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Yongsong Zhang · Heinrich W. Scherer

Mechanisms of fixation and release of ammonium in paddy soils after flooding

II. Effect of transformation of nitrogen forms on ammonium fixation

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Abstract An incubation experiment under aseptic and septic conditions using ^{15}N-labelled NH_4^+-N and NO_3^--N, was carried out to study the effect of N transformations after flooding on NH_4^+ fixation in a paddy soil from China. After flooding ammonification was favoured, providing NH_4^+ for fixation by clay minerals. NH_4^+ fixation was more pronounced under low redox potential (E_h) conditions. Close correlations existed between exchangeable NH_4^+, E_h, and non-exchangeable NH_4^+. Therefore, two major conditions for NH_4^+ fixation induced by flooding in paddy soil were found, namely flooding promoted net production of NH_4^+ due to the deamination of organic N and, in addition, decreased the E_h of the soil. A lower E_h was caused by reduction and dissolution of Fe oxide coating on the clay minerals' surfaces, eliminating the obstacles for NH_4^+ diffusing into or out of the interlayers of clay minerals. A higher concentration of exchangeable NH_4^+ from deamination of organic N would drive NH_4^+ diffusing from the soil solution into the interlayers of clay minerals. ^{15}N-labelled NO_3^- incorporated into the flooded soil was not reduced to NH_3. The addition of NO_3^- retarded the decrease in the soil E_h and, therefore, NH_4^+ fixation.

Key words Ammonium fixation · Rice paddy soil · Nitrogen transformation · Aseptic incubation · Wetland rice

Introduction

NH_4^+ fixation is one of the important transformation processes in which ammoniacal N applied to the soil may be involved. With regard to NH_4^+ fixation in flooded soils, reports are contradictory. According to Keerthisinghe et al. (1984), Scherer and Zhang (1999) and Zhang and Scherer (1999) it is enhanced by flooding. However, the mechanisms governing NH_4^+ fixation and release in paddy soils are not clearly understood. Recently, Schneiders and Scherer (1996, 1998) demonstrated that NH_4^+ fixation is strongly influenced by the redox potential (E_h) of soil, which at low values may result in reduction of the octahedral Fe^{3+} in clay minerals (Stucki et al. 1984) and, therefore, in an increase in the negative charge, accompanied by a higher Coulombic attraction between the interlayer cations and the silicate layers. Further, coating of the surface of clay minerals by Fe oxides has an impact on the diffusion of NH_4^+ into or out of the interlayers of clay minerals (Scherer and Zhang 1999). Because of the reversible oxidation and reduction of Fe oxides in paddy soils this mechanism may be of special importance in sorption and desorption of NH_4^+ and its availability to rice.

The degree of NH_4^+ fixation mainly depends on the concentration of NH_4^+ in the soil solution, which already increases in the first days after flooding (Schneiders and Scherer 1998). Furthermore, according to recent results of Glinski et al. (1992), NO_3^- is expected to be completely reduced to NH_4^+ by 1 week of flooding, which may also influence the NH_4^+ concentration of the soil solution. These N-transformation processes are expected to play an important role in NH_4^+ fixation in flooded soils. The aim of our investigation was to prove the significance of N transformations on NH_4^+ fixation using ^{15}N.

Materials and methods

Soil

The soil was a typical paddy soil (Entisol; degleyed dark paddy soil) widely distributed in Zhejiang Province of China, with the major physico-chemical characteristics: $pH_{(CaCl_2)}$ 6.5; clay 417 g kg^{-1} soil; silt 555 g kg^{-1} soil; smectite 98.8 g kg^{-1} soil; vermiculite

Y.S. Zhang
Department of Soil Science and Agrochemistry, Huajichi
Campus, Zhejiang University, Hangzhou, 310029, China

H.W. Scherer (✉)
Institute of Agrochemistry, University of Bonn,
Karlrobert-Kreiten-Strasse 13, 53115 Bonn, Germany
e-mail: h.scherer@uni-bonn.de

39.5 g kg^{-1} soil; illite 225.2 g kg^{-1} soil; kaolinite 31.6 g kg^{-1} soil; total C 25.6 g kg^{-1} soil; total N 2.9 g kg^{-1} soil; non-exchangeable NH_4^+-N 297.5 mg kg^{-1} soil; exchangeable NH_4^+-N 34.0 mg kg^{-1} soil; NO_3^--N 7.4 mg kg^{-1} soil.

