Effect of Fe (III) Addition on Ammonium Loss and Associated Microbial Gene Expression in Soils

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The combined effects of temperature, Fe (III) contents, NH_4^+-N contents, and soil-liquid ratio were evaluated relative to the loss of NH_4^+-N in soils using a response surface methodology (RSM). The microbial mechanisms were explored for nitrogen transformation by quantifying functional genes related to nitrification and denitrification. According to parameter optimization analysis for prediction equation, the maximum NH_4^+-N loss was 86.1% under the conditions of 17.0 °C, 0.772 g·kg^{-1} Fe (III), 21.9 mg·kg^{-1} NH_4^+-N, and soil: liquid ratio of 1:1. The prediction result was similar to experimental data in the current study, which the NH_4^+-N loss was 83.2% under the condition of 25 °C, 0.723 g·kg^{-1} Fe (III), 20 mg·kg^{-1} NH_4^+-N, and soil-liquid ratio of 1:1. While the N_2O flux reached its minimum value of 8.35 μg·m^{-2}·h^{-1} under the experimental conditions, correlating with gene copy numbers for ammonia-oxidizing bacteria ammonia monooxygenase subunit A gene (AOB-amoA), and nitrite reductase genes (nirK) were maximum values of 4.5×10^5 and 4.8×10^5 copies·g^{-1}, respectively. NH_4^+-N loss resulted from multiple interacting processes beyond ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) mediated oxidation. The research findings can provide insights for reducing nitrogen application to avoid NH_4^+ toxicity and increasing soil planting suitability.

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Keywords: Nitrogen fertilizers; Ammonium; Fe (III); Gene copy numbers

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INTRODUCTION

The use of chemical fertilizers is one of the key strategies to improve soil fertility and crop yield. Nitrate (NO_3^-)- and ammonium (NH_4^+)-based nitrogen fertilizers are commonly employed to pursue high yields in modern intensive agriculture (Li *et al.* 2017), NH_4^+-N is a preferred nitrogen source for most plant species in a certain concentration range, whereas at high concentrations, NH_4^+-N has been found to inhibit the growth of most plants due to NH_4^+ toxicity (Britto and Kronzucker 2002). Characteristics of NH_4^+ toxicity are chlorosis of leaves, root suppression, stunted shoot growth, and so on (Li *et al.* 2014), which can be influenced by a combination of physical conditions, chemical conditions, and microorganisms of the soils, and depend on plant species and varieties (Cruz *et al.* 2011).

In recent years there has been some progress in understanding the mechanisms of NH_4^+ toxicity. Generally, factors such as protein glycosylation defects, inefficient NH_4^+-N cycling, accumulation of reactive oxygen species (ROS), cytoplasm acidification, consumption of inorganic cations and organic acids, disruption of the photosystem, and

interference with hormone signaling, may contribute to NH_4^+ toxicity (Britto and Kronzucker 2002; Liu and Von Wirén 2017; Jia *et al.* 2020; Kong *et al.* 2022). In agricultural contexts, the input of a large amount, or even excessive nitrogen fertilizers for increasing yields, can lead to NH_4^+ toxicity. Therefore, improving nitrogen use efficiency and alleviating NH_4^+ loss is crucial for reducing nitrogen fertilizers input and increasing plant growth.

The nitrogen cycle in soils is mainly derived by the processes of nitrification and denitrification (Pajares and Bohannan 2016). Ammonia oxidation is a crucial step in the nitrification process (Bhambri *et al.* 2025), which correlates with soil microbial ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and different enzymes activity. Denitrification is a microbial process in which nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and finally dinitrogen gas (N_2). Steps in the process are catalyzed by different enzymes, including nitrous oxide reductase, and nitrite reductase, among others. Thus, the abundance of the nitrite reductase genes (nirK and nirS), nitrous oxide reductase gene (nosZ), ammonia-oxidizing archaeal ammonia monooxygenase subunit A gene (AOA-amoA), and ammonia-oxidizing bacteria ammonia monooxygenase subunit A gene (AOB-amoA) affect nitrogen cycling and nitrogen balance (Guo *et al.* 2017; Xu *et al.* 2022). Additionally, Fe (III) affects the diversity and abundance of microorganisms in the soil, specific concentrations of Fe (III) can even impact microbial metabolites and pathways, thereby affecting chemical reactions and material cycles in the soils (Chen *et al.* 2022). The addition of Fe^{3+} can effectively stimulate nitrogen cycling processes by Feammox (Fe (III) reduction coupled to anaerobic ammonium oxidation), where NH_4^+-N can serves as an electron donor while Fe^{3+} can act as a terminal electron acceptor under anaerobic conditions (Wang *et al.* 2025). The addition of Fe (III) in agricultural soils to alter the content of soil NH_4^+ and alleviate the toxicity of NH_4^+ to plants is worthy of further exploration.

