound very fitting to me for the purposes of the paper. I think it might be better to focus on the performance of this crop in comparison to the others. Otherwise, please provide further insights.

A: We agree with reviewer's comment that hypothesis "sorghum is a prospective crop to cultivate in temperate climate" is not very fitting for the purposes of the paper and could not fully proven with presented results. We excluded this hypothesis from the paper.

22. L. 98 It might be better to move this sentence to the section preceding the description of the crop rotation.

A: Done!

23. L.s 104-109 In my opinion, this is the biggest problem of the whole manuscript. Although you specify there are three replicates per treatment, only one is present. This would be a major issue, if it wasn't a long-term field experiment. Therefore, please specify which crops have preceded in the few years before the described experiment started. Also, please specify if fertilization has been applied in the previous years and its entity.

A: Its long-term three-field crop rotation experiment with split-plot design to study effect of mineral and organic fertilisers established in 1989. Crop rotation order: spring wheat – spring barley – sorghum (potato before 2019). All fertilizer treatments have applied continuously from first harvest year in 1990. Manure is applied in every third year to potato/sorghum.

24. In addition, it would be ideal if you could provide further insights about the farmyard manure composition and whether it has been treated somehow (composting or something else).

A: The farmyard manure is cattle dung with straw bedding, freely fermented before use 6-8 months in heap. We have added this information to the Material and Methods section. Additionally, we have added chemical properties (C, N, P, K, dry matter) of the last manure amended in year 2022 and also last ten year average chemical properties of the manure in the Supplementary materials.

25. L. 104 I do not understand where the three replications are. A replicate is a treatment group to which the same levels of factors tested were applied in a way that allows to account for environmental factors' variability. Based on the experimental design you have provided, it seems that you only have one replicate per treatment group. If this is not the case, I think you only have pseudo-replicates.

A: We will include the use of a linear mixed-effects model in the revised version of the manuscript.

26. L.s 107-108 Manure was applied only to sorghum. I think you should make it clear also from the text.

A: Done! We have added following sentence to the revised version of the manuscript: "The farmyard manure treatment was applied only to sorghum."

27. L.s 113-114 As further specified in my comment on the text, it is better to clarify that additional N was applied with manure in the sorghum plots.

A: Done!

28. L. 125 **electron capture and flame ionisation detectors** Probably it is better to provide more details about these two instruments.

A: Done!

29. L.s 129-130 Please provide more details on the soil sampling process implemented (number of samples, rhizosphere or bulk soil).

A: Three auger samples from each point were collected for one composite sample for chemical and microbiological analyses. We have added the information to the Material and Methods section.

30. L.s 131-132 What are the instruments used for these analyses?

A: We have added the information to the Material and Methods section.

31. L. 134 Could you provide the reference for this method?

A: Done! We have provided reference to the Dumas method.

32. L.134 There is no need to use the full term here. It is better to use "C".

A: Done!

33. L.s 131-132 This paragraph should be united with the preceding one. Separating paragraphs discussing the same topic results in difficulty for the reader to understand their meaning.

A: Done! We have consolidated the paragraphs.

34. L. 146 I have some doubts about the term "total". In fact, you sampled roots up to a depth of 18 cm, but all these species' roots can easily reach lower depths.

A: With "total" we mention that above- and below-ground both were accounted. We agree that with the sampling method used some minor portion of root biomass might not be considered, but this would be a limited and probably negligible part.

35. L. 148 **maturity phase** Could you provide insights about the date when the measurement was done for each species?

A: The crops were harvested at maturity phase, and then state that biomass (both above- and belowground) was sampled on the harvest day.

36. L. 150 **Frasier et al. (2018)** This reference is not reported in the cited literature.

A: Thank you for pointing it out! We have added the missing reference.

37. L. 171 **extracted DNA** What is the DNA concentration used?

A: The DNA concentration was usually in the range of 20-35 ng/ μ l, although some samples had slightly lower or higher concentrations.

38. L.s 171, 172 **ml** I really think that here you mean microliter.

A: Done!

