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Effects of urea and $(NH_4)_2SO_4$ on nitrification and acidification of Ultisols from Southern China

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Abstract

The mechanisms for the effects of ammonium-based fertilizers on soil acidification in subtropical regions are not well understood. Two Ultisols collected from cropland and a tea garden in Anhui and Jiangxi Provinces in subtropical southern China, respectively, were used to study the effects of urea and $(NH_4)_2SO_4$ on the nitrification and acidification of soils with incubation experiments. Nitrification occurred at very low pH with no N fertilizer added and led to lowering of the soil pH by 0.53 and 0.30 units for the soils from Jiangxi and Anhui, respectively. Addition of urea accelerated nitrification and soil acidification in both Ultisols; while nitrification was inhibited by the addition of $(NH_4)_2SO_4$, and greater input of $(NH_4)_2SO_4$ led to greater inhibition of nitrification. Ammonia-oxidizing bacteria (AOB) played an important role in nitrification in cropland soil under acidic conditions. Addition of urea increased the soil pH at the early stages of incubation due to hydrolysis and stimulated the increase in the AOB population, and thus accelerated nitrification and soil acidification. At the end of incubation, the pH of Ultisol from Jiangxi had decreased by 1.25, 1.54 and 1.84 units compared to maximum values for the treatments with 150, 300 and 400 mg/kg of urea-N added, respectively; the corresponding figures were 0.95, 1.25 and 1.69 for the Ultisol from Anhui. However, addition of $(NH_4)_2SO_4$ inhibited the increase in the AOB population and thus inhibited nitrification and soil acidification. Soil pH for the treatments with 300 and 400 mg/kg of $(NH_4)_2SO_4$-N remained almost constant during the incubation. AOB played an important role in nitrification of the cropland soil under acidic conditions. Addition of urea stimulated the increase in the AOB population and thus accelerated nitrification and soil acidification; while addition of $(NH_4)_2SO_4$ inhibited the increase in the AOB population and thus inhibited nitrification.

Key words: ammonium-based fertilizer; nitrification; soil acidification; ammonia-oxidizing prokaryotes

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Introduction

Soil pH declines when acidification occurs, accompanied by leaching of base cations and nitrate (NO_3^-) and increasing of active aluminum (Likens et al., 1969; Gahoonia, 1983; Porebska and Mulders, 1994; van Breemen and van Dijk, 1998). Soil acidification can alter the biogeochemical cycling of elements and cause negative effects on biota. Acid deposition and application of excessive ammonium (NH_4^+)-based fertilizers are two serious factors that accelerate soil acidification (Bolan et al., 1991; Vogt et al., 2006; Hu et al., 2007). Under the intensive land use in China, the sharp increase in the application of nitrogen (N) fertilizers in cropping systems has greatly accelerated soil acidification (Zhang et al., 2008, 2009; Guo et al., 2010). From the 1980s to the 2000s, soil pH decreased by 0.13–0.80 units in the major Chinese crop-production areas (Guo et al., 2010). Both nitrification and uptake of NH_4^+ by plants can lead to soil acidification; the key process causing soil acidification by NH_4^+-based fertilizers is nitrification, which is catalyzed by ammonia-oxidizing prokaryotes (Jetten et al., 1997). He et al. (2007) reported that both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) played an important role in ammonia oxidation in subtropical acid soils. During nitrification, the oxidation of 1 mol NH_4^+ leads to the release of 2 mol H^+ into soils (De Vries and Breeuwsma, 1987). It is suggested that the nitrification of NH_4^+-based fertilizers contributes more to soil acidification than acid deposition and other factors (Miegroet and Cole, 1984; Barak et al., 1997; Guo et al., 2010). Soil acidification has become a major problem in Chinese agricultural systems.

Extensive studies have been conducted to investigate the relationship between nitrification and soil acidification (Likens et al., 1969; Van Breemen et al., 1982; De Boer et al., 1992; Zhao and Xing, 2009). According to general opinion (Weber and Gainey, 1962; Robertson, 1982), microbiological oxidation of NH_4^+ to NO_3^- is inhibited in strongly acidic conditions (pH < 5). Results have indicated that nitrification is inhibited in acid soils in subtropical forest and brush-land and thus no acceleration of soil acidi-

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fication occurred (Zhao et al., 2007; Zhao and Xing, 2009). The enhancement of nitrification and acidification of acid upland soils in subtropical areas has been observed, due to the input of NH_4^+ in short-term incubation experiments and long-term field experiments (He et al., 2007; Zhao and Xing, 2009).

