Effect of iron oxide on nitrification in two agricultural soils with different pH

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Abstract. Iron (Fe) affects soil nitrogen (N) cycling processes both in anoxic and oxic environments. The role of Fe on soil N transformations such as mineralization, immobilization, and nitrification depends on its redox activity, which can be regulated by soil pH. We hypothesized that the effect of Fe oxide on N transformation processes would be different in soils as a function of pH. This study aimed to investigate N mineralization-immobilization, especially nitrification, as affected by Fe oxide in soils with different pH. A set of lab incubations under 100 % water holding capacity were carried out to investigate the effect of Fe oxide on N transformation rates in two subtropical agricultural soils with a low pH (pH 5.1) and a high pH (pH 7.8). 15N-labelled ammonium and nitrate were used separately to determine N transformation rates combined with Fe oxide (ferrihydrite) addition. Iron oxide addition stimulated net nitrification in the low pH soil (pH 5.1), while the opposite occurred in the high pH soil (pH 7.8). An explanation for this could be at low pH, Fe oxide increased NH3-N availability by stimulating N mineralization and inhibiting N immobilization. These results suggested that Fe oxide plays an important role in N transformations in soil ecosystem, and the effect of Fe oxide on N transformations depends on soil pH.

Keywords: Fe redox, net nitrification, gross mineralization, microbial immobilization

1 Introduction

Nitrification is the biological process that spans the full range of oxidation states of nitrogen (N) from -3 (NH4+) to +5 (NO3-), including compounds with intermediate oxidation states such as NH2OH and NO2- which are formed to various degrees during nitrification; it is therefore a key process in the global N cycle. The role of iron (Fe) and its oxides affect soil nitrification process both in anoxic (Clément et al., 2005; Yang et al., 2012; Ding et al., 2014) and oxic environments (Jiang et al., 2015a), yet its influence is rarely identified in biogeochemical cycles and models.

The direct participation of Fe in nitrification was first proposed as the Feammox reaction (Sawayama, 2006), referring to anaerobic ammonium (NH4+) oxidation coupled to Fe(III) reduction resulting in nitrate (NO3-) (Luther et al., 1997) or nitrite (NO2-) (Clément et al., 2005; Sawayama, 2006; Shrestha et al., 2009). This process usually occurs in anoxic conditions of wetland or saturated soils (Clément et al., 2005; Shrestha et al., 2009), suggesting that Fe oxides may play a critical role as
an electron acceptor in the absence of oxygen (O\textsubscript{2}) (Schuur and Matson, 2001; Wang and Newman, 2008; Liptzin and Silver, 2009; Park et al., 2009; Ding et al., 2014). Iron is also involved in other soil N transformations (e.g. N mineralization, heterotrophic denitrification, and chemodenitrification) via the Fe reduction-oxidation (redox) cycle, both biotically and abiotically (Li et al., 2012; Zhu-Barker et al., 2015). Oxidation of Fe (II) coupled to NO\textsubscript{3}\textsuperscript{-} reduction can proceed via biotic and abiotic pathways in wetland soils, sediments or anoxic microsites (Senn and Hemond, 2002; Davidson et al., 2003; Straub et al., 2004; Weber et al., 2006; Smolders et al., 2010). The coupling between biotic (e.g. Fe(III)-reducing microorganisms) and abiotic factors (e.g. pH) mediates the redox cycle of Fe that can lead to organic matter decomposition and thus N mineralization under oxic or anoxic conditions (Lovley and Phillips, 1986; Roden et al., 2004; Sahrawat, 2004; Weber et al., 2006; Bauer and Kappler, 2009; Hall and Silver, 2013). The oxidation of Fe (II) stimulates organic matter decomposition are assumed to via two mechanisms: (1) organic matter oxidation (driven by reactive oxygen species) and acidification; (2) releasing the dissolved organic carbon that complexed with Fe. Previous study with diverse West African rice soils showed that NH\textsubscript{4}\textsuperscript{+} production in submerged soils was significantly correlated to reducible Fe (III), suggesting that Fe-organic matter compound is an important factor that influences NH\textsubscript{4}\textsuperscript{+} production in submerged soils (Sahrawat, 2004).

