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Effects of Fe oxide on N transformations in subtropical acid soils

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Subtropical ecosystems are often characterized by high N cycling rates, but net nitrification rates are often low in subtropical acid soils. NO_3^--N immobilization into organic N may be a contributing factor to understand the observed low net nitrification rates in these acid soils. The effects of Fe oxide and organic matter on soil N transformations were evaluated using a ^{15}N tracing study. Soil net nitrification was low for highly acidic yellow soil (Ferralsols), but gross ammonia oxidation was 7 times higher than net nitrification. In weakly acidic purple soil (Cambisols), net nitrification was 8 times higher than in Ferralsols. The addition of 5% Fe oxide to Cambisols, reduced the net nitrification rate to a negative rate, while NO_3^--N immobilization rate increased 8 fold. NO_3^--N immobilization was also observed in Ferralsols which contained high Fe oxides levels. A possible mechanism for these reactions could be stimulation of NO_3^--N immobilization by Fe oxide which promoted the abiotic formation of nitrogenous polymers, suggesting that the absence of net nitrification in some highly acid soils may be due to high rates of NO_3^--N immobilization caused by high Fe oxide content rather than a low pH.

Soils in subtropical and tropical forests are generally characterized by rapid N cycling rates and high N availability[1–3]. However, a few studies indicated that nitrification seems to be absent or a minor process in subtropical acid soils[4–7]. This interpretation was supported by two observations: one is that NO_3^- concentrations are often consistently low in these soils, and that net nitrification rates are low or even negative during incubation assays of soil samples[6–9]. However, this does not necessarily mean that the nitrification does not occur in these subtropical acid soils. Alternatively, it is possible that NO_3^--N produced in these soils during nitrification (ammonia plus organic N oxidation) is immobilized through both abiotic and biotic processes as for observed in coniferous forest soils[10]. For example, high rates of NO_3^--N immobilization into organic N can be responsible for N enrichment in subtropical acid forest soils[3] which could protect the soil N from leaching. Furthermore, NO_3^--N can be converted readily to other inorganic forms through denitrification or dissimilatory NO_3^- reduction to NH_4^+ (DRNA). Previous studies also suggested that N immobilization can be controlled by the concentration of available C[11] and available inorganic N[12,13]. Nugroho et al.[14] observed that net nitrification rates in acid soils can vary about three fold at similar soil pH but with different organic matter content. Of course, both biotic and abiotic processes may be significant or one process may dominate, depending on the soil conditions. However, the causes of variation of N immobilization between soil types are not well known[15].

Subtropical and tropical acid soils are often characterized by high levels of Fe oxides. It has been hypothesized that Fe may play a key role in regulating NO_3^- immobilization in acid forest soils[16]. Likewise, the inhibition of nitrification by high amounts of iron from pyrite (FeS_2) can also occur[17]. Shindo and Huang[18] found that Mn oxide promoted the abiotic formation of nitrogenous polymers. Since Fe and Mn oxides have similar chemical properties, it is possible that Fe oxide can also promote organic N formation from NO_3^- Therefore, Fe oxide may be a primary player in N transformations in subtropical acid soils and may help to explain the low NO_3^- concentrations often observed. Here, we hypothesized that nitrification does occur in subtropical acid soils, and NO_3^--N produced during the nitrification process is immobilized quickly by Fe oxide. A weakly acidic purple soil (Cambisols, Chromic) and a highly acidic yellow soil (Ferralsols, Xanthic) were selected to test this hypothesis. Both soils formed from the same parent material in the same climate but had different Fe oxide

content. The effects of Fe oxide or organic substances addition on N transformations in these soils were estimated using a ^{15}N tracing method and N transformation model[19].

Results

Effects of Fe oxide or organic substances addition on NH_4^+-N and NO_3^--N concentrations during incubation. Half an hour after 50 mg kg^{-1} $^{15}NH_4NO_3$ spiking, NH_4^+-N concentrations for the Ferralsols control averaged 43.1 ± 1.02 mg N kg^{-1}. Organic substance or Fe oxide addition did affect NH_4^+-N concentrations significantly. During the 7 days incubation period, NH_4^+-N concentrations did not change significantly for the Ferralsols control and its two treatments (Figure 1A). However, NH_4^+-N dynamics for the Cambisols differed from Ferralsols, as Fe oxide and organic substances addition caused higher significant effects for Cambisols than Ferralsols. At half an hour, after 50 mg kg^{-1} $^{15}NH_4NO_3$ was introduced, NH_4^+-N concentrations decreased rapidly for the Cambisols control, and further declined during the 7-day incubation ($p < 0.05$). A similar trend was observed when organic substances were added to Cambisols. However, Fe oxide addition did not change NH_4^+-N concentrations throughout the 7-day incubation (Figure 1B).

Half an hour after the addition of 50 mg kg^{-1} NH_4 $^{15}NO_3$, NO_3^--N concentrations for the Ferralsols control averaged 9.89 ± 0.36 mg NO_3^--N kg^{-1}. Addition of organic substances or Fe oxide did not affect NO_3^--N concentrations. During the 7 days incubation period, NO_3^--N concentrations did not change significantly, and did not respond to Fe oxide or organic substances addition in the Ferralsols control (Figure 1C). Conversely, NO_3^--N dynamics in the Cambisols differed were significantly from the one in the Ferralsols. Half an hour, after 50 mg kg^{-1} NH_4 $^{15}NO_3$ application, NO_3^--N concentrations for the Cambisols control averaged 10.5 ± 0.20 mg N kg^{-1}. NO_3^--N concentrations increased significantly for the Cambisols control and organic substances treatment through time, but decreased significantly in the Fe oxide treatment during the 7 days incubation (Figure 1D).