Therefore, this study aimed to systematically investigate the effect of Fe (III) addition on NH_4^+-N loss under different conditions in agricultural soils, which will be helpful for the interpretation of microbial dynamics in iron and nitrogen coupling (Fe-N) cycle and provide reference for reducing the loss of NH_4^+ and improving soil productivity.

EXPERIMENTAL

Materials

Soil sampling

Soil samples (0 to 20 cm depth) were collected from agricultural fields (30°59′4″, 119°13′14″) in Langxi County, Anhui Province, China. The collected soil samples were immediately placed in sterile plastic bags without aeration and transferred to the laboratory as soon as possible. The specimens were then dried at room temperature (25 ± 1 °C) and were subsequently sieved through a 0.15-mm sieve to remove crop residues, gravels, *etc.* The physical and chemical properties of soil samples include moisture content (MC), pH, total phosphorus (TP), soil organic carbon (SOC) content, NH_4^+-N content, NO_3^--N content, and Fe (III) and Fe (II) concentrations were measured.

Methods

After mixing deionized water with soil samples at a liquid-to-soil ratio of 2.5:1 (v/w; L/kg), soil pH was measured using a pH meter (ZXHD/Bante920, China). The MC was determined by heating 50 g soil samples to a constant value at 60 °C, SOC was measured using a potassium bichromate external heating method, total phosphorus determination by the Molybdenum-Antimony-Ascorbic acid colorimetric method (Bao 2005). Soil samples were extracted using a 2 mol·L^{-1} KCl solution at a v/w ratio of 10:1, followed by a 30-minute extraction period at 25 °C. The extracted solution was used for NH_4^+-N and NO_3^--N determination. The NH_4^+-N was determined using the hypochlorite-phenol method, while NO_3^--N was measured using the dual-wavelength spectrophotometric method (Cawse 1967). The soil samples were extracted using a 0.5 mol·L^{-1} HCl solution at liquid-to-soil ratio of 20:1 for a 30-minute extraction period, and the extracted solution was used for soil Fe (II) and Fe (III) determination. Meanwhile, the Fe (II) was measured using the ortho-phenanthroline colorimetric method. Total iron content was determined after reducing Fe (III) to Fe (II) by adding hydroxylamine hydrochloride and employing the same method. The content of Fe (III) was calculated by subtracting the Fe (II) content from the total iron content (Viollier *et al.* 2000). Headspace gas samples were collected from the culture boxes using a 10-mL polyethylene syringe twice each week for N_2O flux analysis. The boxes were sealed with parafilm for 4 h before collection. N_2O flux was measured using a gas chromatograph (GC-2014C, Shimadzu, Tokyo, Japan).

Experimental design

The experiments were conducted in April to December 2023 in a light incubator (GZX-80, China), where 200 g of soil samples were placed in in a series of 400-mL culture boxes. Box-Behnken design (BBD) design experiments was employed to investigate the effects of soil-liquid ratio (w/w), temperature (°C), Fe (III) content (g·kg^{-1}), and NH_4^+-N (mg·kg^{-1}) content on NH_4^+-N loss rate in the soils, and the design of the factors and levels are shown in Table 1. The loss rate of NH_4^+-N (NO_3^--N) was calculated as followed,

$$NH_4^+\text{-N } (NO_3^-\text{- N}) \text{ loss rate } (\%) = \frac{C_1 - C_o}{C_0} \times 100\% \quad (1)$$

where C_0 is the initial concentration of NH_4^+-N or NO_3^--N (mg·kg^{-1}), and C_1 is the concentration of NH_4^+-N or NO_3^--N at predetermined intervals (mg·kg^{-1}).