Nitrification also varies with the type of N fertilizers added. The input of urea into soils can stimulate nitrification and accelerate soil acidification (Martikainen, 1984, 1985; De Boer et al., 1988; Zhao and Xing, 2009); however, elevated $(NH_4)_2SO_4$ in soils may stimulate or inhibit nitrification (Ishaque and Cornfield, 1976; Martikainen, 1984; Zhao et al., 2007; Zhao and Xing, 2009). The mechanisms of the effects of different NH_4^+-based fertilizers on nitrification and acidification of acidic soils in subtropical regions are not well understood. Therefore, urea and $(NH_4)_2SO_4$, which are widely used in agriculture systems in China, were evaluated in the present study for their effects on the nitrification and acidification of two acidic Ultisols from subtropical regions of China. The abundance of ammonia-oxidizing prokaryotes in the soils was determined and used to interpret the different effects of urea and $(NH_4)_2SO_4$ on soil acidification.

1 Materials and methods

1.1 Soil samples

The two Ultisols taken from the topsoil (0–10 cm) were used. One of them was collected from a tea garden located in Yingtan, Jiangxi Province, and another one was collected from a cropland site in Langxi, Anhui Province in southern China. Their parent materials were both quaternary red earths. The soil samples were taken to the laboratory, and green material and roots thicker than about 2 mm were removed. Then the samples were air-dried, ground to pass a 2-mm sieve, and stored at 4°C before use. Some selected physicochemical properties of the soils are listed in Table 1.

1.2 Incubation experiments

Air-dried soil samples were used in incubation experiments (Zhao et al., 2007). The 250 g samples were placed in plastic cups, and urea or ammonium sulfate ($(NH_4)_2SO_4$) was added at rates of 0 (i.e., controls), 150, 300 and 400 mg N/kg soil. Then the soils were mixed with these N fertilizers thoroughly, and deionized water added to adjust soil moisture content to 70% of soil water-holding capacity. All cups were covered with polyethylene film punctured with needle holes to maintain aerobic conditions, and incubated at 25°C. Any water loss from evaporation was replaced using deionized water every 2 days. Soils were sub-sampled at 1, 3, 6, 14, 22, 32, 55, 65 and 75 days for pH and N determination. There were three replicates for each treatment with controls unfertilized. After 75 days of incubation, the soil samples were removed from the cups, air-dried, and ground to pass a 0.25-mm sieve.

1.3 Soil analyses after incubation

Soil pH was measured with fresh samples in soil-water suspensions (1:2.5, *m/V*) using a pH meter with a combined glass electrode. Air-dried samples were used for soil nitrogen determination. Soil NH_4^+-N and (NO_3^- + NO_2^-)-N were extracted with 2.0 mol/L KCl (Pansu and Gautheyrou, 2006), and then measured by a continuous flow analytical system (Skalar San++, The Netherlands). Soil total N content was determined at 1200°C using a Leco CN-2000 analyzer (Leco Corporation, St. Joseph, Michigan, USA). The exchangeable acidity was extracted with 1.0 mol/L KCl, and then titrated with NaOH (Pansu and Gautheyrou, 2006). The soil pH buffer capacity of these samples was determined by the acid-base titration method (Aitken and Moody, 1994). Soil DNA was extracted from 0.5 g soil using MP^{TM} FastDNA SPIN Kit for Soil (France). All real-time experiments were conducted using a Bio-rad C1000^{TM} Thermal Cycler with CFX96^{TM} Real-Time System. The primer sets *amo*A-1F, *amo*A-2R and Arch-*amo*AF, Arch-*amo*AR were used to quantify the abundance of the *amoA* gene of bacteria and *amoA* gene of archaea, respectively. To generate a standard curve four PCR clones were used, and the clones were grown in LB medium. One clone was selected randomly and the plasmid DNA was extracted, purified and quantified. Real-time PCR was performed in triplicate. The 25 μL reaction mixture contained 12.5 μL SYBR®Premix Ex Taq^{TM}, 0.5 μmol/L of each primer, 11 μL PCR-H_2O and 1 μL DNA template, with thermal conditions as described in Table 2. The primer sets *amo*A-1F, *amo*A-2R and Arch-*amo*AF, Arch-*amo*AR (Table 2) were used to quantify the *amoA* gene of bacteria and *amoA* gene of archaea, respectively.

1.4 Statistical analyses

SPSS 15.0 was used for statistical analysis of data. One-way analysis of variance (ANOVA) was undertaken for each time interval of the incubations to determine significant differences between the treatments. Significant effects for various treatments were detected using *t*-test.

Table 1 Selected basic properties of two Ultisols

Location	pH	CEC ($cmol_c/kg$)	Exchangeable acidity ($cmol_c/kg$)	Organic carbon (g/kg)	Total N (g/kg)	pH buffering capacity (mmol H^+/(kg·pH))
Jiangxi	4.53	8.72	4.82	15.32	1.47	32.61
Anhui	4.42	9.36	4.92	9.98	1.31	31.50

CEC: cation exchange capacity.