39. L.s 175-176 Could you specify which are the standard curve ranges used?

A: Yes, we can. We used the standard range of 10^4 to 10^8 for the bacterial 16S rRNA gene. We used the standard range of 10^4 to 10^8 for the archaeal 16S rRNA gene. We used the standard range of 10^2 to 10^8 for the archaeal amoA gene. We used the standard range of 25 to 10^2 for the bacterial amoA gene. We used the standard range of 50 to 10^3 for the comammox amoA gene. We used the standard range of 10^3 to 10^5 for the nirK gene. We used the standard range of 10^4 to 10^6 for the nirS gene. We used the standard range of 50 to 10^3 for the nosZI gene and of 10^6 to 10^8 for nosZII gene.

40. L.180 **analysis** Since it is plural, it would be "analyses".

A: Done!

41. L. 183 **Analysis of variance (ANOVA)** Probably here it is necessary to specify the type of ANOVA used. Please provide me with your opinion.

A: Three-way ANOVA with the factors crop, fertilization rate and manure addition.

42. L. 185 **and** This "and" should be substituted by a comma.

A: Done!

43. L.s 185-187 Later, in the results, you use a different terminology to refer to this term.

A: Yes, we agree. We have unified the terminology in the manuscript to avoid misunderstanding.

44. L. 193 2017 Parentheses are missing here.

A: Done!

45. L.s 199-200 **in the soil** It is redundant to repeat this phrase.

A: Done!

46. L. 207 **Over all** Probably a different conjunction here would make the discussion more fluent.

A: Done!

47. L. 221 **increased** The form of this verb does not seem correct. Probably it is better to say that they "caused an increase in".

A: Done!

48. L. 226 **p < 0.05** This should be written without spaces.

A: Done!

49. L.s 226-227 For biomass yield, the hierarchy of uppercase letters seems to be B>C>A while for N content it seems to be C>D>B>A for yellow bars and A>B>C for the other two colours. I am right? If so, please correct the graph.

A: Yes, this is right. We have changed the hierarchy of uppercase letters to the same order.

50. L. 234 In table S3, N₂ emissions are just an estimation based on N₂O emission if I'm not wrong. If this is the case, please specify it, otherwise it would be misleading.

A: Done! We have specified it.

51. L.s 240-246 Could you please specify in the text which of these differences are significant? It would also be ideal if you could specify the significance of the results with different letters in the table.

A: Done!

52. L. 264-265 Could you rephrase this? It seems quite redundant.

A: Done!

53. L. 286 **The effect of mineral N fertilisation** It would be better to write "mineral N fertilization effect".

A: Done!

54. L. 287 **to effects of crop type** The correct form would be "to the effects of crop type".

A: Done!

55. L. 299 **Feature selection algorithm** Please align the name of this methodology with the one described in the methodology.

A: Done!

56. L. 301 **change** I'm not sure about the term "change". Probably "variations" or "alterations" are better.

A: Done! We have substituted the term "change" with "variations" throughout the entire manuscript.

57. L.s 316-325 I'm not sure if it is really necessary to describe all the significant correlations. Probably it's better to just choose the relevant ones.

A: Done!

58. L.s 333-334 Please, make it clear that you are specifying this in support of what previously stated.

A: Done!

59. L. 342 **indicates the dominance of nitrification over denitrification in N₂O-producing processes** Reference is lacking for the relevance of this ratio between the two genes.

A: We have provided reference for this ratio.

60. L.s 350-351 What does this mean?

A: We have further explained the Discussion section.

61. L. 359 **important** This term is not correct in this context.

A: Done!

62. L.s 362-363 A reference is lacking.

A: Done!

63. L.s 376-388 There is no need to separate this section in two different paragraphs, as the topic discussed is the same. Furthermore, this recurred also in other parts of the manuscript. It would be better to avoid writing short paragraphs with just a few sentences while separating the same topic in multiple paragraphs.

A: Done!