Effects of Fe oxide or organic substances addition on N transformation rates. Our results clearly showed that soil net nitrification rates, gross autotrophic nitrification (ammonia oxidation) rates, NH_4^+ immobilization rates and NO_3^- immobilization rates were significantly affected by Fe oxide addition.

The net nitrification rate for the Ferralsols averaged 0.05 ± 0.01 mg N kg^{-1} soil day^{-1}, which was significantly lower than for Cambisols (0.41 ± 0.04 mg N kg^{-1} soil day^{-1}), and the organic

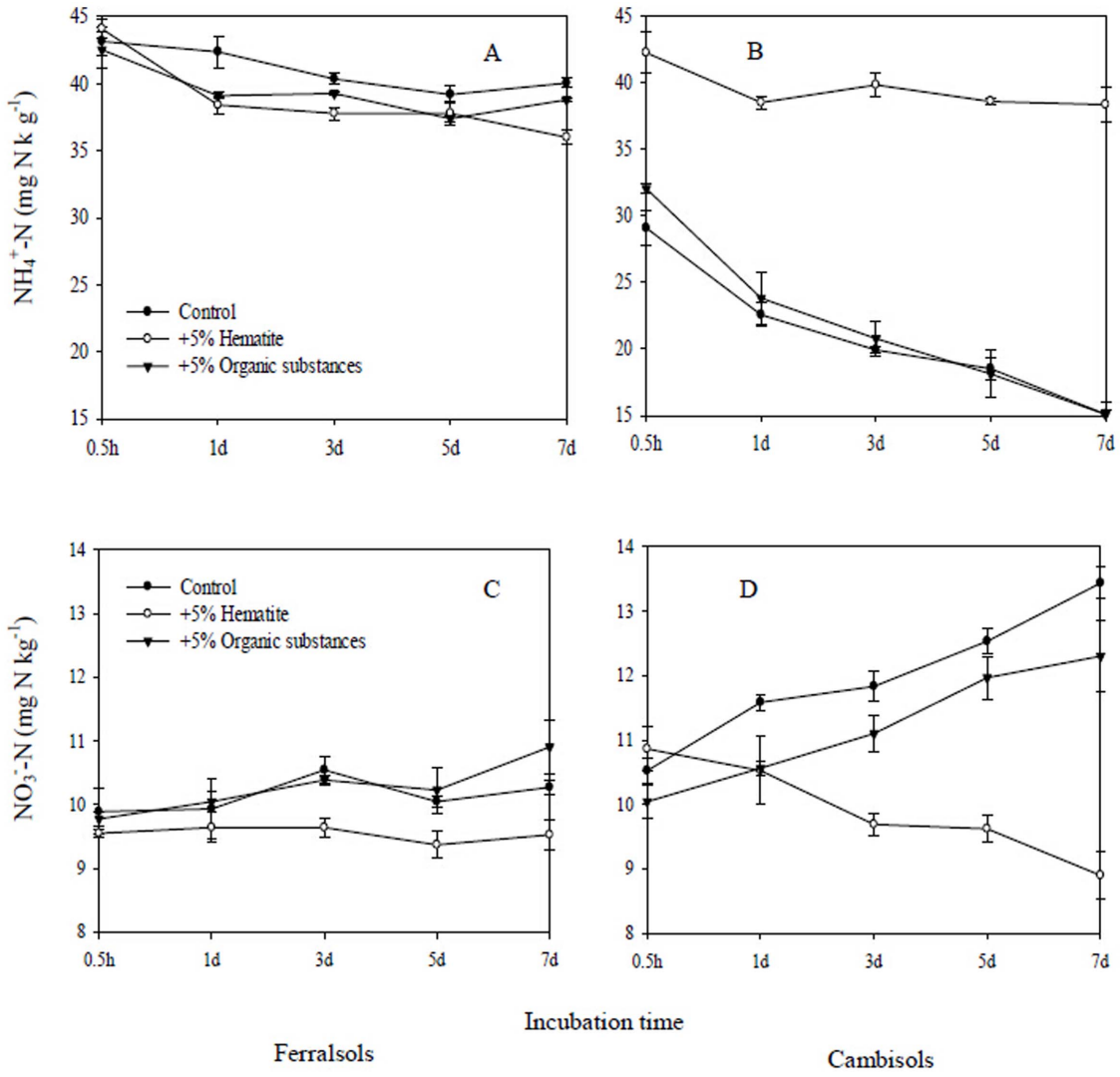

Figure 1 | Effects of Fe oxide or organic substances addition on NH_4^+-N and NO_3^--N dynamics during 7-day's by ^{15}N tracing. (NH_4^+-N concentration was measured following the addition of 50 mg kg^{-1} $^{15}NH_4NO_3$; NO_3^--N concentration was measured following the addition of 50 mg kg^{-1} NH_4 $^{15}NO_3$). Error bars represent standard deviation, n = 3.

substances treatment showed a non-significant decreasing trend in nitrification rates ($p > 0.05$). However, Fe oxide addition decreased the net nitrification rate significantly from 0.41 ± 0.04 to 0.28 ± 0.03 mg N kg^{-1} soil day^{-1} for Cambisols but not for Ferralsols.