Iron oxides can also affect microbial groups and their activities. Meiklejohn (1953) found that small amount of Fe (0.1-6 mg L\textsuperscript{-1}) stimulated growth of nitrifying bacteria and increased the oxidation of NH\textsubscript{3} to NO\textsubscript{2}\textsuperscript{-}, whereas high concentrations of Fe (>112 mg L\textsuperscript{-1}) was toxic to nitrifying bacteria. A recent study showed that the abundance of ammonia-oxidizing bacterial (AOB) and ammonia-oxidizing archaean (AOA) in an acidic forest soil were decreased after the addition of hematite, a type of Fe oxides (Jiang et al., 2015a). Nevertheless, it is difficult to generalize the response of nitrification to iron oxide addition in different pH soils since AOA and AOB occupy different soil niches according to soil pH, i.e., AOA dominates nitrification activity in acidic soils while AOB in alkaline soils (Stopnišek et al., 2010; Gubry-Rangin et al., 2011; Isobe et al., 2012; Jiang et al., 2015b).

We hypothesized that the effect of Fe oxide on N transformation processes would differ depending on soil pH. Two major questions are posed: i) Does the presence of Fe oxide influence the rate and amount of nitrification, N mineralization, and N immobilization in soils with different pH? ii) How does Fe oxide influence these N transformation processes under different pH in soils with 100 % water holding capacity (WHC)? To investigate Fe oxide effects on N dynamics in soils with different pH, a stable isotope (\textsuperscript{15}N) method was used to measure the gross rates of N transformations.

2 Material and Methods

2.1 Site description and soil sampling

Field sites are located at Beibei, Chongqing, China, which have a mean annual temperature of 18.2 °C, annual rainfall of 116 cm, and a frost-free period of 359 days. The soils were derived from a Cenozoic Quaternary Holocene (Q4) alluvium and are classified as Fluvents, Udifluvents (USDA, soil taxonomy) (Soil Survey Staff, 2014). Soil samples were collected from...
agricultural land (29.70° N, 106.38° E) with low pH soil (pH 5.1) and a hill site (29.75° N, 106.40° E) with high pH soil (pH 7.8). The low pH soil sample was collected from maize plots in a rotation system with sweet potato. The high pH soil sample was collected from pear orchard, which was converted from farmland a few years ago and never been fertilized and tilled since then.

Composite soil samples derived from five auger borings to 0–20 cm in depth were brought immediately to the laboratory. Stones, dead plant material, roots, and visible soil fauna were removed. One portion of the soil was slightly air-dried to reach a moisture content of about 15 %, sieved to 2 mm, and stored at 4 °C prior to use. Another portion of the soil was air-dried, passed through a 1 mm sieve and used for chemical analyses. The results of the chemical properties of soils are shown in Table 1.

2.2 Preparation of Fe oxide treatments

Ferrihydrite was used as a precursor to produce Fe oxide. The method of ferrihydrite preparation was modified from the method described by Lovley and Phillips (1986). A mixture of iron (III) sulfate hydrate (Fe$_2$SO$_4$·12H$_2$O) (40 g) and ultrapure water (500 ml) were stirred, followed by pH adjustment to 7–8 with 1 mol L$^{-1}$ KOH and then left to settle until entirely precipitated. The precipitate was centrifuged (2800 g, 5 min) and washed with ultrapure water five times until the suspension had a conductivity of < 20 μS. The particle density of ferrihydrite in the final suspension was 87 g L$^{-1}$. One portion of the suspension was freeze-dried to analyze for ferrihydrite using the X-ray diffractometer (XRD). The remaining ferrihydrite suspension was divided and adjusted to either pH of 5.1 or 7.8 to match the initial pH in the different soils.