Treatments and incubation

1. Soil (control; CK).
2. Soil plus $^{15}NO_3^-$-N.
3. Soil plus $^{15}NH_4^+$-N.
4. Soil (sterilized at 121 °C for 20 min).
5. Soil plus $^{15}NO_3^-$-N (sterilized at 121 °C for 20 min).
6. Soil plus $^{15}NH_4^+$-N (sterilized at 121 °C for 20 min).

To 20-ml test tubes, filled with 2.5 g soil, 4 ml of an N-containing solution as $Ca(^{15}NO_3)_2$ or $(^{15}NH_4)_2SO_4$ (10 atom % excess ^{15}N), respectively, were added to the $^{15}NO_3^-$-N treatments and $^{15}NH_4^+$-N treatments, equal to 500 mg N kg^{-1} soil. These solutions were replaced by 4 ml distilled water in treatments 1 and 4. In order to prevent changes of the N form or concentration of N during sterilization, N solutions and the soil were sterilized separately. N solutions were added to both sterilized and unsterilized soils on an inoculating desk. Afterwards the soils were incubated at 30 °C. Soil samples were taken during the incubation period after 0, 2, 4, 7, 10, 14 and 21 days. The samples of day 0 were taken 1 h after addition of the solutions.

Analyses

Exchangeable NH_4^+-N and followed by NO_3^--N were extracted with 2 N KCl (soil:solution ratio 1:10) steam distillation. Non-exchangeable NH_4^+-N was determined according to the method of Silva and Bremner (1966), modified by Zhang and Scherer (1998).

The E_h was measured daily during the experiment with platinum combination electrodes (PT 4800-M3-S7/510; Mettler-Toledo, Steinbach), which were installed at the bottom of the tube; the measured values were corrected to E_h. In the sterilized treatments, the electrodes were sterilized with ethanol (70%) and fixed on the inoculating desk.

For the measurement of ^{15}N, after distillation and titration the solutions were acidified with HCl and concentrated in an oven before ^{15}N determination by mass spectrometry.

Results and discussion

The relation between E_h, exchangeable NH_4^+-N and NH_4^+ fixation in the flooded soil

After submerging an aerobic soil, it undergoes reduction and its E_h drops. In our experiments flooding had no impact on the E_h in the aseptic treatments (treatments 4–6), while it rapidly decreased in the septic treatments (Fig. 1), where two stages of the change in E_h were observed. The first one was characterized by a sharp drop in the E_h during the first week of flooding, and the second one showed a slow drop from the second week of flooding until the end of the incubation period. As compared to the CK, the addition of NH_4^+ had no impact on the decline of E_h, while application of NO_3^- significantly retarded the decline of E_h.

Confirming the results of Schneiders and Scherer (1998), flooding resulted in a significant increase in the concentration of exchangeable NH_4^+-N in treatments 1 and 2 (Fig. 2). At the end of the incubation period it was nearly 4 times as high as the initial value in treatments 1and 2. Even though organic matter is mineralized at a slower rate in flooded soils than in non-flooded soils, the net amount of NH_4^+ is higher in the former because less N is immobilized by microorganisms (Ponnamperuma 1972) and there is no nitrification of NH_4^+. Especially in soils rich in easily decomposable organic N compounds mineralization is high (Lin and Cheng 1992). Under septic conditions the formation of NH_4^+ ions was only slightly increased (treatments 4 and 5), while in the treatments with a high NH_4^+ application rate (treatments 3 and 6) no net change of the NH_4^+ level could be observed.

