

Response to reviewer

Dear Reviewer:

Thank you very much for your constructive comments and professional advice. These comments are very valuable and helpful for improving our manuscript, as well as the important guiding significance to our research. We have studied the comments carefully and made corrections accordingly.

Editor's and Reviews' comments, **Blue text = authors' reply, line numbers in blue color correspond to the revised manuscript.**

The major issues:

1. What was the rationale to sample soils at the named five locations? What were the selection criteria? Are they representative for larger regions of China or even south/east Asia?

Response: The named five locations are from Hunan and Hubei Province, covering (1) three parent materials: Quaternary red clay (TY, Hunan, and XN, Hubei), Granite (CS, Hunan, and TC, Hubei), and late Pleistocene sediment (DY, Hubei); and (2) five land use types: tea plantation, fruit plantation, vegetable plantation, rice plantation, and wood plantation. Hunan and Hubei have a subtropical continental monsoon climate, which leads to relatively strong physical weathering of the soils and the development of acidic soils. Thus, the objective of the sampling collection was to ensure diversity of soil types and site types. [This information has been added to the methods and materials, line 106 to line 113]

2. Which implications does the application of unrealistically high amounts of reactive N intermediates to the soil have for the generalizability or transferability of results to natural conditions in soils? How are the conversion ratios and abiotic formation fractions biased by these unnaturally high concentrations?

Response: The concentration of NO_2^- (nitrite) used in this study is 50 mg N/kg soil, which can be reached following the application of nitrogen fertilizer (Shen et al, 2003, Chemosphere).

The concentration of the hydroxylamine (NH_2OH) used in this study is 5 mg N/kg soil, however, the natural level of NH_2OH lacks data. It is still unknown that the highest concentration of NH_2OH that can be achieved in local areas when applying nitrogen fertilizer in the soil. Thus, pre-experiment was done with two paddy soils

(which have a low (Soil 1) and high (Soil 2) Mn content, respectively, and four gradients of NH_2OH were set. Here are the conversion rates (%) of each treatment:

| Treatment | Soil 1 | Soil 2 |
|--|---------------|---------------|
| 500 $\mu\text{g N/kg}$ | 2.55 | 24.06 |
| 1 mg N/kg | 3.77 | 19.24 |
| 3 mg N/kg | 3.71 | 20.59 |
| 5 mg N/kg | 5.37 | 34.81 |

We found that higher concentration (5 mg N/kg soil) of NH_2OH stimulated the conversion ratios. Yet, the influence of high concentration of NH_2OH on the activity of relative microorganisms is unknown. We also determined the dominant ammonia oxidizers as AOA, in which the abiotic pathways are largely responsible for the N_2O emissions. Therefore, in this study, the effect of NH_2OH on biological pathways can be overlooked.

Related explanation was added in line 151-155 in the revised manuscript.

3. The novelty of the results should be pointed out more. So far, there are a lot of statements in the discussion like “in line with”, “most of the results fall in the range of earlier reports”, etc.

Response: Thank you. We acknowledge the reviewer’s concern regarding the novelty of the study. We have illustrated the novelties about the results, specified related statements, and polished the MS.

The below comments were from the MS attached.

Line 11-13 These statements can be misleading. Should be more specifically focused on the influence of the parent material from which the soils of interest have formed.

Done. Thanks, we revised the statements as follows: Abiotic pathways may be dominant in N_2O emissions, which have close relationship with soil physicochemical properties influenced by soil parent material and land management. Yet, little is known about the influence of parent material and land use type on abiotic N_2O production in response to NH_2OH and NO_2^- addition. [see line 10-13 in the main text]

Line 13-15 Would be good to have some at least coarse information on the study region, land use and study design.

Done. Coarse information were given as follows: This study investigated fifteen acidic soils covering three parent materials and five land use types, collected from subtropical

continental monsoon climate region in central China. [see line 13-15 in the main text]

Line 17 Replace “notable” by “significant”.

