

Interactive comment on “Effect of iron oxide on nitrification in two agricultural soils with different pH” by Xueru Huang et al.

Xueru Huang et al.

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Dear Reviewers,

We thank you for your most helpful efforts in the evaluation of our manuscript. We have uploaded a revised version of the manuscript that was extensively revised based on the reviewer comments, which we found to be very constructive and useful. Below (*Italic font*) are our point-by-point responses to the reviewers' comments with references to line numbers in the revised version. Please let us know if further information or modifications are needed.

Thank you again for your expert reviewing of our manuscript.

Best wishes,

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Xueru Huang (first author)

Xianjun Jiang (corresponding author)

On behalf of all the authors

Reviewer #2:

This article examines how pH and iron oxides interact while impacting the effective processes of nitrification, mineralization and immobilization in subtropical agricultural soils under anoxic conditions. The science is good, the article is short and concise (which is good), and is typically in the scope of BGS. In particular, targeting how iron oxides impact nitrification, a key process in many soils but especially in tropical soils where the presence of iron is important, is crucial and little addressed in the literature. Overall, the English is to be improved (even if not catastrophic); ask a native English speaker or equivalent to proofread the manuscript. Finally, a number of points are also to be improved, listed below:

Re: We thank the reviewer#2 for your time. We have carefully considered each comment and thoroughly edited the manuscript to address each of them, with point-by-point explanations of how each comment has been addressed below. We have also substantially edited and strived to improve the English throughout the manuscript. Since considerable edits were made, we have not detailed every action here.

ABSTRACT:

1. Line 13: here, and later in the manuscript: please specify the reason why the experiment was done at 100% WHC;

Re: 100% WHC was chosen to create an oxic-anoxic interface, in which the redox cycle of Fe oxide commonly exists. We have explained it in Material and Methods Section. Please see lines 113-114 in the revised manuscript.

2. In the abstract in general: Avoid vague phrases like eg. 'We hypothesized that the

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effect of Fe oxide on N transformation processes would be different' (line 11); be more specific about the expected effects;

Re: We have changed this phrase to "The role of Fe minerals, particularly oxides, on affecting soil N transformation processes depends on soil pH, with Fe oxide often stimulating nitrification activity in the soil with low pH." on lines 11-12 in the revised manuscript.

INTRODUCTION:

3. Here, and in the discussion, there are no details on the potential impact of the processes studied on denitrification rates. Specify in a few lines how your experimental conditions, or the presence of iron in general, likely impact denitrification under anoxic conditions;

Re: We have revised and added the potential impact on denitrification in the Introduction section. Please see lines 27-28 and lines 42-43 in the revised manuscript.

4. Line 23: 'affect'->'affects';

Re: We have deleted the word in the revised manuscript.

5. Lines 23-25: yes, I do agree that the role of iron on nitrification is important and little studied. Also specify how this is especially important for tropical or subtropical ecosystems;

Re: We have added the sentence in Introduction section by "Iron and its oxides are found in abundance in many soils, with large amounts of Fe oxides are typically found in subtropical and tropical soils. Thus, understanding of the relationship between Fe oxides and soil nitrification process is especially important for understanding its influence on N cycling processes." on lines 30-33 in the revised manuscript.

6. Line 38: 'such as humic substances'-> please specify if this parameter's control on nitrification is through quantity, quality, both?;

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Re: In the discussion and revised edition of this manuscript, we had deleted the content “such as humic substances” in Introduction section. Even that, humic substances would control on nitrification through both quantity and quality. First, humic substances would affect soil mineralization rate so that it controls nitrification through quantity. Second, Fe reduction can be facilitated by humus substances that act as “electron shuttles”, especially for dissoluble Fe oxide (e.g. ferrihydrite) reduction (Burgin, et al., 2011), so humic substances also control nitrification through quality.

7. Line 40: ‘Meiklejohn, 1953’-> please find a more recent reference;

Re: We have added the information of another recent reference in Introduction section by “Studies on Fe requirements for ammonia-oxidizing bacterial (AOB) showed that when the Fe concentration in the medium of *Nitrosomonas europaea* culture increased from 0.2 to 10 μM Fe, the activities of both ammonia monooxygenase and hydroxy-lamine oxidoreductase decreased (Wei et al., 2006)” on lines 56-58 in the revised manuscript.