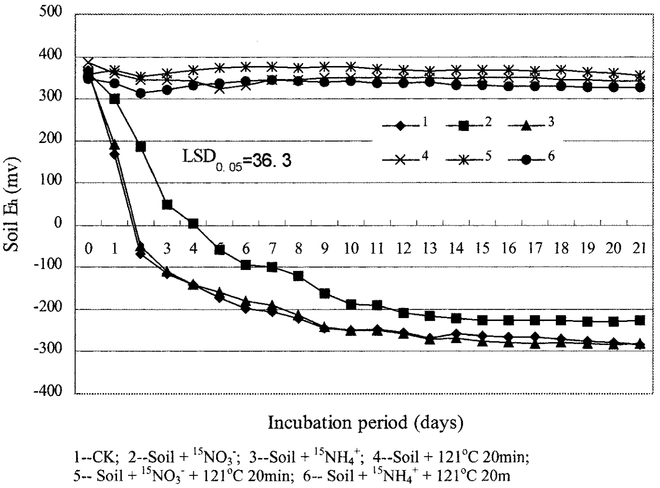


Fig. 1 Dynamics of soil redox potential (*E_h*) in different treatments during flooded incubation. *1* Control, *2* soil plus $^{15}NO_3^-$-N, *3* soil plus $^{15}NH_4^+$-N, *4* soil (sterilized at 121 °C for 20 min), *5* soil plus $^{15}NO_3^-$-N (sterilized at 121 °C for 20 min), *6* soil plus $^{15}NH_4^+$-N (sterilized at 121 °C for 20 min), *LSD* least significant difference

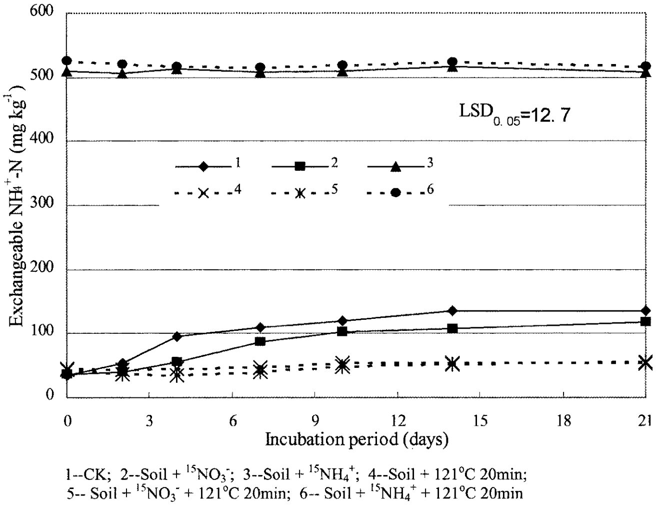
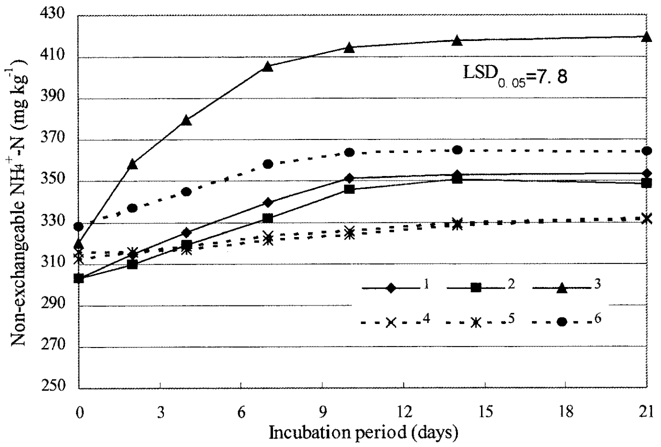


Fig. 2 Dynamics of exchangeable NH_4^+-N in different treatments during flooded incubation. For abbreviations, see Fig. 1

The concentration of non-exchangeable NH_4^+-N distinctly increased during the incubation period in all



1--CK; 2--Soil + $^{15}NO_3^-$; 3--Soil + $^{15}NH_4^+$; 4--Soil + 121°C 20min; 5-- Soil + $^{15}NO_3^-$ + 121°C 20min; 6-- Soil + $^{15}NH_4^+$ + 121°C 20min

Fig. 3 Effect of flooding on non-exchangeable NH_4^+ fixation in the soil of the different treatments. For abbreviations, see Fig. 1

treatments (Fig. 3). The fixation of NH_4^+ was highest in the first week after submerging the soil and was most pronounced in the treatment with NH_4^+ application under septic conditions. It was lowest under aseptic conditions in the treatments with NO_3^- application and without N application.