The gross ammonia oxidation rates were 0.21 ± 0.03 mg N kg^{-1} day^{-1} and 0.74 ± 0.05 mg N kg^{-1} day^{-1} for the Ferralsols control and Cambisols respectively ($p < 0.05$) (Figure 2B). Furthermore, Fe oxide addition significantly decreased the gross NH_4 oxidation rate in Cambisols, whereas the organic substances amendment did not change gross ammonia oxidation ($p > 0.05$). Neither Fe oxide nor organic substances amendments changed gross NH_3 oxidation rates in the Ferralsol (Figure 2A).

Fe oxide or organic substances amendments did not change heterotrophic nitrification rates for the Ferralsols (Figure 2C). However, the Fe oxide treatment increased the heterotrophic nitrification rate for Cambisols (Figure 2D).

NO_3^- immobilization rates for Cambisols were not significantly affected by organic substances addition (Figure 3D) but they significantly increased by Fe oxide addition. NO_3^- immobilization was also observed for the Ferralsol treatments, including the control and both Fe oxide and organic substances treatments. However, Fe and organic substances amendments did not significantly change NO_3^- immobilization rates for Ferralsols compared to the unamended control (Figure 3C).

Gross NH_4^+ immobilization was four times higher for Cambisols than Ferralsols (Figure 3A, B). Hematite addition significantly decreased the NH_4^+ immobilization rate for Cambisols, and the organic substances treatment also showed a decreasing trend but the difference was not significant ($p > 0.05$). NH_4^+ immobilization rates for Ferralsols did not change in response to Fe oxide and organic substances addition.

Gross N mineralization rates did not differ significantly between Ferralsols and Cambisols in the Fe oxide and organic substance treatment ($p > 0.05$), although Fe oxide addition tended to decrease gross N mineralization for both soils (Figure 4).

Ammonia-oxidizing bacterial (AOB) and ammonia-oxidizing archaeal (AOA) *amoA* gene copies. Abundance of AOB and AOA was estimated by quantifying their respective *amoA* gene copy numbers after pre-incubation (Figure 5). The organic substances treatment tended to decrease AOB abundance for both Ferralsols and Cambisols but results were not significant ($p > 0.05$). However, Fe oxide addition significantly decreased AOB *amoA* gene copy numbers by about 50% for Cambisols. For Ferralsols, Fe oxide addition also tended to decrease AOB abundance, but not significantly. The abundance of AOB was significantly higher for Cambisols than Ferralsols, but the difference was not significant after 5% Fe oxide addition.