Table 2 Primers and conditions used for real time PCR of *amoA* gene of bacteria and *amoA* gene of archaea

Primer name	Primer sequence (5′-3′)	Target gene	Thermal profile	Molecular analysis
Arch-*amoA*F	STAATGGTCTGGCTTAGACG	Archaea *amoA*	95°C, 3.0 min; 35× (95°C, 30 sec;	Real-Time PCR
Arch-*amoA*R	GCGGCCATCCATCTGTATGT		55°C, 30 sec; 72°C, 45 sec with plate read);	
			Melt curve 65.0 to 95.0°C ,	
			increment 0.5°C with plate read	
		Bacteria *amoA*	95°C, 3.0 min; 35× (95°C, 30 sec;	
amoA-1F	GGG GTT TCT ACT GGT GGT		55°C, 30 sec; 72°C, 30 sec with plate read);	
			Melt curve 65.0 to 95.0°C ,	Real-Time PCR
amoA-2R	CCC CTC KGS AAA GCC TTC TTC		increment 0.5°C with plate read	

2 Results and discussion

2.1 Soil pH

There were different trends for the changes in soil pH of the two Ultisols among control, urea and $(NH_4)_2SO_4$ systems (Fig. 1). The changes in soil pH with incubation time for the same treatment were similar for the two Ultisols. In controls, soil pH increased slightly at the beginning of incubation due to the mineralization of organic N (De Vries and Breeuwsma, 1987), and then decreased with incubation time from day 3 on. At the end of the incubation, soil pH had decreased by 0.53 and 0.30 units from their initial values for the Ultisols from Jiangxi and Anhui, respectively; the changes of pH were greater for Jiangxi than for Anhui. Nitrification of residual NH_4^+-N in the soils was responsible for the decrease in soil pH.

The treatments with $(NH_4)_2SO_4$ added had lower pH values than controls at the early stage of incubation, due to the input of H^+ from the $(NH_4)_2SO_4$ solution, and greater addition of $(NH_4)_2SO_4$ led to a greater decrease in soil pH. The soil pH for the treatment with 150 mg/kg of $(NH_4)_2SO_4$-N added decreased with incubation time, similar to soil pH for controls. However, the change in pH for the former was less than that for the latter. Soil pH for the treatments with 300 and 400 mg/kg of $(NH_4)_2SO_4$-N