8. Lines 42-43: ‘These findings confirm the relevance of Fe oxides as a key factor in promoting pathways leading to N loss in soils.’-> this is not clear to me. You state in the sentence before that hematite is lowering AOB and AOA, so it is likely not promoting but lowering N loss, as denitrification should be reduced due to lower nitrification. . . ;

Re: in the discussion and revised edition of this manuscript, we had deleted the content of “These findings confirm the relevance of Fe oxides as a key factor in promoting pathways leading to N loss in soils.” We had improved the paragraph in Introduction section. Please see page 2 lines 54-63 in the revised manuscript.

9. Lines 47-49: I don’t understand the difference between the two questions: ‘Does the presence of Fe oxide influence the rate and amount of nitrification, N mineralization, and N immobilization in soils with different pH?’; and ‘How does Fe oxide influence these N transformation processes under different pH in soils with 100 % water holding capacity (WHC)?’. Please be more specific.

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Re: We have changed the second question by “ii) What is the mechanism of Fe oxide that influenced N transformations under different soil pH?” Please see lines 66-67 in the revised manuscript.

MATERIAL AND METHODS:

10. Line 54: ‘days->‘days per year’;

Re: We have revised it on line 72 in the revised manuscript.

11. Lines 53-59: please be more precise with the description of the studied sites. - Soils are classified as Fluvents, Udifluvents: both (agricultural land vs. hill)? Please describe what it means; - Precise soils management (possibly by citing previous papers on these sites); especially for the high pH site: how many years (‘a few’) after conversion? What was the amount of N fertilizer before? - Precise the dates of sampling;

Re: Both agricultural land and hill are classified as Fluvents, Udifluvents. The low pH soil was sampled from maize plots in a rotation system with sweet potato under conventional cultivation over ten years. In spring maize and autumn sweet potato growing seasons, N fertilizers were conventionally applied as urea at rates of 75 and 225 kg N ha⁻¹, respectively. The high pH soil was sampled from a pear orchard, which was converted from cropland three years ago and never been fertilized or tilled since the conversion. We sampled the soils on March 2015. Please see lines 72-78 in the revised manuscript.

12. Line 62: ‘and stored at 4 °C prior to use’-> for how long exactly?;

Re: Soil samples were stored at 4 °C prior to use within two months. We revised it. Please see line 81 in the revised manuscript.

13. Line 63: ‘passed through a 1 mm sieve’->why not 2 mm?

Re: We have added reference (Jiang et al., 2015a) to support the method of passing

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soil samples through a 1 mm sieve for measuring soil chemical analyses. Please see line 82 in the revised manuscript.

14. Lines 63-64: 'The results of the chemical properties of soils are shown in Table 1.'-> please put this sentence at the beginning of the 'Soil chemical parameter' paragraph;

Re: We have revised it on line 84 in the revised manuscript.

15. Line 71: 'XRD': please specify the brand of device;

Re: We have added the brand information of device in the "2.3 Preparation of Fe oxide treatments" section by "One portion of the suspension was freeze-dried and then analyzed for ferrihydrite using the X-ray diffractometer (XRD) (PANalytical B.V., Holland) (Fig. 1)" on lines 96-97 in the revised manuscript.

16. Line 73 and below: please choose a constant term in the manuscript between 'amended with Fe' vs. '+Fe' vs. 'Fe treated';

Re: We choose "Fe treated" as the constant term in the manuscript. Even that, we choose "+Fe" as the constant term in the pictures for convenience.

17. Line 77: why sieving at 2mm again? (the second time);

Re: The incubation soils were generally sieved at 2 mm, please refer to Jiang, X. J. et al. Effects of Fe oxide on N transformations in subtropical acid soils, Sci. Rep-UK, 5, 8615, 2015.

18. Line 82: 'free Fe oxides'-> what are they, 'available Fe' in Table 1? If not, they are lacking in Table 1 and 'available Fe' are not described;

Re: "available Fe" but not "free Fe oxides" is the result of the soils chemical property in the table. We have replaced "free Fe oxides" with "available Fe" and described the analysis of available Fe in the Material and Methods section. We have added the information on the measurement of available Fe "Available Fe was extracted using the diethylenetriamine penta-acetic acid (DTPA) method and analyzed by Inductively

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Coupled Plasma Optical Emission Spectrometry (ICP-OES).” on page 3 lines 88-89 of the revised manuscript.