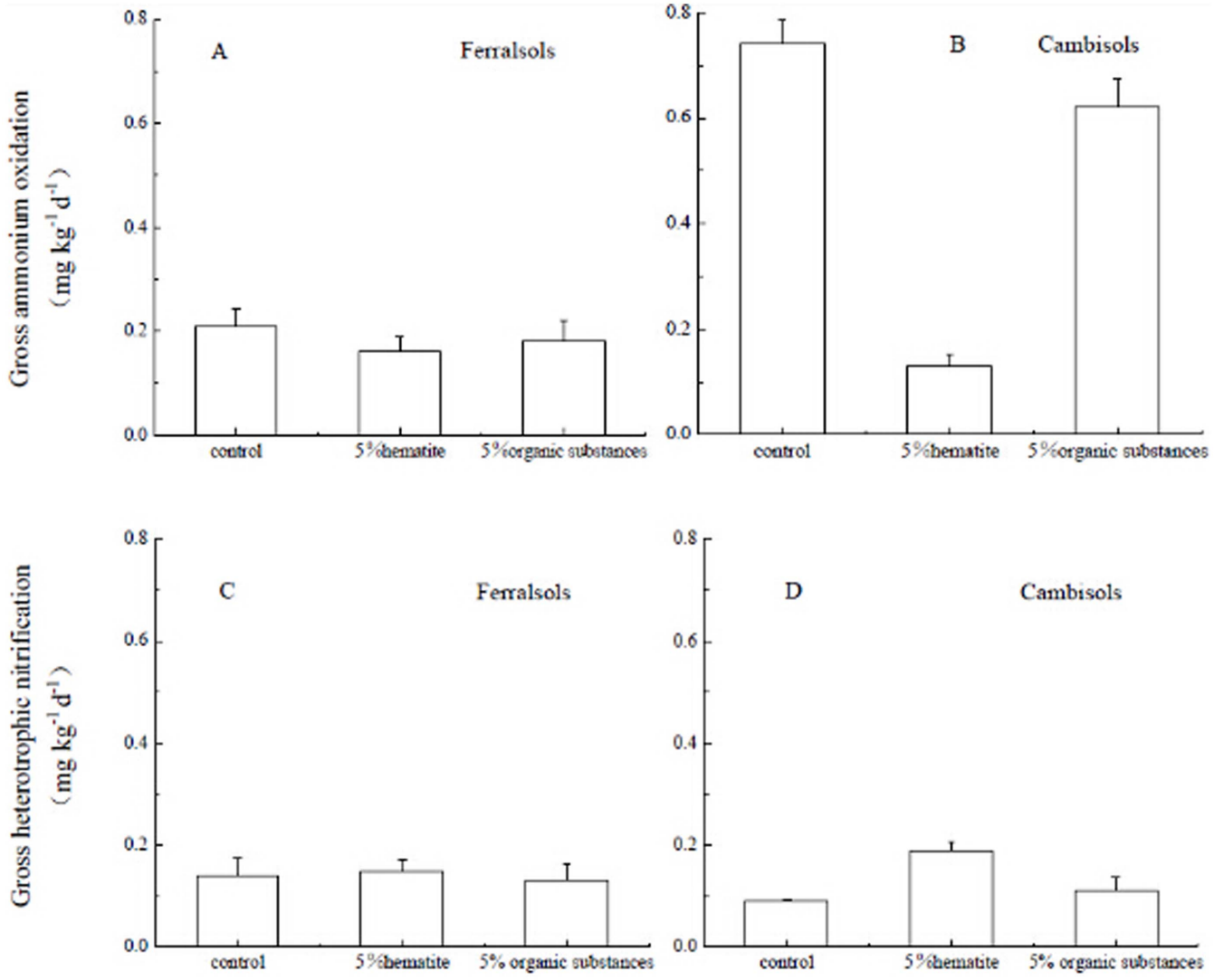

Figure 2 | Effects of Fe oxide or organic substances addition on gross heterotrophic nitrification and gross ammonia oxidation in Ferralsols and Cambisols estimated by the ^{15}N tracing. Error bars represent standard deviation, n = 3.

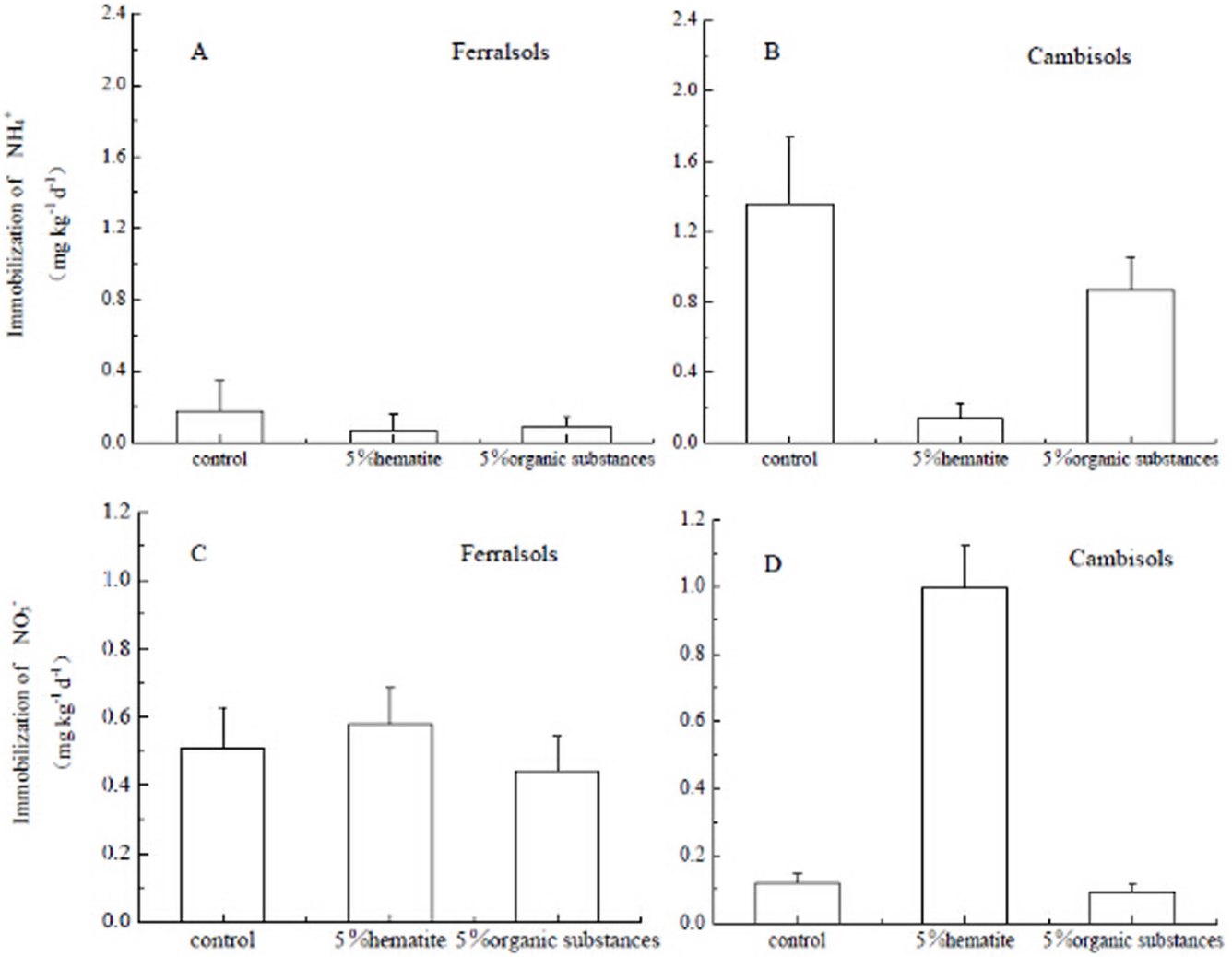

Figure 3 | Effects of Fe oxide or organic substances addition on the immobilization of NO_3^- and NH_4^+ (total of two immobilization rates including immobilization into recalcitrant and immobilization into labile organic N) (mg kg^{-1} d^{-1}) in Ferralsols and Cambisols estimated by the ^{15}N tracing model. Error bars represent standard deviation, n = 3.