For each soil, two Fe oxide treatments were applied: non-Fe (control) and amended with Fe (adjusted the pH of ferrihydrite to match the soil pH). Ferrihydrite was added at 3 % (w/w). The low pH soil without or with the ferrihydrite amendment was designated as pH 5.1 control or pH 5.1+Fe, while the high pH soil without or with Fe oxide amendment was designated as pH 7.8 control or pH 7.8+Fe. The suspension of ferrihydrite was added and mixed well with soils, then the soil mixtures were slightly air-dried to reach a moisture content of about 15 %, passed through a sieve of 2 mm and stored at 4 °C before use.

2.3 Soil chemical analyses

Soil pH was measured using a soil to water ratio of 1:2.5 (v/v) by a DMP-2 mV/pH detector (Quark Ltd, Nanjing, China). Total N (TN) and soil organic matter (SOM) contents were determined by a Macro Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Total soil Fe and free Fe oxides were extracted with HNO$_3$-HF-HClO$_4$ and Na$_2$S$_2$O$_3$-Na$_3$C$_6$H$_5$O$_7$-NaHCO$_3$, respectively. The concentration of Fe was measured by atomic absorption spectrophotometry with a graphite furnace (GFAAS) using a model Z-8200 spectrophotometer.
2.4 Experimental design and \(^{15}\)N addition

In this study, a set of \(^{15}\)N tracing experiments were conducted to quantify process-specific and pool-specific N transformation rates. For each soil, 20 g (dry mass) Fe treated or non-Fe treated soils were weighed into 150-ml conical flasks. Two N treatments were applied, \(^{15}\)N enriched (NH\(_4\)\(_2\)SO\(_4\) (10 atom% excess) or \(^{15}\)N enriched KNO\(_3\) (10 atom% excess). Each N treatment received 50 mg N kg\(^{-1}\) of soil and replicated three times. Soils in all the treatments were adjusted to 100 % WHC and incubated for 6 days at 28 °C. All the flasks were covered with polyethylene film punctured with needle holes to maintain oxic conditions in the headspace.

2.5 Soil extraction and soil N analysis

For soil mineral N analysis, soils (three replicates for each treatment) were extracted with 2 mol L\(^{-1}\) KCl (5 to 1 extractant volume to soil mass ratio) at hour 0 and 0.5, and days 1, 3, and 6 after the application of N. The extracted soils were centrifuged at 1200 rpm and the supernatants were frozen at –20 °C until analysis. The contents of NH\(_4^+\) and NO\(_3^-\) were analyzed using colorimetric methods (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 (Feast and Dennis, 1996; Zhang et al., 2011, 2013).

The \(^{15}\)N isotopic composition in NH\(_4^+\)-N and NO\(_3^-\)-N were analyzed using an automated C/N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK).

For soil microbial biomass N (MBN) analysis, soils were fumigated or unfumigated at time 0 and 6 days after N application, then extracted with 0.5 mol L\(^{-1}\) K\(_2\)SO\(_4\) (5 to 1 extractant volume to soil mass ratio) (Brookes et al., 1985; Breland and Hansen, 1996; Dempster et al., 2012). The extracts were filtered, and the supernatant was stored at –20 °C until analysis. The total dissolved N (TDN) in the extractant was separated by distillation with 25 mol L\(^{-1}\) NaOH solution (Brooks et al., 1989). The \(^{15}\)N isotopic composition in TDN were analyzed using an automated C/N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK). Microbial biomass N (calculated from 1 day CHCl\(_3\)-N) was calculated as the difference of TDN between fumigated and un-fumigated soils (Brookes et al., 1985; Dempster et al., 2012).