Table 1. Experimental Design of Variables Factors and Levels

Factor	Encode	Level		
		-1	0	1
Temperature (°C)	A	15	25	35
Fe (III) (g·kg^{-1})	B	0.434	0.723	0.868
NH_4^+-N (mg·kg^{-1})	C	20	40	60
Soil-liquid ratio (v/w)	D	0.4:1	0.6:1	1:1
Note: The addition of Fe (III) is based on 1 to 3 times the background value of soil, NH_4^+-N content gradient design is also referring to the background value of soil. Soil-liquid ratio design is based on soil aerobic, anoxic, and anaerobic conditions.				

DNA extraction and bacterial PCR gene amplification

Using a headless plastic syringe (50 mL, tip cut off), three replicate soil samples were collected from the culture boxes. Soil DNA extraction using a PowerSoilTM DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturers' instructions, and quantitative polymerase chain reaction (PCR) targeting the denitrifying genes (nosZ, nirK) and nitrifying genes (AOA-amoA, AOB-amoA).

To amplify the target genes, AOA-amoA (635 bp) was amplified using the forward primer Arch-amoAF (STA ATG GTC TGG CTT AGA CG) and the reverse primer Arch-amoAR (GCG GCC ATC CAT CTG TAT GT) (Francis *et al.* 2005). The standard curve had a slope of -3.257, an R^2 of 0.9992, and a PCR efficiency of 102.8%. AOB-amoA (491bp) was amplified using forward primer amoA-1F (GGG GTT TCT ACT GGT GGT) and reverse primer amoA-2R (CCC CTC KGS AAA GCC TTC TTC) (Rotthauwe *et al.* 1997). The standard curve had a slope of -3.353, an R^2 of 0.9931, and a PCR efficiency of 98.76%. NirK (165bp) was amplified using forward primer nirK876F (ATY GGC GGG VAY GGC GA) and reverse primer nirK1040R (GCC TCG ATC AGR TTR TGG GTT) (Henry *et al.* 2004), the standard curve had a slope of -3.583, an R^2 of 0.9979, and a PCR efficiency of 90.2%. NosZ (267bp) was amplified using forward primer nosZ2FHenry (CGC RAC GGG CAA SAA GGT SMS SGT) and reverse primer nosZ2RHenry (CAK RTG CAK SGC RTGG CAG AA) (Henry *et al.* 2006). The standard curve had a slope of -4.012, an R^2 of 0.9971, and a PCR efficiency of 77.5%. DNA purity and concentration were measured using a Micro Drop spectrophotometer (SMA4000, Beijing Meilin Hengtong Technology Co., Ltd., Beijing, China).

Statistical analyses

All experiments were performed in triplicate, such that reported results represent the mean of the triplicates. Design-Expert (version 13, Stat-Ease Inc., Minneapolis, MO, USA) and Origin2022 (Inc., OriginLab, Northampton, MA, USA) software were used to assess the correlation between Fe (III) and soil physic-chemical properties, abundance of AOA-amoA, AOB-amoA, nosZ, and nirK. The significance level was set at $P < 0.05$.

RESULTS

Physico-chemical Characteristics of Agricultural Soils

The physico-chemical characteristics of the agricultural soil samples are shown in Table 2, including soil moisture, NH_4^+-N, NO_3^--N, TP, SOC, pH, Fe (III) content, and Fe (II) content.

Table 2. Physico-chemical Characteristics of Sample Soil

Soil moisture (%)	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	SOC ($g \cdot kg^{-1}$)	pH	Fe (III) ($g \cdot kg^{-1}$)	Fe (II) ($g \cdot kg^{-1}$)
0.02	14.92	16.25	0.83	6	5.5	0.289	0.041