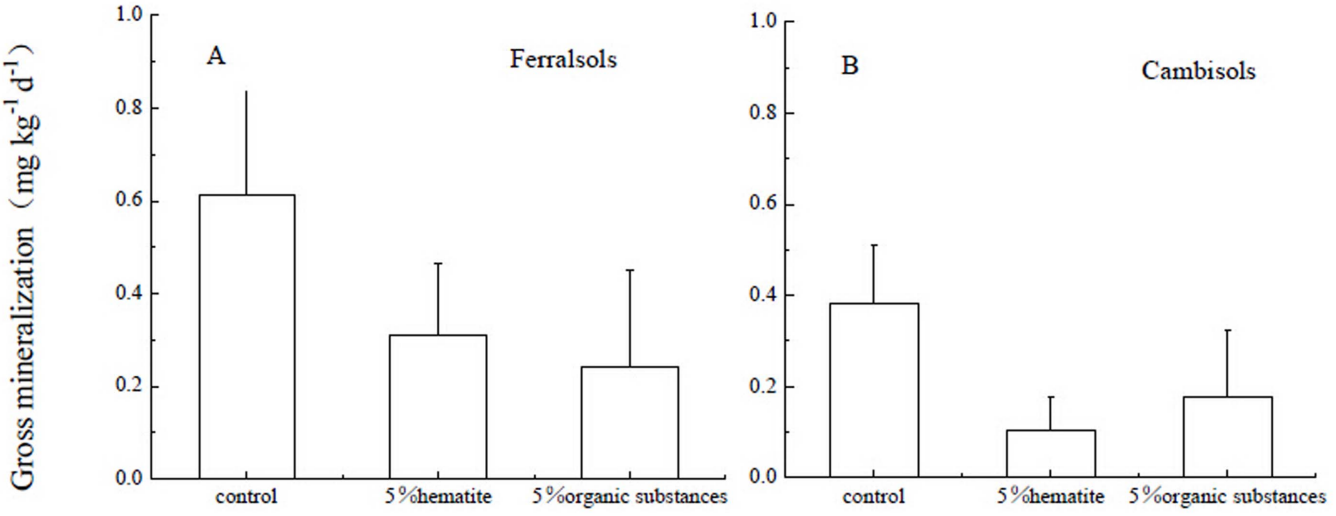

Figure 4 | Effects of Fe oxide or organic substances addition on gross mineralization (mineralization of recalcitrant organic N and labile organic N to NH_4^+) (mg kg^{-1} d^{-1}) in Ferralsols and Cambisols estimated by the ^{15}N tracing model. Error bars represent standard deviations, n = 3.