The data from the septic treatments were used to calculate the correlations between E_h, exchangeable NH_4^+-N and non-exchangeable NH_4^+-N. Confirming the results of Schneiders and Scherer (1998), we found with the same soil a very close linear correlation between E_h and non-exchangeable NH_4^+-N ($R^2 = 0.8454$) (Fig. 4a). However, a better correlation ($R^2 = 0.9399$) was obtained by using a multinomial function (Fig. 4b). Our results further prove that the decrease in the E_h after flooding is an important factor controlling the fixation of NH_4^+ ions, regardless of whether they are produced by the decomposition of organic N compounds or applied as mineral fertilizer.

A very high logarithmic correlation between E_h and the concentration of exchangeable NH_4^+ ($R^2 = 0.9681$) in the flooded soil was found (Fig. 4c). However, based on the knowledge of reactions in anaerobic zones of paddy soils, the influence of flooding on this high correlation between both parameters was expected.

Effect of N mineralization and the concentration of exchangeable NH_4^+-N on NH_4^+ fixation in the flooded soil

NH_4^+ fixation (Fig. 4d) was closely correlated to the concentration of exchangeable NH_4^+ ($R^2 = 0.938$). Therefore, it may be assumed that the degree of NH_4^+ fixation mainly depends on the concentration of exchangeable NH_4^+. However, we found (Fig. 2) that the concentration of exchangeable NH_4^+-N in treatment 1 was 2.5 times as high as that in treatment 4 at the end of the incubation period. Because treatment 1 was not sterilized, this increase resulted from the mineralization of organic N compounds. This increase in exchangeable NH_4^+, resulting from the deamination of organic amino compounds and from the lack of nitrification of NH_4^+, is an important characteristic in flooded soils. Nevertheless, the concentrations of exchangeable NH_4–N in treatments 3 and 6 stayed almost at the same level during the whole incubation period (Fig. 2), but the concentration of non-exchangeable NH_4^+-N in the septic treatment was much higher than that in the aseptic treatment (Fig. 3). In the experiment where we used ^{15}N (Fig. 5a), at the end of the incubation period 18.2% of non-exchangeable NH_4^+-N was derived from the $^{15}NH_4^+$ added in the septic treatment (treatment 3) but only 8.5% in the aseptic treatment (treatment 5). From these results it may be deduced that higher NH_4^+ fixation, induced by flooding in paddy soil, depends on both a higher concentration of exchangeable NH_4^+ as well as a low E_h. According to Zhang and Scherer (1999), the reduction and dissolution of Fe oxides coat-

Fig. 4 Correlations between soil E_h and non-exchangeable NH_4^+-N (**a, b**), E_h and exchangeable NH_4^+-N (**c**) and exchangeable and non-exchangeable NH_4^+-N (**d**)