2.6 Analysis of Fe(II) production

Reduced iron, Fe(II), was quantified using the ferrozine assay method (Stookey, 1970). Briefly, 0.1 g soil was extracted with 0.5 mol L\(^{-1}\) HCl (Lovley and Phillips, 1987) and 100 μl of extracts was added to 4 ml of color reagent (1 g L\(^{-1}\) Ferrozine in 50 mmol L\(^{-1}\) HEPES buffer pH 8). After the color was developed (approximately in 15 s), the ferrous concentration was spectrophotometrically determined immediately by measuring the absorbance of the ferrozine-Fe(II) complex at 562 nm. Standards of ferrous iron for the ferrozine assay were prepared with ferrous ethylene diammonium sulfate dissolved in 0.5 mol L\(^{-1}\) HCl (Lovley and Phillips, 1986).
2.7 Data calculation

Gross N mineralization rate was calculated with the equation described by Kirkham and Bartholomew (1954) and Davidson et al. (1991). Net nitrification rate was calculated from the net increase of NO$_3^-$ concentration in the (NH$_4$)$_2$SO$_4$ treatment during the incubation period (Davidson et al., 1992). Microbial biomass $^{15}$N (MB$^{15}$N) was calculated as MB$^{15}$N = F$^{15}$N/0.54 (Brookes et al., 1985), where F$^{15}$N = (TD$^{15}$N in the digested fumigated sample) – (TD$^{15}$N in the digested non-fumigated sample). Total dissolved $^{15}$N (TD$^{15}$N) of fumigated and un-fumigated soils were calculated by multiplying the atom% excess TD$^{15}$N and the amount of N in the form of TDN (Shen et al., 1984; Brookes et al., 1985).

2.8 Statistical analyses

Data were subjected to one-way ANOVA and mean values were separated using Duncan’s New Multiple Range Test at $P < 0.05$. All statistical analyses were performed by SPSS statistical package.

3 Results

3.1 Soil inorganic N concentrations during the incubation

The dynamics of soil inorganic N concentrations during the 6-day incubation are shown in Fig. 1. In both low and high pH soils, the NH$_4^+$-N concentrations showed a significant decrease after the application of (NH$_4$)$_2$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. The NO$_3^-$-N concentrations increased significantly in all (NH$_4$)$_2$SO$_4$ treatments during the incubation.

3.2 Gross N mineralization and net nitrification rates

The gross N mineralization rate in the high pH soil was significant higher in the control than in the Fe oxide amended soils, whereas the opposite phenomenon was found in the low pH soil (Fig. 2a) ($P < 0.05$). During the entire incubation, 22.4 and 7.80 mg NH$_4^+$-N kg$^{-1}$ were mineralized in the high pH soil without and with the Fe oxide, while 5.88 and 7.32 mg NH$_4^+$-N kg$^{-1}$ were mineralized in the low pH soil without and with Fe oxide, respectively. No difference in gross N mineralization rate was found between the low and high pH soils with the Fe oxide, but the non-Fe oxide (control) low pH soil had significantly lower gross N mineralization than the high pH soil. The net nitrification rate in the high pH soil without the Fe oxide was 6.02 mg N kg$^{-1}$ soil day$^{-1}$, the highest among all the treatments, while the smallest net nitrification rate was 2.41 mg N kg$^{-1}$ soil day$^{-1}$ in the low pH soil without the Fe oxide. The application of Fe oxide decreased the net nitrification rate in the high pH soil by 22.7 %, whereas the net nitrification rate in the low pH soil was increased 27.1 % with Fe oxide (Fig. 2b).
3.3 Microbial nitrogen immobilization

The (15NH4)2SO4 amended soils were used to determine microbial N immobilization as affected by Fe oxide in the 6-day incubation (Fig. 3). The 15N content in the microbial biomass (MB15N) was 0.17 mg N kg⁻¹ soil in the low pH soil with Fe oxide, which was significantly lower than in the high pH soil with Fe oxide (0.65 mg N kg⁻¹ soil). The addition of Fe oxide had no significant influence on MB15N but slightly decreased it in the low pH soil, while MB15N content in the high pH soil was 3.7 times higher in the Fe oxide treatment than in the control (Fig. 3a). In the low pH soil, the total N in the microbial biomass (MBN) was 13.8 mg N kg⁻¹ soil in the control, which was significant lower than it in the high pH soil without Fe oxide (15.2 mg N kg⁻¹ soil). The addition of Fe oxide caused a significant decrease in MBN in the low pH soil with Fe oxide, while the opposite occurred in the high pH soil with Fe oxide (P < 0.05) (Fig. 3b).