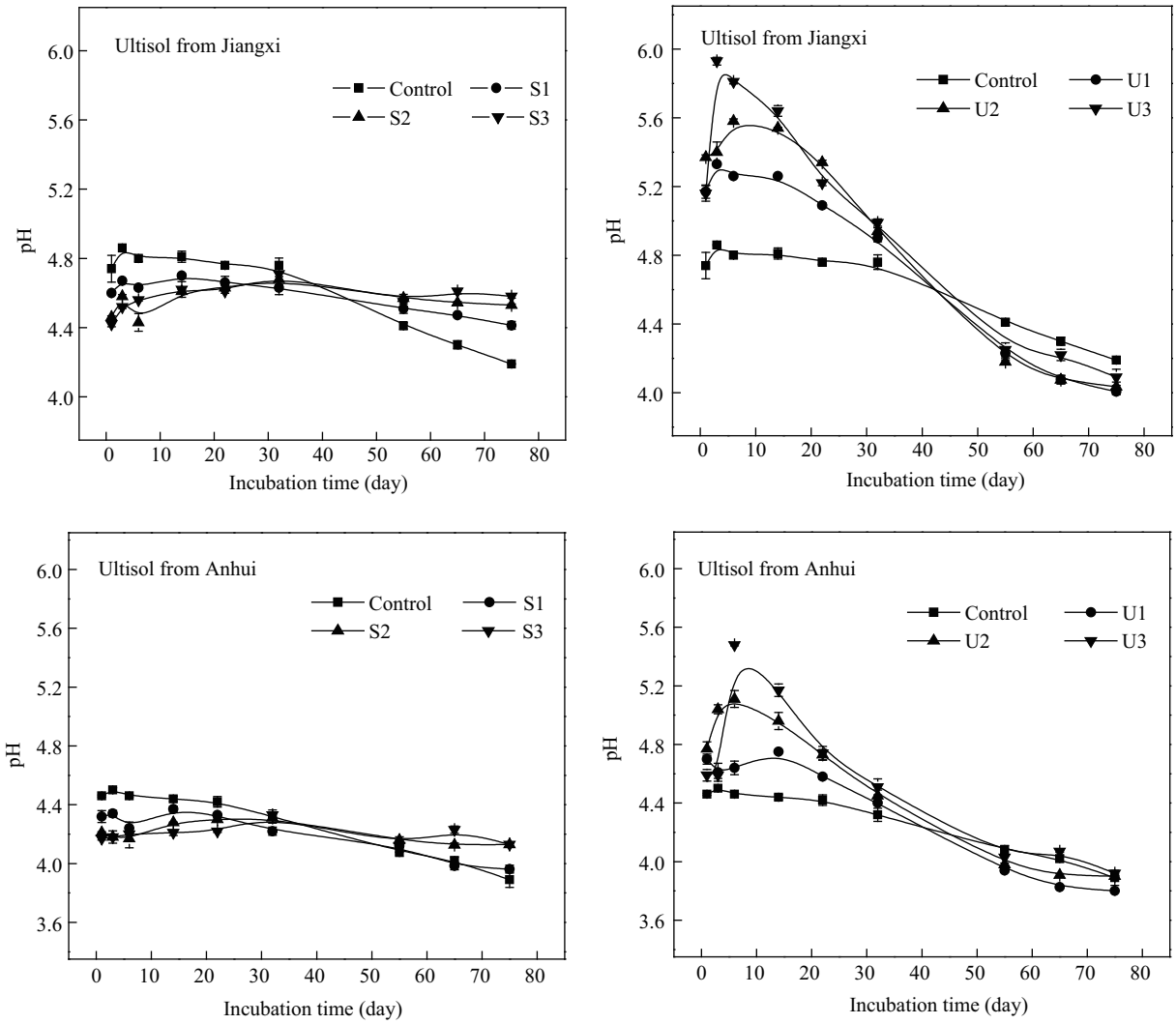

Fig. 1 Dynamics of soil pH during the incubation of two Ultisols with $(NH_4)_2SO_4$ and urea added. S1, S2 and S3: 150, 300 and 400 mg/kg $(NH_4)_2SO_4$-N, respectively; U1, U2 and U3: 150, 300 and 400 mg/kg urea-N, respectively.

added remained almost constant during the incubation.

The greatest changes in soil pH were for treatments with urea added. Soil pH was higher for the treatments with urea added than for controls, and the more urea added the greater was the increase in soil pH for both Ultisols at the early stage of incubation. The increase in soil pH for urea-added systems was ascribed to the consumption of H^+ during hydrolysis of urea in the soils (De Vries and Breeuwsma, 1987). However, soil pH decreased sharply during 6–14 days and reached very low values at the end of the incubation and even lower than that of controls. At the end of incubation, the pH of Ultisol from Jiangxi had decreased by 1.25, 1.54 and 1.84 units compared to the maximum values for the treatments with 150, 300 and 400 mg/kg of urea-N added, respectively; the corresponding data were 0.95, 1.25 and 1.69 for the Ultisol from Anhui. For the same amount of urea added, pH in the Ultisol from Jiangxi changed more than that from Anhui.

2.2 NH_4^+-N and (NO_2^- + NO_3^-)-N in soils

The contents of soil (NO_2^-+ NO_3^-)-N were below the detection limit at the beginning of incubation for both soils, and only data from day 14 onward is reported in Fig. 2. In control systems of both Ultisols, the content of NH_4^+-N increased slightly with incubation time at the beginning of incubation and reached a maximum value at day 6, and then decreased with increased incubation time. In contrast to soil NH_4^+-N, soil (NO_2^- + NO_3^-)-N continuously increased with incubation time (Fig. 2). The dynamics of soil NH_4^+-N and (NO_2^-+NO_3^-)-N were consistent with the change of pH with incubation time. These results suggested that there was nitrification of residual NH_4^+ in soils, which lowered the soil pH. The slight increase of soil NH_4^+-N and pH at the beginning of incubation was ascribed to the mineralization of soil organic N.

When urea was added, soil NH_4^+-N and (NO_2^- + NO_3^-)-N changed with incubation time similarly to the control. Hydrolysis of urea in soil led to increased NH_4^+-N at the beginning of incubation, and then nitrification led to decreased NH_4^+-N and increased (NO_2^- + NO_3^-)-N. At the later stage of incubation, the (NO_2^- + NO_3^-)-N content was much higher in treatments with urea added than in control and more urea added led to greater increases in soil (NO_2^- + NO_3^-)-N. These results indicated that the addition of urea accelerated nitrification and resulted in greater changes in soil pH during the incubation compared with control.