Done. See line 18.

Line 18 Replace “intrigued by” by “following”; “a markedly higher abiotic N₂O production” should be more precise, and mention some numbers.

Specifically, 2.55 μg g⁻¹ soil of abiotic N₂O-N was produced from soils developed from Quaternary red clay, five times higher than those derived from granite. [see line 18-21 in the main text]

Line 20 Replace “than” by “compared to”.

Removed.

Line 22 Replace “in comparison to” by “than”.

Done. See line 22.

Line 22-24 Mention also conversion ratios and abiotic N₂O fractions in the abstract.

Done. Supplemental information were involved as follows: In addition, the conversion ratios of NH₂OH-N to N₂O-N were 56.6%, 12.7% and 40.8% in soil developed from Quaternary red clay, granite and late Pleistocene sediment, respectively. [see line 25-27 in the main text]

Line 23 Dismiss “the” before “abiotic”.

Done. See line 24.

Line 26 Replace “aligning” by “in line”.

Done. See line 29.

Line 26 The ranges of SP of N₂O should be more precise.

Done. “with the expected ranges characterizing ammonia oxidation (22.2-34‰) and chemodenitrification processes (16.8-30.1‰).” [see line 29-30 in the main text]

Line 43 Replace “were” by “are:”.

Done. See line 46.

Line 44 This sentence is repetitive and can be omitted.

Done.

Line 48 “Microorganisms” should be brought forward.

Done. See line 51.

Line 52 Start a new paragraph here.

Done. See line 56.

Line 62-71 You should also elaborate on the potential influence of parent material on Mn and Fe content of the soil developed from that parent material.

Done. Supplementary information were given as follows: Specifically, parent material containing various sedimentary rocks present different chemical composition. For example, basalt has the largest proportion of Fe₂O₃ and FeO; peridotite has the most largest proportion of MnO (Gray and Murphy, 2002). [see line 67-70 in the main text]

Line 79 Replace “purely cultured” by “pure culture of”.

Done. See line 85.

Line 81 Replace “exhibit” by “produce N₂O with”.

Done. See line 87.

Line 83 Replace “presented a low SP range” by “produce N₂O with a low SP”.

Done. See line 90.

Line 84 Replace “scope” by “range”.

Done. See line 90.

Line 86 Replace “has” by “have”.

Done. See line 92.

Line 88 Remove “that”.

Done. See line 95.

Line 95 Replace “yielded” by “derived” and move “NO₂” afterward the “chemodenitrification”.

Done. See lines 101 and 102.

Line 99 Specify the distribution of sampling locations.

Done. “Fifteen soils were collected from five sites derived from three parent materials and five land use types in Hunan and Hubei province in mainland China (Fig. 1): Changsha (CS, 113°19'E, 28°33'N, granite), Tongcheng (TC, 113°46'E, 29°13'N,

granite), Taoyuan (TY, 111°31'E, 29°14'N, Quaternary red clay), Dangyang (DY, 111°48'E, 30°41'N, late Pleistocene sediment) and Xianning (XN, 114°22'E, 30°0'N, Quaternary red clay) were collected for this study. These soils also represented five land use types: tea plantation, fruit plantation, vegetable plantation, rice plantation, and wood plantation for over 10 years' plantation." [see line 106-113 in the main text]

Line 102 Provide information about land use and parent materials.

Done. See line 106-113 in the main text.

Line 105 Dried at which temperature? Also room temperature?

Yes, all soils were naturally dried in a well-ventilated room at room temperature, almost 25°C. [see line 116 in the main text]

Line 108 How were the samples treated? With aqua regia digest?

Before the measurement, the samples were all digested with a microwave. Briefly, 100 mg of sample material were mixed with 3 ml HNO₃ and 2 ml H₂O₂, heated in the microwave at 800 W for 30 min. The mixtures were subsequently filled up to 14 ml and diluted 10-fold with deionized water followed by the ICP-OES measurement. [see line 121-125 in the main text]

Line 112 Provide information on analyzer type.