Response Surface Design Results

The effects of Temperature (A), Fe (III) content (B), NH_4^+-N content (C), and soil-liquid ratio (D) on the loss rate of NH_4^+-N (Y) are shown in Table 3. The data were processed and regression analyzed using the software Design Expert 13.0, and the prediction equation for the loss rate of NH_4^+-N was obtained as follows:

$$Y = 257.34272 - 1.83296A - 58.36061B - 2.51492C - 1.60374D + 0.447421AB + 0.008438AC + 0.007169AD - 0.029211BC - 0.16818BD - 0.003135\ CD - 0.006934A^2 + 9.064717B^2 + 0.032288C^2 + 0.007769D^2$$

Table 3. Experimental Design and Result for NH_4^+-N Removal by RSM

Serial Number	Level of Factors				Y:NH_4^+-N Removal Rate (%)
	A: Temperature (°C)	B: Fe (III) ($g \cdot kg^{-1}$)	C: NH_4^+-N ($mg \cdot kg^{-1}$)	D: soil-liquid Ratio (w/w)	
12	35	0.723	40	1:1	72.54
11	15	0.723	40	1:1	68.6
6	25	0.723	60	0.4:1	81.1
13	25	0.434	20	0.6:1	81.28
18	35	0.723	20	0.6:1	67.29
19	15	0.723	60	0.6:1	75.02
22	25	0.868	40	0.4:1	73.78
21	25	0.434	40	0.4:1	76.24
16	25	0.868	60	0.6:1	82.32
2	35	0.434	40	0.6:1	50.78
28	25	0.723	40	0.6:1	64.78
5	25	0.723	20	0.4:1	79.02
26	25	0.723	40	0.6:1	65.56
7	25	0.723	20	1:1	83.19
10	35	0.723	40	0.4:1	70.79
1	15	0.434	40	0.6:1	70.68
3	15	0.868	40	0.6:1	74.85
23	25	0.434	40	1:1	59.78
8	25	0.723	60	1:1	78.32
4	35	0.868	40	0.6:1	63.29
14	25	0.868	20	0.6:1	84.58
27	25	0.723	40	0.6:1	62.28
15	25	0.434	60	0.6:1	81.97
9	15	0.723	40	0.4:1	71.38
25	25	0.723	40	0.6:1	55.53
29	25	0.723	40	0.6:1	65.28
17	15	0.723	20	0.6:1	75.02
20	35	0.723	60	0.6:1	74.04
24	25	0.868	40	1:1	69.78

Table 4. ANOVA of Model Equation for NH_4^+ -N Removal

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1712.84	14	122.35	5.26	0.0024
A	103.69	1	103.69	4.46	0.0547
B	177.28	1	177.28	7.62	0.0162
C	2.91	1	2.91	0.1253	0.7290
D	52.52	1	52.52	2.26	0.1568
AB	48.16	1	48.16	2.07	0.1738
AC	11.39	1	11.39	0.4897	0.4964
AD	19.86	1	19.86	0.8541	0.3722
BC	1.66	1	1.66	0.0715	0.7934
BD	130.95	1	130.95	5.63	0.0338
CD	33.48	1	33.48	1.44	0.2516
A^2	8.23	1	8.23	0.3539	0.5621
B^2	187.44	1	187.44	8.06	0.0140
C^2	910.52	1	910.52	93.15	< 0.0001
D^2	268.29	1	268.29	11.54	0.0048
Residual	302.36	14	23.26	-	-
Lack of Fit	231.61	10	25.73	1.46	0.3813

Pure Error	70.75	4	17.69	-	-
Cor Total	2015.20	28	-	-	-
R^2 = 0.8500		R^2_{Adj} = 0.6884		CV = 6.72%	
Note: "-" means no data available					

The results of the final model analysis of variance (ANOVA) are shown in Table 4. The model was highly significant (P = 0.0024), the effect of B, BD, B^2, C^2, and D^2 on Y are significant (P < 0.05), while other terms did not yield a significant effect on Y.

Analysis of interaction effects of impact factors

Figure 1(a) shows the combined effect of temperature and Fe (III) on NH_4^+-N loss. NH_4^+-N loss increased significantly with increasing Fe (III) content when the temperature was constant, but the relatively flat curvilinear shows that the NH_4^+-N loss was not significantly affected by temperature when the Fe (III) content was constant.