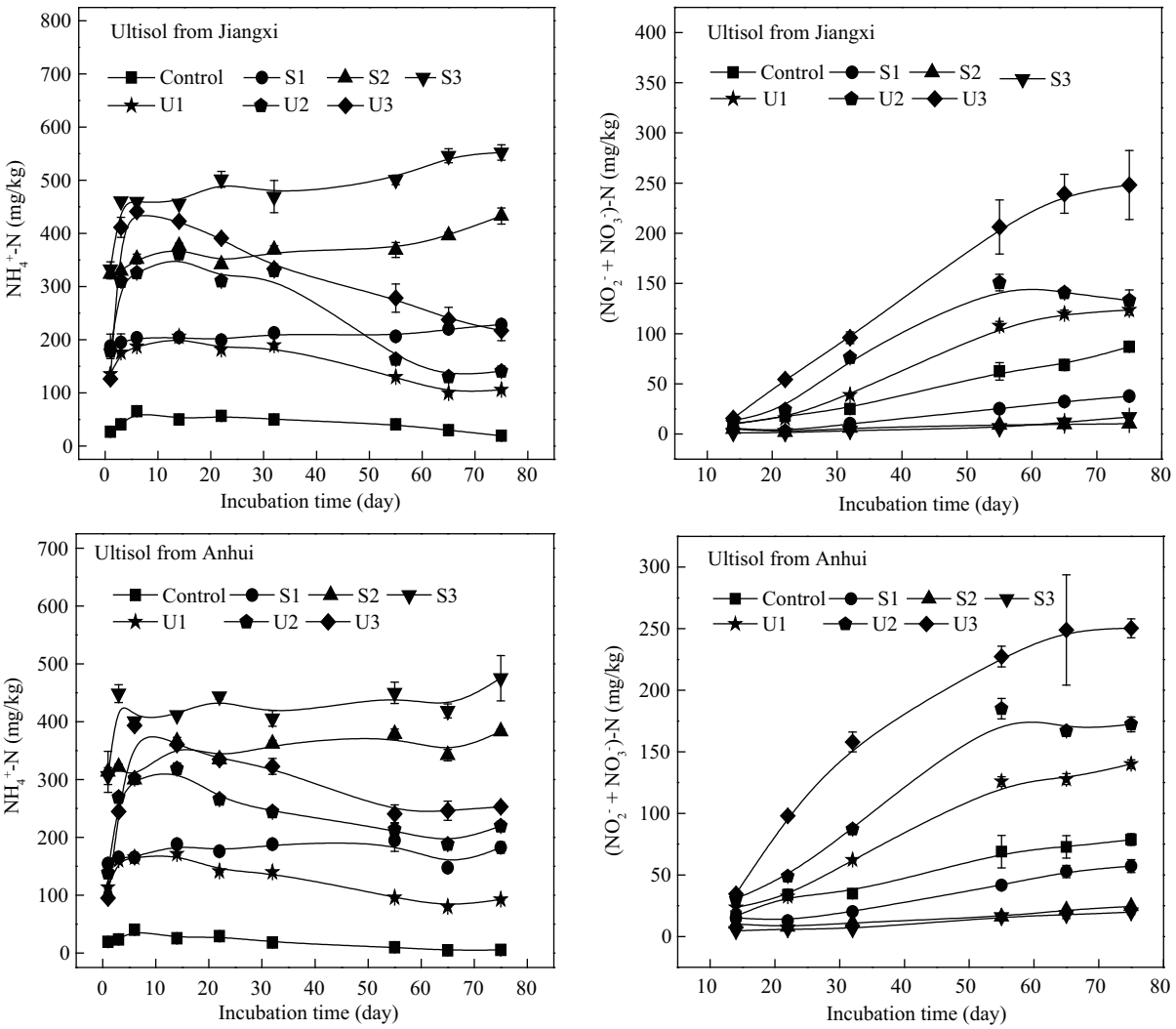

Fig. 2 Dynamics of soil NH_4^+-N and (NO_3^- + NO_2^-)-N during the incubation of two Ultisols with $(NH_4)_2SO_4$ and urea-N added. S1, S2 and S3: 150, 300 and 400 mg/kg $(NH_4)_2SO_4$-N, respectively; U1, U2 and U3: 150, 300 and 400 mg/kg urea-N, respectively.

In contrast to control and treatments with urea added, the contents of soil NH_4^+-N increased slightly with incubation time for treatments with $(NH_4)_2SO_4$ added and were higher than the NH_4^+ contents for inputs of $(NH_4)_2SO_4$. The contents of soil (NO_2^- + NO_3^-)-N for treatments with $(NH_4)_2SO_4$ added were much lower than that for controls. These results suggested that $(NH_4)_2SO_4$ addition did not accelerate nitrification in the two soils, but inhibited it to some extent. The inhibition of nitrification by $(NH_4)_2SO_4$ was increased with increased amount of $(NH_4)_2SO_4$ added. Nitrification in treatments with 300 and 400 mg/kg $(NH_4)_2SO_4$ added was almost completely inhibited.

2.3 Net nitrification

Net nitrification was defined as the accumulation of NO_3^- and NO_2^- during the incubation. The net nitrification was the greatest in urea-treated soils followed by the controls, and was the lowest in the $(NH_4)_2SO_4$-treated soils (P < 0.05) (Fig. 3). The hydrolysis of added urea increased the soil pH at the early stage of incubation (Fig. 1), which may have stimulated the activity of ammonia oxidizers. The effective supply of NH_4^+ provided enough substrate for nitrification. Therefore, there was a great nitrification potential in urea-treated soils. It is interesting that some net nitrification occurred in controls, even though the pH of both soils was very low, and this accelerated the further acidification of both soils. At the end of the incubation, almost all NH_4^+-N was transformed to (NO_2^- + NO_3^-)-N in controls (Fig. 3). Some of the NH_4^+ came from mineralization of organic N during the incubation, thus the amount of (NO_2^- + NO_3^-)-N was much higher than the initial content of soil NH_4^+-N in controls (Fig. 2). Therefore, nitrification can occur in cropland soils of subtropical regions under strongly acidic conditions and can accelerate the acidification of these soils.