Ion chromatography (Dionex, Sunnyvale, CA, USA). [see line 128 in the main text]

Line 113 This is a very untypical way of determining soil pH. Usually, either water or diluted (0.01 M CaCl₂ solution) is used for soil extraction. The extraction with 1 M KCl will yield significantly lower pH values.

We are sorry for making a mistake here. Distilled water was used to determine soil pH. [see line 128-130 in the main text]

Line 116 Provide more details: spectrophotometer type, wavelength, calibration.

Spectrophotometer: UV 1900 (Shanghai, China); wavelength: 220nm and 275nm; distilled water was used as control. [see line 132-133 in the main text]

Line 120 Replace "under" by "with".

Done. See line 137.

Line 128 Add number of replicates here.

Done. See line 147 in the main text.

Line 131 Which concentration? Which pH? Which solvent? Were buffers used?

Done. The added NH_2OH and NO_2^- concentration were $5 \text{ mg N kg}^{-1}\text{soil}$ and $50 \text{ mg N kg}^{-1} \text{ soil}$, respectively. However, the pH were not determined. The used solvent was distilled water. And for the sterilized samples, these solution were all filtered. No buffers were used. [see line 145 in the main text]

Line 132 Which volume and total amount of NH_2OH and NaNO_2 were applied?.

It depended on the water holding capacity of the soils and it differed among these samples. The final concentration of NH_2OH and NaNO_2 will reach $5 \text{ mg N kg}^{-1}\text{soil}$ and $50 \text{ mg N kg}^{-1} \text{ soil}$, respectively. [see line 145-146 in the main text]

Line 133 This sentence should be mentioned earlier.

Done. See line 145.

Line 135 Replace “achieve” by “be achieved”.

Done. See line 148.

Line 136 Replace “and” by “but”.

Removed.

Line 138 and 142 The concentration of NO_2^- and NH_2OH are artificially high. How realistic/transferrable are your results to natural conditions?

Relative information is given in the revision of major issues.

Line 140 Provide supplier details (city, state, country).

Done. Gas chromatograph (GC-7890A, Agilent, USA). [see line 164 in the main text]

Line 152 It was not really an isotope experiment (where one would expect the addition of isotopically labeled substances), but isotope analysis.

Done. See line 175.

Line 157 Provide details of the IRMS (type, periphery, company, city, state, country).

Done. IRMS (Isoprime 100, Isoprime Company, Manchester, Britain). [see line 180 in the main text]

Line 158 Add the delta symbol (small, not capital) before each ^{15}N , also in equation 3 before $^{15}\text{N}^{\text{bulk}}$.

Done. $\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{15}\text{N}$. [see line 183 in the main text]

Line 159 The equation is wrong. $\delta^{15}\text{N}^{\text{bulk}}$ needs to be multiplied with 2. The full equation reads either: $\delta^{15}\text{N}^{\text{bulk}} = (\delta^{15}\text{N}^{\text{alpha}} + \delta^{15}\text{N}^{\text{beta}})/2$.

Done. The equation(3) is corrected. [see line 183 in the main text]

Line 169 The correct citation usually is "R Core Team (YEAR). The year has to correspond with the version used. See information on the internet how to cite R.

Done. R (version 4.1.1; R Core Team; 2021). [see line 192 in the main text]

Line 174 As commented already above, the parent materials have to be introduced in the Materials and Methods section. Replace “have” by “had”, and “largest amount of” by “highest”.

Done. See line 198.

Line 177 Replace “least” by “smallest”.

Done. See line 200.

Line 180 Also here, land use types of this study have to be introduced in the M&M section.

Done. [see line 106-113 in the main text]

Line 181 Replace “least” by “smallest”.

Done. See line 205.