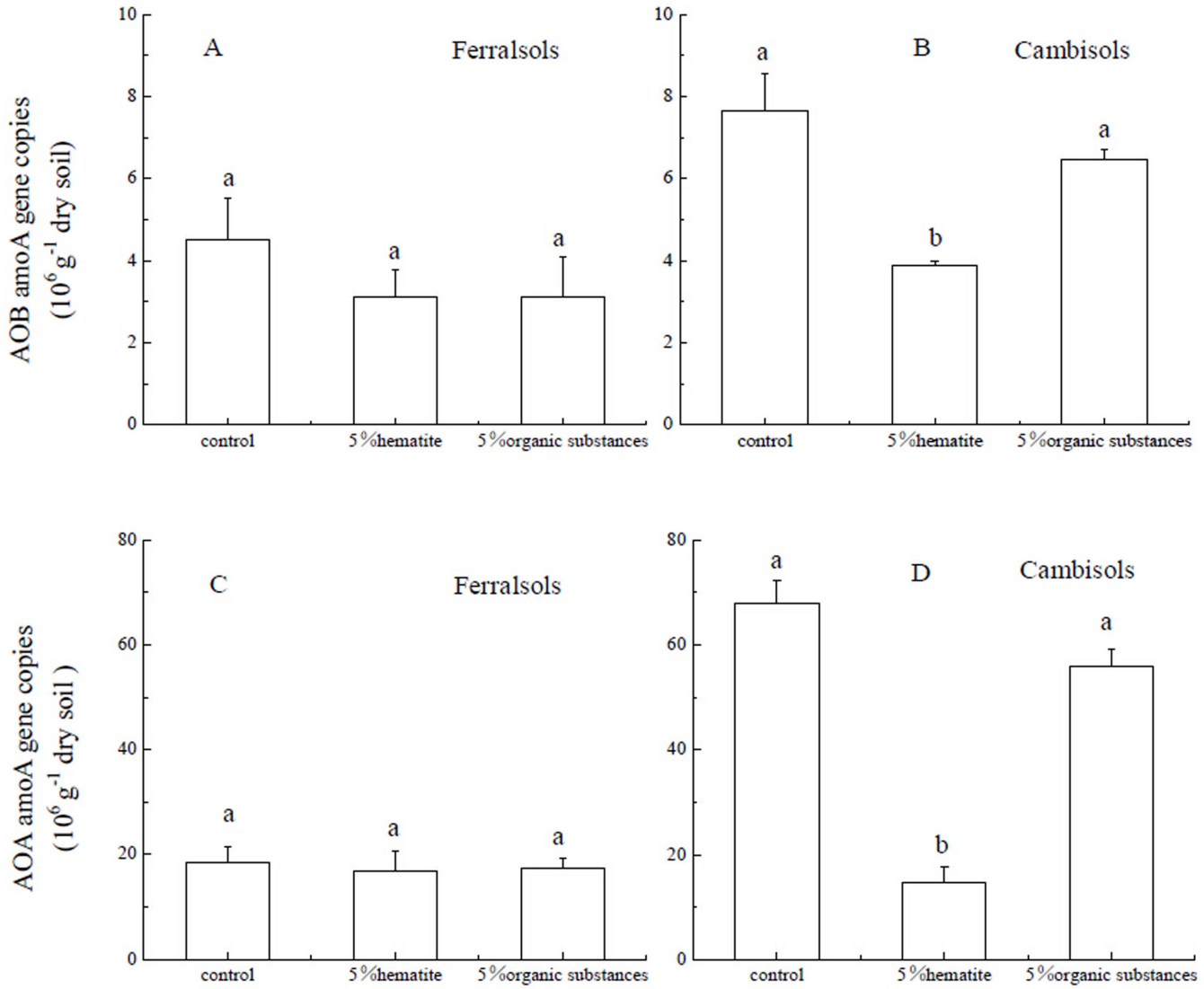

Figure 5 | Effects of Fe oxide or organic substances addition on abundance of ammonia-oxidizing bacterial (AOB) and ammonia-oxidizing archaeal (AOA) *amoA* gene copies. Lowercase letters indicate statistically significant differences ($p < 0.05$). Error bars represent standard deviations, $n = 3$.

AOA *amoA* gene copy numbers were significantly higher for Cambisols than Ferralsols (Figure 5). Fe oxide addition decreased AOA *amoA* gene copy numbers for Cambisols to a similar level in Ferralsols. For Ferralsols, Fe oxide addition also did not change AOA abundance significantly. Similar to AOB, AOA abundance showed a non-significant decreasing trend after the organic substances addition for both Ferralsols and Cambisols ($p > 0.05$).

Discussion

While net nitrification was low for the highly acidic Ferralsols, gross autotrophic nitrification rates were about three times higher than net nitrification rates. The net nitrification rate was 8 times higher for weakly acidic Cambisols than highly acidic Ferralsols. However, the net nitrification rate decreased to a negative value for the weakly acidic Cambisols after Fe oxide addition. Results clearly showed that soil net nitrification rate was significantly inhibited by Fe oxide addition. However, only the evaluation of the associated individual gross N rates sheds light whether the oxidation to NO_3^- or immobilization of NO_3^- was responsible for this result.

Nitrification could be retarded by high amounts of Fe from pyrite (FeS_2). Blaise[17] reported that nitrification was inhibited by 40.3% with the addition of 10000 mg pyrite kg^{-1} soil at the end of a 30-day incubation. However, they were unable to identify the exact mechanism by which pyrite inhibits nitrification/immobilization. Furthermore, postulated that the effect of pyrite could be due to the toxic action of any one or a combination of the following: (1) sulphides[20]; (2) the oxidized forms of the sulphides, or (3) presence of Fe^{2+} ions[21]. Compared to their results, a stronger inhibition of net nitrification by 50000 mg hematite kg^{-1} soil was observed in the present study. However, the mechanisms they postulated for pyrite inhibition of nitrification did not seem to be applicable for the present study since S or sulphides were not involved. Therefore, other mechanisms must be responsible for ferric iron inhibition of nitrification.

The toxicity of Fe to nitrifying microorganisms may partly contribute to the depression of nitrification. A pioneer study by Meiklejohn[22] indicated that small amounts of Fe (0.1–0.6 mg L^{-1}) stimulated growth of nitrifying bacteria and increased the oxidation of NH_3 to NO_2^-, whereas high concentrations of Fe (>112 mg L^{-1}) was toxic to nitrifying bacteria. The present results clearly demonstrated that both *amoA* genes for the ammonia monooxygenase (AMO) of AOB and AOA decreased two or three times after Fe oxide addition to Cambisols, and the soil gross ammonia nitrification rate also decreased. Higher *amoA* gene copy numbers of AOB and AOA

were observed for Cambisols than for Ferralsols. Since Fe oxide content of Ferralsols (50.1 mg kg^{-1} soil) was significantly higher than for Cambisols (16.6 mg kg^{-1} soil), AOB and AOA were likely inhibited by high Fe oxide content. However, Fe toxicity to AOB and AOA cannot explain the decrease of gross ammonia nitrification completely, because the average activity was estimated around 40 fmol NH_3 $cell^{-1}$ day^{-1} for AOA and AOB[23,24]. The gene copy numbers of AOB and AOA after Fe oxide addition still have potential nitrification up to 20 mg N kg^{-1} soil day^{-1}.