Figure 1(b) shows the combined effect of temperature and NH_4^+-N concentration on NH_4^+-N loss. NH_4^+-N loss initially decreased, followed by an increase with NH_4^+-N concentration when the temperature was constant. The flat curvilinear shows NH_4^+-N loss was not significantly affected by temperature when NH_4^+-N concentration was constant.

Figure 1(c) shows the combined effect of temperature and soil-liquid ratio on NH_4^+-N loss. As shown, the overall variation was small, indicating that the interaction of temperature and soil-liquid ratio did not have a significant effect on the NH_4^+-N loss.

Figure 1(d) shows the combined effect of Fe (III) and NH_4^+-N concentration on NH_4^+-N loss. It can be seen that NH_4^+-N loss increased with increasing Fe (III) content when the NH_4^+-N was constant, but NH_4^+-N loss showed a tendency of decreasing and then increasing with NH_4^+-N concentration when the Fe (III) content was constant, and the maximum NH_4^+-N loss rate was appeared when the Fe (III) content was 0.868 $g \cdot kg^{-1}$ and NH_4^+-N content was 20 $mg \cdot kg^{-1}$

. Figure 1(e) shows the combined effect of Fe (III) and soil-liquid ratio on NH_4^+-N loss. The overall magnitude of the curves varied steeply, which means NH_4^+-N loss was significantly affected by the interaction of Fe (III) and soil-liquid ratio, which was consistent with the results reflected in the statistical significance of individual interactions (Table 4). In all interaction combinations, the combined effect of Fe (III) and NH_4^+-N concentration on NH_4^+-N loss was significantly higher than other interaction combination ($P<0.05$).

Figure 1(f) shows the combined effect of NH_4^+-N concentrations and soil-liquid ratio on NH_4^+-N loss. It can be seen NH_4^+-N loss showed a tendency of decreasing and then increasing with NH_4^+-N concentration when soil-liquid ratio was constant. The minimum NH_4^+-N loss rate was achieved when soil-liquid ratio was 0.6:1 and NH_4^+-N content was 40 $mg \cdot kg^{-1}$.

Optimization analysis for NH_4^+-N loss

According to parameter optimization analysis for prediction equation, the optimal NH_4^+-N loss condition was obtained as: Temperature of 17.0 °C, 0.772 $g \cdot kg^{-1}$ Fe (III), 21.9 $mg \cdot kg^{-1}$ NH_4^+-N, and soil-liquid ratio of 1:1. It was assumed that NH_4^+-N loss was 86.1% under these conditions. The prediction result was similar to the current study, where the NH_4^+-N loss was 83.2% under the condition of 25 °C, 0.723 $g \cdot kg^{-1}$ Fe (III), 20 $mg \cdot kg^{-1}$ NH_4^+-N, and soil-liquid ratio of 1:1.