Line 183 Add “content” after “TC and TN”.

Done. See line 207.

Line 184 Replace “with” by “after”.

Done. See line 209 and 212.

Line 188 Replace “have” by “had”.

Done. See line 213.

Line 189 Replace “with” by “after”.

Done. See line 214.

Line 192 Replace “difference” by “differences”.

Done. See line 217.

Line 194 The meaning of “an approximate range”?

Deleted in the main text.

Line 195 Replace “with” by “after”.

Done. See line 220.

Line 198 Replace “was” by “were”.

Done. See line 222.

Line 199 Add “converted” after “NH₂OH”.

Done. See line 223.

Line 200 Replace “ratios” by “corresponding fractions”.

Done. See line 224.

Line 202 Add “conversion” before “ratios”.

Done. See line 226 and 227.

Line 205 Replace “with” by “after”.

Done. See line 230.

Line 207 Replace “with” by “after”.

Done. See line 232.

Line 208 Add “after NH₂OH addition” after “N₂O production”; Replace “to” by “with”.

Done. See line 233 and 235.

Line 209 Delete “with NH₂OH addition”; Add “after NO₂⁻ addition” after “N₂O production”.

Done. See line 234.

Line 210 Replace “to” by “with”; Delete “with NO₂⁻ addition”.

Done. See line 239 and 235.

Line 211 Add “after NH₂OH addition” after “N₂O production”.

Done. See line 236.

Line 212 Delete “with NH₂OH addition”.

Done. See line 238.

Line 213 Add “with NO₂⁻ addition” after “N₂O production”; delete “with NO₂- addition”.

Done. See line 238.

Line 214 Add “with NO₂⁻ addition” after “N₂O production”; delete “with NO₂- addition”; replace “to” by “with”.

Done. See line 239.

Line 215 Remove “positively” forward “correlated”; Replace “to” by “with”.

Done. See line 248 and 253.

Line 217 The named samples should be specified in the M&M section. Why specifically these four samples selected to analyse SP values? What were the selection criteria?

Done. To reveal the influence of γ sterilization on N₂O formation, Soils named DY1, CS1, TC3, TC5, showing different N₂O production trend between non-sterile and sterilized treatment, were used for stable isotopic signature determination. [see line 109-110, and line 242-244 in the main text]

Line 218 Replace “with” by “after”.

Done. See line 245.

Line 219 Replace “falls” by “was”, and “scope” by “range”; Add “of N₂O” after “SP values”.

Done. See line 245-246.

Line 220 Replace “value” by “values”; replace “with” by “after”.

Done. See line 246-247.

Line 221 Replace “falls” by “was”, and “scope” by “range”; Add “of N₂O” after “SP values”; Change “ γ ” to “Gamma”.

Done. See line 247-248.

Line 224 Replace “with” by “after”. Change “value ranged” to “values ranging”.

Done. See line 249-252.

Line 225 Replace “with” by “after”.

Done. See line 252.

Line 226 Change “also fell in the scope” to “was in the range”.

Done. See line 253.

Line 227 Change “value” to “values”; Replace “with” by “after”.

Done. See line 254.

Line 228 Replace “in” by “for”.

Done. See line 255.

Line 230 Change “materials”, “types”, and “influences” to “material”, “type”, and “influence”, respectively.

Done. See line 257.

Line 241 Replace “generated” by “developed”.

Done. See line 268.

Line 246 Replace “aligned” by “in line”.

Done. See line 272.

Line 248 There is a contradict here for data not shown, but it was actually mentioned before; Change “were fallen” to “falling”; replace “scope” by “range”.

Done. A misunderstand is made here. That “The conversion rates of NH_2OH and NO_2^- in the sterilized acidic soils” means the conversion ratios calculated with all acidic soils. In the MS Table 3, what was shown is the average conversion ratios divided by either parent material or land use type.

Line 250 Change “varied” to “varying”.

Done. See line 277.