3.4 Fe (II) production

The concentration of Fe (II) (0.5 mol L⁻¹ HCl extractable) in the soils with Fe oxide before and after 6 days incubation are shown in Fig. 4. In the low pH soil amended with Fe oxide, the concentration of Fe (II) was increased from 0.44 to 1.28 mg Fe kg⁻¹ soil after the 6 days incubation. In the high pH soil, the concentration of Fe (II) did not change between day 0 and days 6 after the addition of Fe oxide.

4 Discussion

The effect of Fe oxide on the nitrification rate varied in different pH soils under 100 % WHC, supporting our hypothesis in this study. The addition of Fe oxide stimulated the net nitrification rate in the low pH soil (pH 5.1) (F = 63.13; P = 0.048), but suppressed it in the high pH soil (pH 7.8).

Nitrification is primarily dependent on NH₃ availability and the activities of nitrifying microorganisms. Factors that affect NH₃ availability and nitrifying microorganisms can directly influence the nitrification process. Studies estimating relationships among the rates of N transformation using regression analyses on data from 100 studies on nearly 300 different organic and mineral soil materials also showed that nitrification rate was controlled by the rate of ammonia release following the mineralization of organic matter (Booth et al., 2005). In the present study, the addition of Fe oxide increased the net nitrification rate by 20.8 % in the low pH soil. This could be explained by changes in the gross N mineralization rate, which increased significantly in the low pH soil with Fe oxide. The increased N mineralization R–NH₂ → NH₃ rate at low pH increased the substrate availability for nitrification in presence of Fe oxide. Generally, both AOA and AOB play roles in nitrification, but it is difficult for AOB to sustain ammonia oxidation in soil with low pH due to the high pKa of ammonia (NH₃ + H⁺ → NH₄⁺; pKa = 9.25) (Kuroiwa et al., 2011). Since AOA have much higher affinity for NH₃ than AOB (Martens-Habbena et al., 2009), it dominates nitrification activity in acidic soils (Stopnišek et al., 2010; Gubry-Rangin et al., 2011; Isobe et al., 2012; Prosser and Nicol, 2012; Jiang et al., 2015b).
The high solubility of Fe(III) in low pH soil could also promote scavenging of hydroxylamine (NH$_2$OH), an intermediate in nitrification (Vajrala et al., 2013), by the chemical reaction 2Fe$^{3+}$ + 2NH$_2$OH → 2Fe$^{2+}$ + N$_2$ + 2H$_2$O + 4H$^+$ (Zhu-Barker et al., 2015). The significant increase in the Fe (II) concentration at the end of the incubation in the low pH soil with Fe oxide supported this assumption (Fig. 4). Under low pH, Fe(III) solubility is higher (Weber et al., 2006). It was not absolutely certain that the 100 % WHC condition in our incubation conditions provided a complete anoxic environment for the occurrence of anaerobic NH$_4^+$ oxidation into NO$_3^-$ or NO$_2^-$ by coupled Fe(III) reduction (Feammox). Thus, the process of Feammox cannot be used to explain the increase of the net nitrification rate in the low pH soil. Further studies are needed to fully understand the process of Feammox in the low pH soil.

In the high pH soil, iron oxide significantly decreased net nitrification rate. Immobilization of inorganic N increased due to the addition of Fe oxide in the high pH soil, might have contributed to the decrease in net nitrification (Fig. 3b). Furthermore, the toxicity of Fe oxide on nitrifying microorganisms could be an important reason for the decrease in nitrification in the high pH soil. Meiklejohn (1953) indicated that high concentrations of Fe (> 112 mg L$^{-1}$) was toxic to nitrifying bacteria.