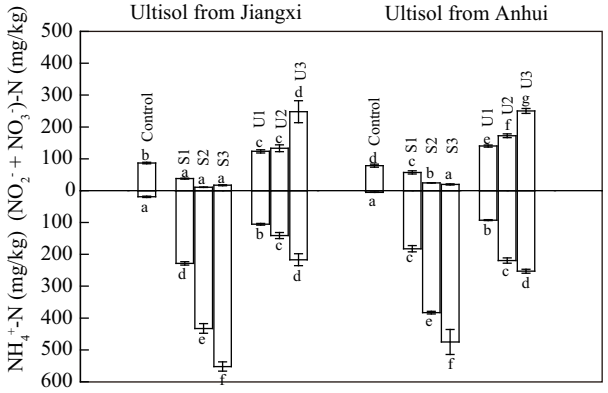

Fig. 3 Soil NH_4^+-N and (NO_3^- + NO_2^-)-N at the end of the incubation of two Ultisols with $(NH_4)_2SO_4$ and urea added. S1, S2 and S3: 150, 300 and 400 mg/kg $(NH_4)_2SO_4$-N, respectively; U1, U2 and U3: 150, 300 and 400 mg/kg urea-N, respectively. Means followed by the same letters above or below data columns are not significantly different (P < 0.05).

Net nitrification was much weaker in treatments with $(NH_4)_2SO_4$ added compared with control and treatments with urea added (P < 0.05); and the increased input of $(NH_4)_2SO_4$ led to more inhibition of nitrification in the two Ultisols. The inhibition of nitrification in acid soils by the addition of NH_4^+ salts has been reported previously (Ishaque and Cornfield, 1976). Zhao et al. (2007) also found that adding NH_4^+-N did not accelerate nitrification and acidification of acidic forest soils in subtropical regions. The low initial soil pH was not the main reason for the inhibition of nitrification by addition of $(NH_4)_2SO_4$, because there was obvious nitrification in controls with very low soil pH (Fig. 2). The mechanism for the inhibition of nitrification by addition of NH_4^+-N in acid soils remains to be determined in the future.

2.4 Abundances of *amoA* gene copies of AOB and AOA and their relation with nitrification

The ranges in number of *amoA* gene copies in AOB was 3.41×10^3 to 3.69×10^5 and in AOA was 1.09×10^3 to 3.86×10^6 for the Ultisol from Jiangxi; the corresponding data were 1.98×10^3 to 1.52×10^6 and 7.42×10^3 to 2.16×10^5 for the Ultisol from Anhui (Fig. 4). From the data (Fig. 4), the ratios of *amoA* gene copies of AOA to AOB were calculated and had ranges of 0.56–1.73 and 0.70–1.58 for the Ultisols from Jiangxi and Anhui, respectively. The AOA were more abundant than AOB in control and

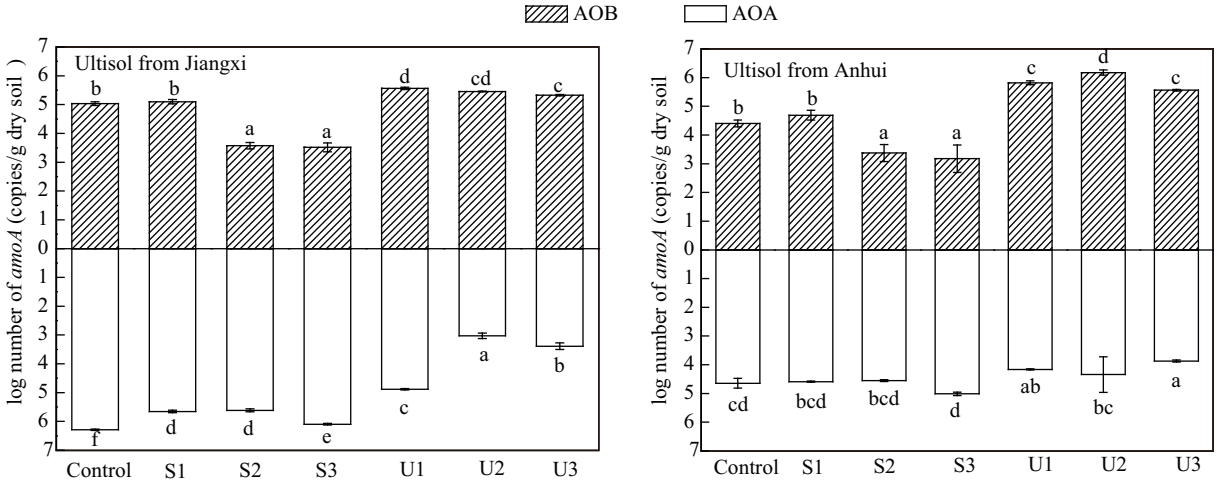

Fig. 4 Abundances of archaeal and bacterial *amoA* genes in two Ultisols at the end of the incubation with $(NH_4)_2SO_4$ and urea added. S1, S2 and S3: 150, 300 and 400 mg/kg $(NH_4)_2SO_4$-N, respectively; U1, U2 and U3: 150, 300 and 400 mg/kg urea-N, respectively. Means followed by the same letter above or below data columns are not significantly different (P < 0.05).