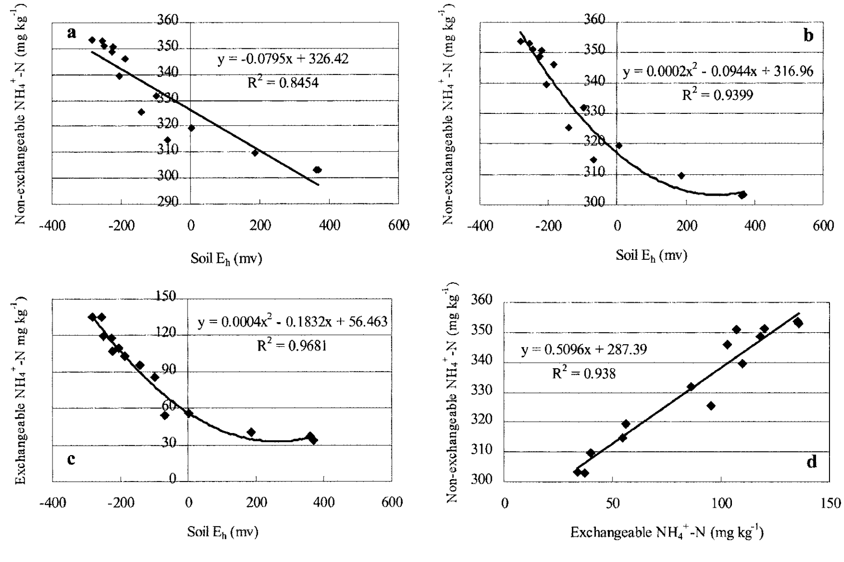
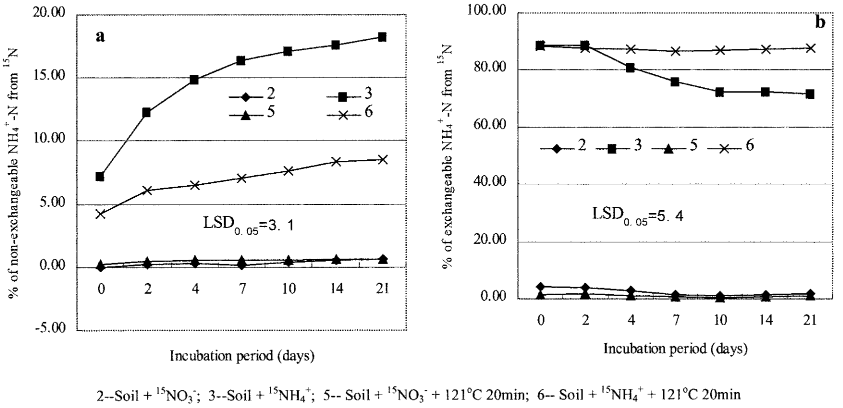


Fig. 5 Dynamics of ^{15}N in non-exchangeable (**a**) and exchangeable (**b**) NH_4^+-N in various treatments during flooded incubation. For abbreviations, see Fig. 1



ing the surfaces of clay minerals induced by a low E_h after flooding favour the diffusion of NH_4^+ ions from the soil solution into the interlayers of the clay minerals. The reduction of Fe^{III} of Fe oxides on the clay mineral surface caused by a low E_h followed by its dissolution, eliminates the obstacles for NH_4^+ ions diffusing into or out of the interlayers of clay minerals. A high concentration of NH_4^+ in the soil solution derived from ammonified N drives the diffusion of NH_4^+ ions from the soil solution into the interlayers of clay minerals.

In all aseptic treatments the E_h and the concentration of exchangeable NH_4^+ remained stable during the whole incubation period (Fig. 2), while the concentration of non-exchangeable NH_4^+-N increased slightly but significantly during the incubation (Fig. 3). This phenomenon may be explained by the facilitated diffusion of NH_4^+ ions into the interlayers of clay minerals under flooding conditions.

Our results proved the assumption that non-exchangeable NH_4^+ increased by flooding is due to the deamination of organic N. Therefore, part of the N produced via mineralization will be fixed by clay minerals under flooding conditions. However, the method of flooding and incubation in a closed system has been widely used to determine the rate of N mineralization in soils, in which only water-soluble and exchangeable NH_4–N were determined (Feng and Ying 1992). Therefore, we suggest that it is necessary to give consideration to the quantity of NH_4^+ fixed by clay minerals when using the above-mentioned method to measure the rate of N mineralization in a soil. Otherwise, the rate of ammonification is underestimated.

In treatment 3 the concentration of exchangeable NH_4^+-N almost remained at the same level during the whole incubation period (Fig. 2), while the increase in the concentration of non-exchangeable NH_4^+-N amounted to 116 mg kg^{-1} (Fig. 3). Therefore, the question of the origin of this N arises. From the changes of the ^{15}N ratio in the non-exchangeable and exchangeable NH_4^+ fraction in treatment 3 (Fig. 5a) it could be calculated that 76 mg kg^{-1} of this increase was derived from the addition of $^{15}NH_4^+$-N. This amount is almost equal to the decrease of 80 mg kg^{-1} in the labelled, exchangeable $^{15}NH_4^+$-N. While in the aseptic treatment (treatment 6) the abundance of ^{15}N of the exchangeable NH_4^+-N remained stable (Fig. 5b), it decreased in the septic treatment (treatment 3) by about 17%, which meant that the $^{15}NH_4^+$-N was diluted by NH_4^+-N from other sources, for example from the ammonification of organic N compounds.