The gross NO_3^--N immobilization rate for Cambisols was 8 times higher in the 5% Fe oxide compared to the control treatment. In general immobilization of NO_3^- is assumed to be assimilated into microbial biomass. However, higher NO_3^--N immobilization induced by Fe oxide in the present soils can not be explained by the assimilation of NO_3^--N into microbial biomass. This was supported by the fact that NH_4, rather than NO_3, is the preferred form of N for assimilation by soil microorganisms[25–27]. Further evidence was that the gene abundance of nitrifying microorganisms decreased after Fe oxide addition (Figure 5). Results from upland tropical forest soils in America also showed that microbial biomass ^{15}N did not increase after NO_3^- addition[28]. Positive charges on the soil surface may also increase NO_3^--N immobilization under field conditions[29–31]. Anion exchange capacity (AEC) increased significantly after Fe oxide addition (Table 1). However, it cannot explain why NO_3^--N concentrations kept decreasing with time after Fe oxide addition because most of the NO_3^--N absorbed together with cations should have been released through ion exchange by the extractant (2 M KCl).

A potential mechanism of abiotic immobilization of NO_3^- has been postulated, and the authors suggested that Fe plays a key role in NO_3^--N immobilization by promoting organic N formation from inorganic N[16]. Shindo and Huang found that Mn oxide significantly promoted the abiotic formation of nitrogenous polymers in the pH range 4 to 8 within 24 hours[18]. Since Fe and Mn oxides have similar chemical properties, it is possible that Fe oxide also functions similarly and can thus promote organic N formation. The mechanism for this organic N formation may explain the N dynamics for the two soils evaluated in our study. In a recent study, Zhang et al. reported that the gross autotrophic nitrification ranged from 0 to 0.5 μg N $g^{-1}d^{-1}$ and the NO_3^--N immobilization rate into organic N ranged from 0.11 to 1.71 μg N $g^{-1}d^{-1}$ in a subtropical acid forest soil[3]. Their results indicated high rates of NO_3^--N immobilization into organic N were responsible for the N enrichment in humid subtropical forest soils which contained large amounts of Fe and Mn oxides. A higher net nitrification rate occurred for weakly acidic Cambisols than for highly acidic Ferralsols in our study, and the NO_3^--N immobilization was higher for Ferralsols than for Cambisols. The Fe oxide content of Ferralsols (50.1 g kg^{-1} soil) was higher than for Cambisols (16.6 g kg^{-1} soil), which implied that the absence of nitrification in some highly acid soils may due to high amounts of NO_3^--N immobilization stimulated by high Fe oxide concentrations.

Soil net nitrification, gross NH_4^+ oxidation, total gross NH_4^+ mineralization and NO_3^--N immobilization did not change significantly by organic substances addition for both soils. The insignificant response of N transformations by C/N ratio or organic substances addition may be due to the short incubation period which was not enough to highlight possible effects.

Cambisols and Ferralsols formed from the same parent material in the same climate, but differing in pH. While net nitrification occurred for the slightly acidic Cambisols, no obvious net nitrification was observed for highly acidic Ferralsols. The soil net nitrification rate strongly decreased to a negative value after Fe oxide addition to the slightly acidic Cambisols. However, significant autotrophic nitrification was observed using ^{15}N tracers for both soils. Fe oxide greatly increased NO_3^--N immobilization, probably by promoting the abiotic formation of nitrogenous polymers. This suggests that the absence of net nitrification in some highly acid soils may due to high amounts of NO_3^--N immobilization caused by the high Fe oxide content rather than the low pH.

Methods

Site description and soil sampling. The yellow soil samples (Ferralsols, Xanthic) were collected from a pine forest soil derived from a sandstone parent material in Jingyun Mountain, Chongqing, China (29°83′N,106°39′E). In this region, the annual mean temperature is 19.6°C and annual rainfall is 1611 mm. Middle subtropical evergreen broad-leaved forests characterize the vegetation of this area. Purple soils (Cambisols, Chromic) were collected at a nearby hill. The Ferralsols samples were highly weathered and experienced a long developing process. To the contrary, Cambisols were slightly weathered and have not experienced a long developing process because of soil erosion. Selected soil properties for two soils and each treatment were listed in Table 1.

Four field replicates were taken at each site to a 0–20 cm depth in October, 2012, with each replicate being composed of seven individual soil cores (diameter was 5.5 cm), which were pooled and homogenized to reduce heterogeneity. Samples for each of the four replicates per site were were air-dried and separated into two parts. One was ground to pass a 2-mm sieve and used to make subsamples for incubation, another was ground to pass a 1-mm sieve and used for chemical analyses.

Preparation of Fe oxide and organic substances treatments. The precipitates of hematite were prepared from ferric chlorite of at least 98% purity, and solutions containing 100 g of $FeCl_3 \cdot 6H_2O$/L were prepared. Ferric oxides were precipitated by the addition of NH_3 to the $FeCl_3$ solution until pH 7.0. The resultant suspension was thoroughly dialyzed for 24 hours at which time no further Cl^- and Fe^{3+} was detected in the external solution, followed by aging for 48 hours in a 160°C oven[32]. Obtained