64. L. 386 **manure enhances the activity of soil microbes** It is better to not directly give this conclusion. It would be ideal if you propose this as one of the possible hypotheses.

A: We have changed the wording of the sentence and proposed it as one of the possibilities.

65. L.s 389-390 **with emissions increasing slower than linearly with the fertilisation rate** I don't think that this phrase describes correctly the observed trend.

A: Yes, we agree. We have removed it.

66. L.s 390-391 Are you sure this conclusion can be derived from just three points you have?
L. 394 **N₂O emissions often grow exponentially when the applied N exceeds the necessary amount for crops** I don't understand what you mean by "often". I think that whether the growth is exponential or not depends on the number of N doses tested and their entity. I'm not sure about this so please let me know.

A: Yes, we agree with the reviewer's comment. We have removed sections from the manuscript that refer to a linear, exponential or any other response between fertilisation rate and N₂O emissions.

67. L. 397 **positive linear response** Here the same comment as before applies. Please provide further explanation about the linear or exponential response.

A: As mentioned in the previous comments, we have removed sections from the manuscript that refer to a linear, exponential or any other response between fertilisation rate and N₂O emissions.

68. L.s 400-405 I don't think that discussing this part can provide useful insights to this work.

A: Thank you for the comment!.We have removed the paragraph.

69. L. 406 **indirectly affecting** Probably it is better to say: "as it directly affects".

A: Done!

70. L. 410 Based on my personal experience, this information does not seem very fitting.
Could you please check that it is correct?

A: **We checked and elaborated on the possible reasons further, and changed it accordingly.**

71. L.s 411-412 This sentence has no reference, and it is not very clear as it is not specified how water scarcity might enhance N gases emissions.

A: We have added suitable references and specified the link between gaseous N emissions and water scarcity.

72. L. 414 **N₂O management should align with crop yield** It is not very clear here what is intended for "align".

A: Yes, we agree that it needs clarification. We mean that N₂O management should be coordinated with futher crop yield objectives to sustain fastly growing human population. We have rephrased the sentence.

73. L. 414 Biomass It would be better to say "biomass production".

A: Done!

74. L. 416 **fertilisation rate 160 kg N ha⁻¹** "of" is missing here.

A: Done!

75. L.s 416-417 **but our study shows increasing N₂O emissions at higher fertilisation rates (Figure 6A), suggesting potential overfertilisation.** I don't understand what the connection with the previous sentence is.

A: **We have rephrased it.**

76. L.s 419-421 If this sentence is added to sustain the previously discussed results, I think it is better to specify it. Otherwise, it does not seem very clear.

A: Done!

77. L. 422 **rate 160 kg N ha⁻¹** "of" is missing here.

A: Done!

78. L. 422 **increase** Probably it is better to use the past tense, as you are describing results you observed.

A: Done!

79. L. 423 **The fertilisation rate 80 kg N ha⁻¹** "of" is missing here.

A: Done!

80. L.s 433-434 There are some typos in this sentence.

A: Done!

81. L.s 437-438 As you studied the abundance of microbial functional groups based on a DNA approach, I think that this speculation is too strong. What you can say is that the nitrification potential was higher than the denitrification one, but not that one process prevailed over one other.

A: We agree that it might be too strong to use word "dominance." We have corrected this throughout the entire manuscript. For example, we included the following sentence in the Conclusions section:

"N₂O emissions were mostly caused by nitrification, with potential contribution from denitrification, comammox and DNRA processes."

We included following sentence in the Discussion section: "The significant positive correlation between the ratio of *amoA/nir* and N₂O emissions ($\rho = 0.20$, $p < 0.001$) indicates that nitrification potential was higher than denitrification potential and thereby N₂O emissions were mainly related to nitrification in the soil."

82. L. 438 **N cycle** A hyphen is required.

A: Done!

83. L.s 442-443 **fertilisation rate 80 kg N ha⁻¹** "of" is missing here.

A: Done!

84. L. 444 **positioning** This term is not very fitting here. Please, change it.

A: Done!