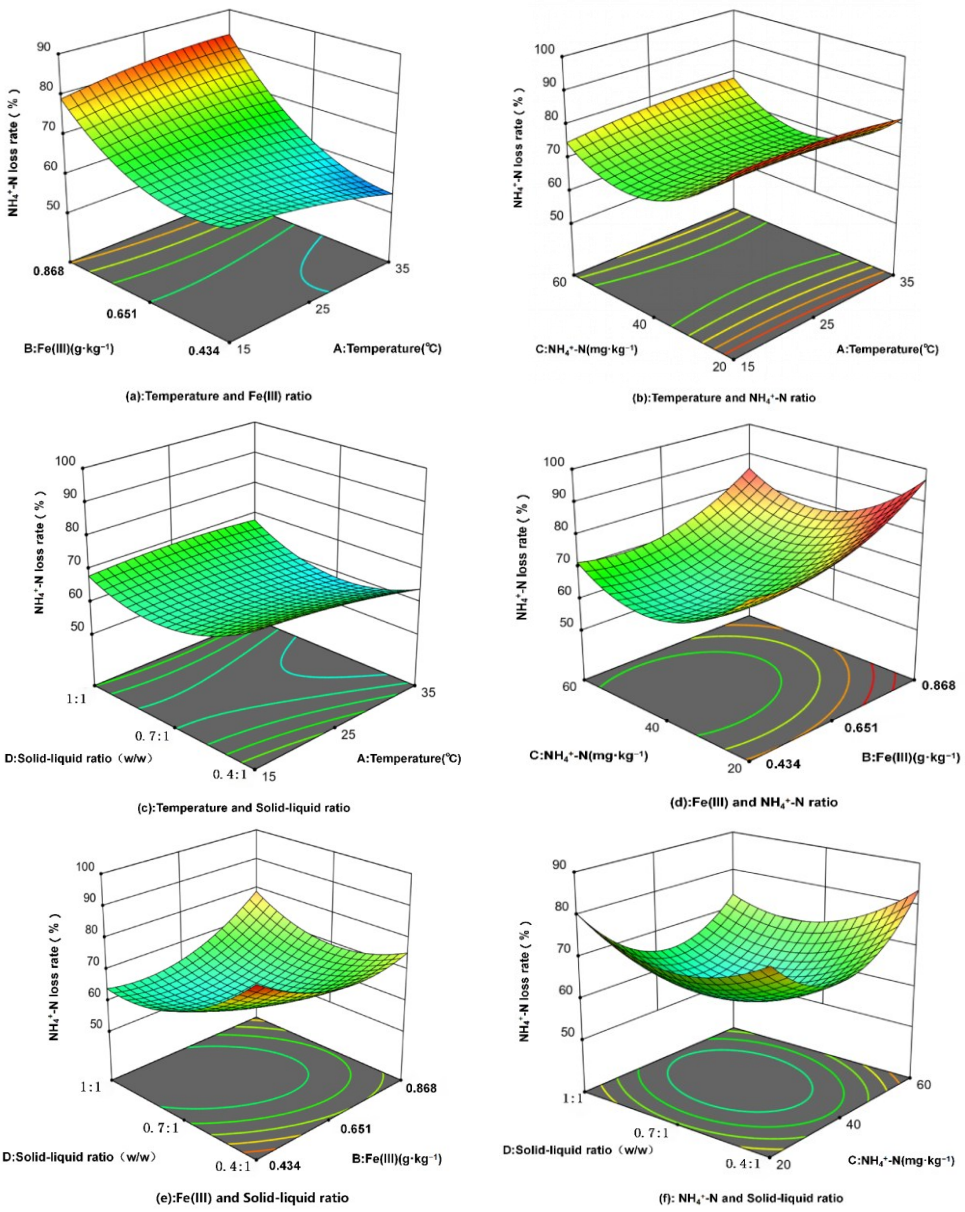

Fig. 1. Response surface plot and contour plot showing the effect of (a) A and B, (b) A and C, (c) A and D, (d) B and C, (e) B and D, and (f) C and D on the loss of NH_4^+-N

DISCUSSION

Iron (Fe) is a key factor in the regulation of soil nitrification, denitrification processes, and nitrogen turnover. The nitrogen and the Fe cycle are coupled in soil environments (Kampschreur *et al.* 2011). Changes of nitrogen and Fe (III) concentrations can lead to shifts in soil microbial communities (Chen *et al.* 2022), which will impact nitrification and denitrification processes, thereby affecting nitrogen distribution and transformation in soils (Davidson *et al.* 2003; Heckman *et al.* 2013; Park and Novak 2013; Cai *et al.* 2014). In this study, the maximum NH_4^+-N loss of 84.6% occurred when the addition of Fe (III) was 0.868 $g \cdot kg^{-1}$ in soils (Table 5). It was found that gene copy

numbers for AOB-amoA, nirK, and nosZ under that condition increased 44%, 87.6%, and 0.48% compared with control, respectively. However, the gene copy numbers for AOA-amoA dropped 46.5%, simultaneously. The decrease in AOA-amoA abundance alongside the increase in AOB-amoA might result from AOB could inhibit AOA through rapid growth under resource-limited condition (Guo *et al.* 2021). The gene copy numbers for nirK and nosZ, which have been categorized as denitrifying genes, have higher population abundance than nitrifying genes in the soils. Table 5 further indicated that N_2O flux from control and test 2 were significantly higher than those in the other tests but were not correlated to population abundance of denitrifying genes (nirK + nosZ). A possible explanation is that the loss of NH_4^+-N in this system is not only caused by AOA and AOB interactions, but also by other reaction processes, such as anaerobic ammonium oxidation, the oxidation of NH_4^+ coupled with Fe (III) reduction (Van der Star *et al.* 2007), *etc*. Feammox is a promising technology that can proceed without oxygen and minimize N_2O production. Previous studies have also shown that Fe (III)-mediated Feammox was positively correlated with NH_4^+-N loss (Zhu *et al.* 2021; Hu *et al.* 2022).