Table 1 | Selected soil properties

Treatments	Texture	Parent material	pH	SOM (g/kg)	TN (g/kg)	CEC (cmol/kg)	AEC (cmol/kg)	Total Fe (g/kg)	Free Fe oxide (g/kg)	C/N
Ferralsols	Sandy loam	Purple sandstone	4.2	16.0 ± 0.34[b]	1.96 ± 0.01[a]	7.50 ± 0.86[b]	1.91 ± 0.23[b]	50.1 ± 4.33[a]	4.47 ± 0.47[b]	4.73
Ferralsols + 5% hematite			4.0	16.1 ± 0.69[b]	1.86 ± 0.06[a]	7.30 ± 0.77[b]	3.77 ± 0.17[a]		6.37 ± 0.84[a]	5.02
Ferralsols + 5% organic substances			4.1	20.9 ± 1.19[a]	1.85 ± 0.11[a]	7.37 ± 3.00[b]	2.24 ± 0.15[b]		4.68 ± 0.86[b]	6.55
Cambisols	Sandy loam	Purple sandstone	5.8	14.6 ± 1.62[b]	0.96 ± 0.10[b]	35.9 ± 2.20[a]	1.04 ± 0.47[c]	16.6 ± 1.21[b]	1.52 ± 0.10[c]	8.82
Cambisols + 5% hematite			5.4	14.3 ± 0.69[b]	0.81 ± 0.04[b]	33.3 ± 0.73[a]	1.93 ± 0.35[b]		5.84 ± 0.68[ab]	10.2
Cambisols + 5% organic substances			5.6	19.1 ± 1.39[a]	0.82 ± 0.09[b]	32.5 ± 3.29[a]	1.38 ± 0.22[c]		1.60 ± 0.23[c]	13.5

[a]SOM reprents soil organic matter; TN reprents total nitrogen; CEC reprents cation exchange capacity; AEC reprents anion exchange capacity.
[b]Values behind ± represent standard deviation.
[c]Mean values in a same column not followed by the same letter are different, $p < 5\%$.

hematite was checked by X-ray powder diffractometer (XRD) (see Supplementary figure 1). The organic substances were collected from the organic layer of the Ferralsols, and sieved to pass a sieve <2 mm to remove coarse roots, mixed thoroughly and then air dried (20°C) for 3 days before use.

Subsamples were obtained by adding 5% hematite or 5% organic substances into Ferralsols or Cambisols, respectively. Six subsamples were obtained as follows: Ferralsols + 5% hematite, Ferralsols + 5% humic substances, Cambisols + 5% hematite and Cambisols + 5% humic substances. Subsamples were air-dried, and separated into two parts. One was ground to pass a 2-mm sieve and stored at 4°C for 2 months before use, another was ground to pass a 1-mm sieve and used for chemical analyses.

Physical and chemical analyses. Soil pH was measured in 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 was extracted with HNO_3–HF–$HClO_4$, and free Fe oxides were extracted with $Na_2S_2O_4$–$Na_3C_6H_5O_7$–$NaHCO_3$. Fe content was measured by atomic absorption spectrophotometry with a graphite furnace (GFAAS) using a model Z-8200 spectrophotometer. Soil cation exchange capacity (CEC) and anion exchange capacity (AEC) were determined after extraction with 0.0215 mol (NH_4NO_3) L^{-1} solution using the method described by Gillman & Sumpter[33].

Experimental design and ^{15}N addition. The soil subsamples were adjusted to 60% water holding capacity (WHC) and pre-incubated for 7 days at 25°C. We employed a combination of ^{15}N tracing experiments and full process-based N cycle models to quantify process-specific and pool-specific N transformation rates, which is the standard method for the quantification of N dynamics in soils[19,34–37]. For all samples, ^{15}N tracing studies were carried out under controlled conditions. For the ^{15}N tracing experiments, we employed two NH_4NO_3 treatments (each with three replications). In the first, NH_4 ($^{15}NH_4NO_3$) was labeled with ^{15}N at 20 atom% excess, and in the second, NO_3 ($NH_4\,^{15}NO_3$) was labeled. For each soil sample, a series of 250-ml conical flasks was prepared, each containing 30 g of fresh soil. 2 ml of $^{15}NH_4NO_3$ or $NH_4\,^{15}NO_3$ solution was added to each conical flask at a rate of 7.14 μmol N g^{-1} soil (50 μg NH_4^+-N g^{-1} soil and 50 μg NO_3^--N g^{-1} soil). The soil was adjusted to 60% water-holding capacity (WHC) and incubated for 7 days at 25°C. All bottles were covered with polyethylene film punctured with needle holes to maintain aerobic conditions. The soils (three replications for each treatment) were extracted at 0.5, 24, 72, 120 and 168 hours after fertilizer application to determine the concentrations and isotopic composition of NH_4^+ and NO_3^-.

For isotopic analysis, NH_4^+ and NO_3^- were separated by distillation with Mg oxide and Devarda's alloy[38,39] (see Supplementary information). The isotopic composition of NH_4^+ and NO_3^- were measured using an automated C/N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK). Simultaneous gross N transformations in soil were quantified using a process-based ^{15}N tracing model[19](see Supplementary information).

DNA extraction & quantitative PCR assay. Right after pre-incubation, 4 replicates of each treatment were randomly selected to extract DNA and *amoA* genes were analyzed by quantitative PCR (qPCR). The DNA was extracted for three sub-samples from 0.50 g of soil with the FastDNA Spin Kit for soil (MP Biomedicals, United States), according to the protocol of the manufacturer. The quality and quantity of the DNA extracts were determined with a spectrophotometer (Nanodrop, PeqLab, Germany), and were pooled and stored at −20°C until use. Quantitative PCR of *amoA* genes was performed to estimate the abundance of the ammonia-oxidizing bacterial and archaeal communities, respectively (see Supplementary information).

Statistical analyses. Data (measured or calculated) 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.

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Author contributions

X.J. wrote the manuscript and carried out data analysis; X.X. and J.Z. prepared the

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