19. Please put paragraph ‘2.3 Soil chemical analyses’ before paragraph ‘2.2 Preparation of Fe oxide treatments’;

Re: We have revised it. Please see lines 83-89 and lines 90-105 in the revised manuscript.

20. Paragraph ‘2.4 Experimental design and 15N addition’: please specify the total N of samples. Is it $2 \times 2 \times 2 \times 3 = 24 \times 5$ (time kinetics) = 120?

Re: Yes, the total experimental units is 120. We have revised it on lines 111-113 of the revised manuscript.

21. Line 90: ‘incubated for 6 days at 28 °C.’-> in the dark?

Re: Yes, all the treatments were incubated in dark. We have revised it on line 111 of the revised manuscript.

22. Lines 96-98: please specify in few words the techniques used for colorimetry and diffusion, and the model of machines;

Re: The contents of NH_4^+ and NO_3^- were quantified colorimetrically on a GENESYS 10 UV spectrophotometer (ThermoScientific, Madison, WI) using the salicylate method and the single reagent method, respectively (Verdouw et al., 1978; Doane and Horwath, 2003). Isotope analysis of NH_4^+ and NO_3^- was performed on aliquots of the extracts using a diffusion technique, by which NH_4^+ was distilled with Mg oxide and NO_3^- was converted to NH_4^+ by Devarda’s alloy and then distilled with Mg oxide. The NH_3 volatilizes were trapped using a boric acid solution (Feast and Dennis, 1996; Zhang et al., 2011, 2013). The ^{15}N isotopic composition in the trapped NH_3 volatilizes were then analyzed using an automated C/N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK). We have revised it on lines 119-126 of the revised manuscript.

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23. Line 100: 'MBN'. As for the other acronyms, once you defined them, use them always in the subsequent text;

Re: We have revised it on lines 132, 179, 180, 395 and 419 of the revised manuscript.

24. Line 104: 'were'->'was';

Re: We have revised it on line 131 of the revised manuscript.

25. Line 110: 'approximately'->' approximately';

Re: We have revised it on line 137 of the revised manuscript.

26. Paragraph '2.8 Statistical analyses': please state how you have checked the normality and homoscedasticity prior to ANOVA; please also specify the post-hoc tests you used;

Re: We have revised it by "Differences in soil NH_4^+ and NO_3^- content, net nitrification rate, gross mineralization rate, and MBN content among different treatments were assessed by analysis of variance (ANOVA). Prior to any statistical analysis, the normality of the data was evaluated by Shapiro–Wilk test and appropriate transformation (e.g. natural log-transformation) of the data was carried out if the transformation improved the normality. Post hoc Tukey's honestly significant difference multiple comparisons of means or paired t tests were used when appropriate to verify significant differences ($P < 0.05$) between treatments. All statistical analyses were performed by SPSS statistical package." on lines 149-154 of the revised manuscript.

RESULTS:

27. Lines 126-128: 'In both low and high pH soils, the NH_4^+ -N concentrations showed a significant decrease after the application of $(\text{NH}_4)_2\text{SO}_4$, at both 30.9 and 15.6 mg NH_4^+ -N kg^{-1} soil at day 1 and 6, respectively, in the higher pH soil with the Fe oxide amendment' -> not clear, please rephrase;

Re: We have revised this phrase by "The dynamics of soil inorganic N concentrations

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during the 6-day incubation are shown in Fig. 2. In both low and high pH soils with $(\text{NH}_4)_2\text{SO}_4$ application, the $\text{NH}_4^+\text{-N}$ concentrations significantly decreased over the course of incubation. For example, in the high pH soil with Fe oxide and $(\text{NH}_4)_2\text{SO}_4$ were applied, the $\text{NH}_4^+\text{-N}$ concentrations were 30.9 and 15.6 mg N kg^{-1} soil at day 1 and 6, respectively ($F=39.1$, $P=0.003$)." on lines 157-160 of the revised manuscript.

28. Lines 126-128: please describe the +K15NO₃ figures and results;

Re: We have added the results that "The NO₃—N concentrations increased significantly in all the $(\text{NH}_4)_2\text{SO}_4$ treatments during the incubation. However, the NO₃—N concentrations in all the KNO₃ treatments did not fluctuated significantly during the course of incubation ($P > 0.05$) (Fig. 2c and 2d)." Please see lines 160-162 in the revised manuscript.