In addition, it has been widely reported that pH is also an important factor influencing the distribution and transformation of N in soils (Clement *et al.* 2005; Jiang *et al.* 2022). The current results are consistent with this, compared with control, the pH increased from 5.5 to 6.0 as the loss of NH_4^+-N increased from 25% to 84.6% after 14 d incubation experiments. A possible explanation is the loss of NH_4^+-N was related with the decrease of pH due to the ammonia oxidation process, but the increase of NO_3^- caused by nitrification might enhance the denitrification (Chen *et al.* 2016), thereby increasing the pH with higher loss rate of NH_4^+-N.

The authors also found that the loss of NH_4^+-N was increased from 79.0% to 83.2% with the soil-liquid ratio increased from 0.4:1 to 1:1 under the same condition of temperature, Fe (III) addition, and NH_4^+-N content, while the gene copy numbers for AOB-amoA and nirK were increased 68.5% and 45%, the gene copy numbers for AOA-amoA and nosZ were decreased 32.4% and 3.35% (Table 5), respectively.

Table 5. Changes in Abundance of Functional Genes and N Turnover in Soils

Test	Soil Cultivation Condition			N Turnover			Gene Copies			
	Fe (III) Content ($g \cdot kg^{-1}$)	NH_4^+-N Content ($mg \cdot kg^{-1}$)	Soil-liquid Ratio (w/w)	Loss of NH_4^+-N (%)	Loss of NO_3^--N (%)	N_2O Flux ($\mu g \cdot m^{-2} \cdot h^{-1}$)	$\times 10^3$ g^{-1} Soil	$\times 10^5$ g^{-1} Soil		
							AOA-amoA	AOB-amoA	NirK	NirZ
1	0.868	20	0.6	84.58	63.28	9.26	4.14	1.93	3.19	16.63
2	0.723	20	0.4	79.02	62.78	9.66	6.71	2.67	3.31	19.20
3	0.868	60	0.6	82.32	70.03	9.03	3.53	0.80	1.12	12.82
4	0.723	20	1	83.19	62.53	8.35	4.53	4.50	4.80	18.75
5	0.723	60	0.4	81.1	64.28	8.37	4.88	0.98	1.56	13.58
6	0.289	14.92	0.4	25	62.03	9.96	7.74	1.34	1.70	16.55

Note: Test 6 were control tests, where Fe (III) content, NH_4^+-N content and Soil-liquid ratio are the background values of soils

This means that NH_4^+-N loss was not significantly affected by the soil-liquid ratio. A possible explanation is the decrease soil-liquid ratio leads to an increase in aerobic conditions in the soil, thereby promoting nitrification reactions. However, it is difficult to explore the specific mechanisms in depth because the various processes can be mixed and interact with each other (Maag and Vinther 1999; Xu *et al.* 2003; Hu *et al.* 2010). Future research should focus on investigating the interrelationships between microbial interactions and Fe–N cycle, to promote its application in broader soil types.

CONCLUSIONS

1. In this study, the authors systematically analyzed the combined effects of temperature, Fe (III) contents, NH_4^+-N contents, and soil-liquid ratio on the magnitude of loss of NH_4^+-N in soils using response surface methodology (RSM). The results showed that Fe (III) addition affected NH_4^+-N loss by various processes that interact with each other, which could contribute significantly to nitrogen management and soil productivity enhancement.
2. The N_2O flux reached its minimum value, correlating with gene copy numbers for AOB-amoA and nirK were maximum values, respectively. Nitrogen turnover was not correlated with corresponding functional gene abundance.
3. Iron-nitrogen interaction in soils play a fundamental role in plant growth by influencing nitrogen turnover, water retention, and microbial activity. Further research is needed for NH_4^+-N loss mechanisms with Fe (III) addition.

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