$(NH_4)_2SO_4$-treated soils; while AOB were more abundant than AOA in urea-treated soils.

Copy numbers of the AOB *amoA* gene in both Jiangxi and Anhui soils were significantly higher in treatments with urea added than in control ($P < 0.05$); while they were significantly lower in treatments with 300 and 400 mg/kg of $(NH_4)_2SO_4$-N added than in control and the treatments with 150 mg/kg of $(NH_4)_2SO_4$-N added ($P< 0.05$). There was no significant difference in copy numbers of the AOB *amoA* gene between the treatments with 150 mg/kg of $(NH_4)_2SO_4$-N added and control. DNA-stable isotope probing was used to identify the function of AOA and AOB (Jia and Conrad, 2009), which showed that AOB rather than AOA functionally dominate ammonia oxidation in agricultural soil, despite the fact that archaeal versus bacterial *amoA* genes are numerically more dominant. The greater population size of AOB in the treatments with urea added was responsible for the higher nitrification potential in the treatments. Higher pH and sufficient NH_4^+-N at the early stage of the incubation with added urea provided appropriate conditions to increase the AOB population and thus accelerated nitrification and acidification. Smaller AOB populations in the treatments with 300 and 400 mg/kg of $(NH_4)_2SO_4$-N added may be the reason for the inhibition of nitrification in these treatments.

In contrast to AOB, the population size of AOA in the treatments with urea added was lower than in control and $(NH_4)_2SO_4$-treated soils ($P< 0.05$). These results suggested that AOB played a greater role in soil nitrification than AOA in treatments with urea added.

The *amoA* gene of the ammonia monooxygenase (the key enzyme for nitrification) was found in AOB and AOA (Hatzenpichler et al., 2008; De La Torre et al., 2008). Quantitative analysis of the *amoA* genes has shown that AOA predominated among ammonia-oxidizing prokaryotes in soils (Leininger et al., 2006; Nicol and Schleper, 2006; Chen et al., 2008; Shen et al., 2008). He et al. (2007) reported that both AOB and AOA played an important role in ammonia oxidation in tropical acid soils. However, Jia and Conrad (2009) reported that AOB rather than AOA dominate microbial ammonia oxidation in agricultural soil, consistent with observations in treatments with urea added in the present study. This was confirmed by the positive correlation between AOB population size and $(NO_2^- + NO_3^-)$-N accumulation (Table 3). Therefore, AOB dominated the microbial ammonia oxidation in soils in the present study. Intense nitrification resulted in the decline of soil pH and thus soil acidification. In contrast, there was a negative correlation between AOA and accumulation of $(NO_2^- + NO_3^-)$-N. This result suggested that high $(NO_2^- + NO_3^-)$-N concentrations may feed back to AOA and inhibit the increase of the AOA population size in the soils.

Table 3 Coefficients of correlation between abundances of archaeal and bacterial *amoA* genes and soil pH and $(NO_2^-+NO_3^-)$-N accumulation

Type of microorganisms	AOA		AOB	
	Jiangxi	Anhui	Jiangxi	Anhui
$(NO_2^- + NO_3^-)$-N accumulation	–0.832*	–0.899**	0.707	0.844*
Soil pH	0.657	0.695	–0.827*	–0.920**

* Significant correlation was between AOA and pH, and $(NO_2^-+NO_3^-)$-N, AOB and pH, and $(NO_2^-+NO_3^-)$-N, respectively ($P<0.05$); ** significant correlation was between AOA and pH, and $(NO_2^-+NO_3^-)$-N, AOB and pH, and $(NO_2^-+NO_3^-)$-N, respectively ($P<0.01$), df = 6.

2.5 General discussion

Soil N cycling is accompanied by the absorption and release of protons. There is 1 mol H^+ consumed when 1 mol NH_4^+ is released through ammonification; while 2 mol H^+ are released when 1 mol NH_4^+ is oxidized to NO_3^-, and 1 mol H^+ is released or absorbed when 1 mol NH_4^+ or NO_3^- is assimilated by organisms (De Vries and Breeuwsma, 1987). The amount of inorganic N increased in all treatments except for the Ultisol from Jiangxi with 300 mg/kg of urea-N added, which indicated that appreciable ammonification occurred during the incubation due to the low ratio of C to N (Table 1). Greater levels of net nitrification in treatments with urea added and controls were responsible for most of the declines in soil pH.

Correlation analysis was conducted between soil pH and contents of $(NO_2^- + NO_3^-)$-N during the incubation. There was a significant negative correlation between the soil pH and contents of $(NO_2^- + NO_3^-)$-N (Table 4), confirming that nitrification in soils was the main reason for accelerated soil acidification.