Line 251 Replace “fell” by “were”.

Done. See line 278.

Line 252 Replace “scope” by “range”.

Done. See line 279.

Line 254 Add “soils from” before “late Pleistocene”.

Done. See line 281.

Line 255 Add “soils from” before “granite”; replace “yield” by “production”.

Done. See line 282.

Line 256 Change “high” to “higher”.

Done. See line 283.

Line 257 Replace “suggested” by “indicated”.

Done. See line 285.

Line 258 Replace “to” by “with”.

Done. See line 286.

Line 259 Replace “shown by” by “of”.

Done. See line 287.

Line 261 Remove “has”.

Done. See line 288.

Line 264 Replace “that” by “who reported”.

Done. See line 291.

Line 265 Replace “ranged from” by “in the range of”; replace “demonstrated” by “reported”.

Done. See line 292.

Line 266 Replace “with” by “after”.

Done. See line 293.

Line 270-272 Be more specific. These generic statements (“Further work is necessary...”) are not really helpful. Which aspects should this further work cover? Why did you not cover these aspects in your present work?

Related statements were replaced by “However, SP values of N₂O produced from microbial and abiotic processes share the same range, and further work can focus on new method to differentiate SP values of N₂O produced from different pathways.” [see line 297-300 in the main text]

Line 273 Remove “regarding the conversion of NO₂⁻ to N₂O”.

Done. See line 301.

Line 274 Replace “lined” by “line”.

Done. See line 302.

Line 279 Replace “revealed” by “reported”.

Done. See line 306.

Line 281 Replace “difference” by “differences”; replace “was” by “were”.

Done. See line 308.

Line 282 Remove “soil”.

Done. See line 310.

Line 283 Add “soil” before “organic matter”.

Done. See line 310.

Line 283-284 Move this sentence to the beginning of the next paragraph and merge it with the first sentence of that paragraph; delete "Nonetheless" and "In supporting" (L288).

Done. See line 314.

Line 286 Add “gamma” before “irradiation”.

Done. See line 312.

Line 293 Replace “whereas” by “in contrast”.

Done. See line 319.

Line 295 But in Heil et al. (2014), all reactions involved hydroxylamine, also the reactions with nitrite. In that work, N₂O was not solely the product of nitrite reduction. We have reconfirmed that the SP values were obtained from nitrite reduction involved hydroxylamine. This citation was removed in the revised MS.

Line 296 Depends on the substrate: for N₂O from NH₂OH = yes; from NO₂⁻ = no (but intermediate range, see Wei et al.).

Yes, this statement is kind of absolute. Relevant statement will be removed.

Line 300 This sentence should be moved to the Conclusion. Again, this statement is too generic. What exactly did we learn? Which new knowledge was generated?

The main idea of this MS is: Parent material has an influence on Mn and Fe content, which can exert a significant influence on soil abiotic N₂O production from chemical oxidation of NH₂OH. Whereas, soil land use type can not be a good indicator in the difference on abiotic N₂O production. Related statements were replaced in Conclusion.

Line 303 Replace “materials” by “material”.

Done. See line 329.

Line 304 Replace “contents” by “content”.

Done. See line 330.

Line 305 Replace “to” by “of”; remove “the”.

Done. See line 331.

Line 306 Replace “amplifies” by “in soil enhance”.

Done. See line 332.

Line 307 Which soil properties exactly? Name them; replace “contribution” by “dominant role”.

Done. Mn and Fe content. [see line 334 in the main text]

Line 308 Remove “additionally”.

Done. See line 337.

Line 310 But that's what you have done already, haven't you? By comparing non-sterile and sterile soils.

In this study, we used acidic soils only for the reality that abiotic processes play a dominant role in N₂O production. Further study may focus on the microbial activity and composition, which are also influenced by parent material and land use type. At the same time, distinction of biotic and abiotic processes can also be done.

Thanks for the suggestions again.