Effect of NO_3^- reduction on NH_4^+ fixation in the flooded soil

In treatment 2 amended with NO_3^--N, NO_3^--N was almost completely reduced by 2 weeks of flooding (Fig. 6), which is in agreement with the results obtained by Glinski et al. (1992).

In flooded soils anaerobiosis starts with the disappearance of O_2 and the microbiological reduction of NO_3^- to N_2 and N_2O (Mengel and Kirkby 1987), while Buresh and Patrick (1978) assume that part of the NO_3^- in flooded soils reaches the NH_4^+ form. According to

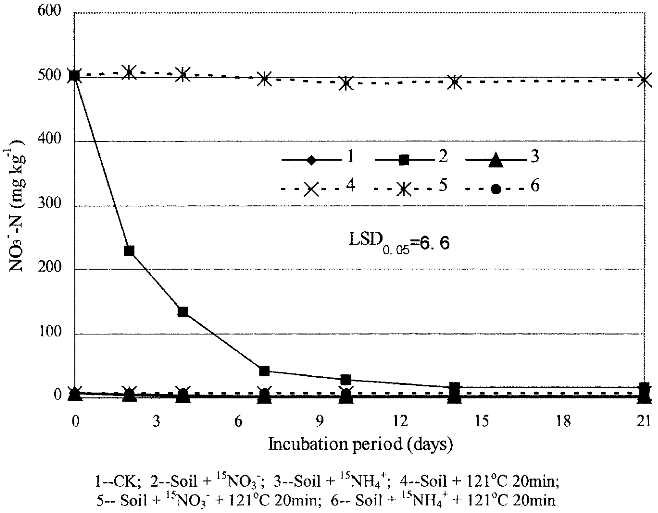


Fig. 6 Dynamics of NO_3^--N in the different treatments during flooded incubation. For abbreviations, see Fig. 1

Delwiche (1978), the reduction of NO_3^- to NH_4^+ would be more thermodynamically efficient than the formation of N_2 under the conditions of abundant organic substrate and limited availability of electron acceptors.

In this context enzymatic reactions and mere chemical reactions should be distinguished between the former, characterized by high reaction rates, while chemical reactions may proceed slowly. Since N_2 and N_2O are volatile they quickly disappear and therefore may not be available for further chemical reactions (Savant and De Datta 1982; Reddy and Patrick 1986; De Datta and Buresh 1989). Denitrification comprises several steps catalysed by microbial enzymes (Mengel 1991). The importance of microorganisms for this reaction sequence is evident from data of Fig. 6, showing that in the sterile treatment no NO_3^- was reduced. Under non-sterile conditions the electrons originating from microbial respiration may be scavenged by NO_3^- and used for denitrification and, therefore, the reduction of Fe^{III} and higher valency Mn compounds is retarded. This is the reason why in the presence of NO_3^- the decrease in the E_h proceeded at a lower rate, as shown in Fig. 1.

However, the question of whether NO_3^- may partially be reduced to NH_4^+, and as such plays a role in further NH_4^+ fixation in flooded soils, is still open to question. In our experiment with ^{15}N-labelled NO_3^-, almost all the NO_3^- added to the soil (treatment 2) seemed to be completely lost by denitrification as the percentage of ^{15}N in the exchangeable NH_4^+ fraction was the same as that in the aseptic treatment (treatment 5). Furthermore, the concentration of non-exchangeable NH_4^+-N in treatment 2, where NO_3^- was added, was unexpectedly lower than that in treatment 1 (Fig. 3). This result may have been caused by the retarding effect of NO_3^- on the decrease in the soil E_h (Fig. 1). Therefore, it can be concluded that NO_3^- application may retard the fixation of NH_4^+ ions in paddy soil.

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