If the soil acidification rate is defined as the change in pH multiplied by the pHBC, then the values of acidification rate for both Ultisols can be calculated from the data shown in Fig. 1. The ranges in acidification rate were –30~580.3 and 16.8–379.2 μmol H^+/(kg·day) for the Ultisols from Jiangxi and Anhui, respectively. If the net nitrification rate is defined as the change in $(NO_2^- + NO_3^-)$-N content, then it can be calculated from the data in Fig. 2. The range in net nitrification rate was 9.9–236.2 and 18.8–238.4 μmol/(kg·day) for Ultisols from Jiangxi and Anhui, respectively. The correlation analysis between acidification rate (y) and nitrification rate (x) was conducted and regression equations obtained as follows:

$$\text{Ultisol from Jiangxi: } y = 32.011 + 2.607x, R^2 = 0.681, P < 0.05 \quad (1)$$

$$\text{Ultisol from Jiangxi: } y = 60.858 + 1.518x, R^2 = 0.657, P < 0.05 \quad (2)$$

Soil acidification rate was linearly related to nitrification rate, and the slope of the line represents production of protons when 1 mol $(NO_2^- + NO_3^-)$-N accumulated; the

Table 4 Coefficients of correlation between soil pH and contents of $(NO_2^- + NO_3^-)$-N for control and two fertilizers added

Treatment	Coefficient	
	Ultisol from Jiangxi	Ultisol from Anhui
Control	–0.993** df=5	–0.971** df=5
$(NH_4)_2SO_4$	–0.769** df=17	–0.820** df=17
Urea	–0.826** df=17	–0.768** df=17

** Correlation between soil pH and contents of $(NO_2^- + NO_3^-)$-N was significant ($P < 0.01$).

theoretical value should be two (De Vries and Breeuwsma, 1987). In the present study it was 2.607 and 1.518 for Ultisols from Jiangxi and Anhui, respectively. The slope values for the two Ultisols were not constant, but varied around the theoretical value, suggesting that the effect of nitrification on acidification varied with soils tested. For the Ultisol from the tea garden, there are the other proton resources in addition to nitrification, which led to the higher slope of the line.

Nitrification, which is catalyzed by ammonia-oxidizing prokaryotes, is sensitive to environmental conditions (Dancer et al., 1973; Nishio and Fujimoto, 1990; Stephen et al., 1998; Oved et al., 2001; Kowalchuk and Stephen, 2001). According to traditional opinion, nitrification is inhibited in acidic conditions (Weber and Gainey, 1962; Robertson, 1982); however, nitrification has been observed in many natural acidic environments (Troelstra et al., 1990; De Boer et al., 1992; Pennington and Ellis, 1993). In the present study, nitrification occurred at very low pH in both Ultisols with no N fertilizer added. Addition of urea accelerated the nitrification in both Ultisols, but addition of $(NH_4)_2SO_4$ inhibited nitrification to some extent.

The increased concentration of substrate provided by adding N fertilizer should stimulate nitrification. However, in some cases, input of NH_4^+-N does not accelerate nitrification under acidic conditions (De Boer et al., 1988; Killham, 1990; Stams et al., 1990; Strong et al., 1997). We found that nitrification was stimulated by addition of urea and that more addition of urea led to greater nitrification in Ultisols from both cropland and tea garden, which is similar to previous reports (Martikainen, 1984, 1985; De Boer et al., 1988; Zhao and Xing, 2009). In contrast, Zhao et al. (2007) found that addition of urea did not stimulate nitrification in forest soils in subtropical regions. For forest soils, N fertilizers are not usually applied, which may be a disadvantage for the development of ammonia-oxidizing prokaryotes. In the cropland soil, the hydrolysis of urea increased soil pH at the early stage of incubation and stimulated the increase in AOB population. Therefore, addition of urea promoted nitrification and soil acidification. Addition of $(NH_4)_2SO_4$ inhibited the increase in AOB population and thus the nitrification in soils.

3 Conclusions

Nitrification catalyzed by ammonia-oxidizing prokaryotes is affected not only by the concentration of substrate, but also by the soil properties, types of N fertilizers, and activity of ammonia-oxidizing prokaryotes. In this study, there was nitrification at very low pH in both Ultisols with no N fertilizer added. Addition of urea accelerated the nitrification and thus soil acidification in both the Ultisols from subtropical regions. However, nitrification was inhibited by addition of $(NH_4)_2SO_4$, and higher input of $(NH_4)_2SO_4$ led to greater inhibition of nitrification. AOB played an important role in nitrification of the cropland soil under acidic conditions. Addition of urea stimulated the increase in AOB population and thus accelerated nitrification and soil acidification; while addition of $(NH_4)_2SO_4$ inhibited the increase in AOB population and thus inhibited nitrification and soil acidification.

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