29. Lines 137-140: please state what is significant. . . ;

Re: We have added the significant sentence in the end "Compared with the control, the addition of Fe oxide significantly decreased the net nitrification rate in the high pH soil by 22.7 %, whereas 27.1% of net nitrification rate was increased by Fe oxide in the low pH soil ($F = 63.1$; $P = 0.048$) (Fig. 3b)." on lines 172-174 of the revised manuscript.

30. Line 145: 'but slightly decreased it in the low pH soil' -> no, it is not significant so there is no decrease;

Re: We have deleted it in the revised manuscript.

DISCUSSION:

31. Line 158: 'suppressed' -> too strong. 'lowered'?

Re: We have deleted the word in the revised manuscript.

32. Line 168: 'Kuroiwa et al., 2011' -> reference lacking;

Re: We have added the information of "Kuroiwa, M., Koba, K., Isobe, K., Tateno, R.,

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Nakanishi, A., Inagaki, Y., Toda, H., Otsuka, S., Senoo, K., Suwa, Y., Yoh, M., Urakawa, R. and Shibata, H.: Gross nitrification rates in four Japanese forest soils: heterotrophic versus autotrophic and the regulation factors for the nitrification, J. For. Res., 16, 363–373, 2011.” in Reference section. Please see lines 287-289 in the revised manuscript.

33. Line 169: ‘it dominates nitrification’->’ it generally dominates nitrification’;

Re: We have revised it on line 203 of the revised manuscript.

34. Line 176: ‘occurance’->’ occurrence’;

Re: We have revised it on line 210 of the revised manuscript.

35. Generally in the Discussion: please discuss the MBN15N results. . . ; and discuss the impact on denitrification process;

Re: We have revised it by “The addition of Fe oxide had no influence on MB15N in the low pH soil, whereas in the high pH soil, 3.7 times higher MB15N was found in the Fe oxide treatment than in the control (Fig. 4a). The high MB15N content in the high pH soil with Fe oxide addition was probably related to the low activity of Fe oxide in the high pH soil due to the low solubility of Fe(III) oxide (Weber et al., 2006). Further research is needed to explore the mechanism of how the addition of Fe oxide increases microbial N assimilation in the high pH environment.” on lines 225-230 in the revised manuscript.

36. Line 183: ‘Jansson et al., 1955’-> please find a more recent reference;

Re: We have replenished the references by “(Jansson et al., 1955; Recous et al., 1990; Zhang et al., 2013)” and added the information of these references. Please see lines 217-218, lines 310-311 and lines 352-354 in the revised manuscript.

TABLE 1:

37. Legend: ‘studied soils’ too vague, precise; precise what are fluvent/udifluvents subsamples. . . the two sites?

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Re: We have changed “studied soils” to “the two soils with low pH and high pH”. We have revised the table information, and the soil type of the two sites is fluvent/udifluvents. Please see lines 363-364 in the revised manuscript.

38. The statistical data are lacking here! Specify as said above what is ‘available Fe’;

Re: We have replenished the statistical data in the table on line 364. Available Fe is the Fe which can be absorbed by soil microorganisms and plants and is extracted by DPTA (e.g. Wang, C., Ji, J., Yang, Z., Chen, L., Browne, P., and Yu, R.: Effects of soil properties on the transfer of cadmium from soil to wheat in the yangtze river delta region, China—a typical industry-agriculture transition area, Biological Trace Element Research, 148, 264-274, 2012).

FIGURE 1:

39. Legend, line 357: ‘moisture of’->‘moisture at’; ‘concentration’->‘concentrations’;

Re: We have revised it on lines 390, 393, 396, 399, 408, 414, 420 and 427 of the revised manuscript.

FIGURE 2:

40. Legend: specify Fig 2a and Fig2b after mineralisation and nitrification;

Re: We have added the information by “gross mineralization rate (a) and net nitrification rate (b)”. Please see lines 392 and 413 in the revised manuscript.

41. Are you sure that for Fig 2b pH5.1 control and pH 5.1 +Fe are statistically different?

Re: We have checked the calculation process and statistical analysis results and come to the conclusion that there is a significant difference between pH 5.1 control and pH 5.1+Fe treatments ($F = 63.1$, $P = 0.048$).

FIGURE 3:

42. Legend: specify acronyms + Fig 3a and Fig3b after 15N and N.

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Re: We have revised it by “MB15N (a) and MBN (b)” on lines 395 and 419 of the revised manuscript.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2016-170/bg-2016-170-AC2-supplement.pdf>

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-170, 2016.

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