Previous research showed that soil microbial communities prefer NH$_4^+$ to NO$_3^-$ as a source of N (Jansson et al., 1955). However, NO$_3^-$ immobilization was found to be high in undisturbed forest soils suggesting the microbial biomass maybe flexible in utilizing N sources (Stark and Hart, 1997). Besides the factors of inorganic N (NH$_4^+$ or NO$_3^-$) availability and microbial activity (Stark and Hart, 1997), Fe oxide is another factor that affects microbial N immobilization. In the low pH soil, Fe oxide addition caused a significant decrease in MBN, while the opposite was found in the high pH soil (Fig. 3b). This could indicate that the high solubility of Fe oxide at low pH could impair the assimilation of N by the microbial biomass.

While Fe oxide in the low pH soil decreased microbial N assimilation, the Fe(III) reduction process could release Fe-bound N and lead to N mineralization and ammonification, thus increasing nitrification potential. Compared with the low pH soil, the activity of Fe oxide in the high pH soil was low due to the low solubility of Fe(III) oxide (Weber et al., 2006). Thus, no or low Fe(III) reduction likely occurred in the high pH soil.

5 Conclusions

The addition of Fe oxide stimulated net nitrification and gross N mineralization rates but reduced microbial N immobilization in the low pH soil. The opposite was observed in the high pH soil. These findings indicated that Fe oxide has an important role in N transformations. The effect of Fe oxide on N transformations varies according to pH. Further studies should focus on Fe redox in different pH soils to elucidate mechanisms on how Fe oxide changes N mineralization and nitrification through abiotic and biotic-related processes to influence the production of N$_2$, N$_2$O and NO$_2^-$.
Author contributions

Xueru Huang and Xianjun Jiang designed the experiments and Xueru Huang carried them out. Xueru Huang prepared the manuscript with contributions from co-authors. Xianjun Jiang, Xia Zhu-Barker, William R. Horwath and Sarwee J. Faeflen helped with discussion and language checking.

Acknowledgements

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References


### Table 1. Chemical properties of studied soils. Different letters represent statistically significant between treatments at $P < 0.05$. 

<table>
<thead>
<tr>
<th>Subsamples</th>
<th>pH</th>
<th>Organic matter (g kg$^{-1}$)</th>
<th>Total N (g kg$^{-1}$)</th>
<th>Total Fe (g kg$^{-1}$)</th>
<th>Available Fe (mg kg$^{-1}$)</th>
<th>NO$_3^-$-N (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>NH$_4^+$-N (mg N kg$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
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<td>Fluvents</td>
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<td>132</td>
<td>10.3 a</td>
<td>1.54 b</td>
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<tr>
<td>Udifluvents</td>
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<td>13.9</td>
<td>0.68</td>
<td>27.5</td>
<td>5.64</td>
<td>4.68 b</td>
<td>2.44 a</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1: Effects of Fe oxide on NH$_4^+$-N and NO$_3^-$-N dynamics during 6-day by $^{15}$N tracing incubation at 28 °C with soil moisture of 100 % WHC. NH$_4^+$-N and NO$_3^-$-N concentration were measured following the addition of 50 mg N kg$^{-1}$ ($^{15}$NH$_4$)$_2$SO$_4$ (a and b) and K$^{15}$NO$_3$ (c and d). Error bars represent standard deviation, n = 3.

Figure 2: Effects of Fe oxide on gross mineralization rate and net nitrification rate during 6-day for incubated soil samples incubation at 28 °C with soil moisture of 100 % WHC. Error bars represent standard deviations, n = 3. The different letters above the columns indicate a significant difference ($P < 0.05$).

Figure 3: Effects of Fe oxide on microbial biomass $^{15}$N and microbial biomass N pools during 6-day with ($^{15}$NH$_4$)$_2$SO$_4$ treatment incubation at 28 °C with soil moisture of 100 % WHC. Error bars represent standard deviations, n = 3. The different letters above the columns indicate a significant difference ($P < 0.05$).

Figure 4: Effects of Fe oxide on concentration of Fe(II) (0.5 mol L$^{-1}$ HCl extractable) before and after 6-day with ($^{15}$NH$_4$)$_2$SO$_4$ treatment incubation at 28 °C with soil moisture of 100 % WHC. Error bars represent standard deviations, n = 3. The different letters above the columns indicate a significant difference ($P < 0.05$).
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