The influence of long-term animal manure and crop residue application on abiotic and biotic N immobilization in an acidified agricultural soil

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ABSTRACT

Long-term application of organic fertilizers in acidified agricultural soils could alter soil nitrogen (N) immobilization by providing carbon (C) source and alleviating soil acidification. However, an understanding of the relative importance of abiotic and biotic N immobilization in acidified agricultural soils following long-term organic fertilizer application is largely lacking. Generally, the application of crop straw, which has a higher C/N ratio than animal manure, results in higher biotic N immobilization in soils. In contrast, the application of animal manure can result in greater stimulation of soil microbial activity due to a higher capacity to alleviate acidification. Resolving this contradiction is critical for predicting microbial N retention capacity and the effects of different types of organic fertilizer in acidified agricultural soils. A laboratory ^{15}N tracer experiment was carried out to examine the effects of long-term animal manure and crop straw application on abiotic and biotic N immobilization in an acidified agricultural soil in China. Animal manure application had a higher stimulation effect on abiotic and biotic N immobilization than crop straw. Microbial NH_4^+-N immobilization, as opposed to abiotic NH_4^+-N immobilization, largely contributed to the retention of NH_4^+-N. In contrast, abiotic NO_3^--N immobilization had a more significant role in NO_3^--N retention than microbial NO_3^--N immobilization under elevated C conditions. Animal manure-induced increases in microbial NH_4^+-N and NO_3^--N immobilization could be attributed to both increased C availability and enhanced soil microbial activity due to an increase in soil pH. The results suggest that long-term inputs of organic material to acidified agricultural soils could enhance abiotic and biotic N immobilization capacity.

1. Introduction

Overuse of chemical nitrogen (N) fertilizer has caused severe soil acidification in intensive Chinese agricultural systems (Guo et al., 2010). Soil pH can markedly decrease from 5.7 to 4.2 in naturally acidic soils following long-term application of chemical N fertilizer (Cai et al., 2015). Soil acidification is commonly associated with phosphorus deficiency, aluminum toxicity, and reduced biodiversity and productivity (Blake et al., 1994; Zhou et al., 2014). Liming has been widely employed to neutralize soil acidity. However, it is a strenuous activity and

is costly for farmers in China (Guo et al., 2010). Organic fertilizer application has been shown to counteract soil acidification (Cai et al., 2015; Wang et al., 2017a) while enhancing soil fertility (Zaman et al., 2004; Mallory and Griffin, 2007). Since carbon (C) and N turnover are tightly coupled, the use of organic fertilizers as C sources could alter soil N transformations (Edmeades, 2003). However, it is largely unknown how organic fertilizers influence soil N retention capacity in acidified agricultural soils.

Soil N retention mechanisms include abiotic and biotic N immobilization. Biotic N immobilization is highly dependent on the

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quality of organic fertilizer used (Gentile et al., 2008). A key organic fertilizer quality parameter is the C/N ratio. Organic fertilizers with low C/N ratios exhibit higher N mineralization rates compared to those with higher C/N ratios, with the latter mostly causing N immobilization during decomposition (Mary et al., 1996). The C/N ratio of crop straw is often higher than that of animal manure. Therefore, lower biotic N immobilization could be expected in soil treated with animal manure compared to soil treated with crop straw (Cheng et al., 2017). In addition, biotic N immobilization could be inhibited by soil acidification due to a relative decrease in bacterial abundance and diversity (Rousk et al., 2010; Zhalnina et al., 2015). Compared with crop straw, animal manure, which often has higher alkalinity, has been demonstrated to be more efficient in mitigating soil acidification (Meng et al., 2012). Therefore, the application of animal manure could result in higher increases in microbial activity compared to crop straw (Thiele-Bruhn et al., 2012). Consequently, the inconsistent results suggest that the effects of organic fertilization on biotic N immobilization and their underlying control mechanisms are unclear in acidified agricultural soils.

Abiotic N immobilization has been mainly investigated in forest soils probably due to higher C availability in forest soils compared to agricultural soils (Johnson et al., 2000; Davidson et al., 2003; Zhang et al., 2010). Abiotic N immobilization accounts for between 6% and 90% of total N immobilization in forest soils (Johnson et al., 2000). Abiotic NH_4^+-N immobilization has largely been associated with 2:1 clay minerals (Davidson et al., 1991) and phenolic compounds (Nömmik, 1970). In addition, abiotic NO_3^--N immobilization is limited to C-rich forest soils (Dail et al., 2001). Therefore, abiotic N immobilization could increase with increasing soil organic matter (SOM) content due to the input of organic fertilizer in agricultural soils. Since both abiotic and biotic N immobilization are regulated by C availability, it is largely unknown which process is dominant in acidified agricultural soils under elevated C conditions following long-term organic fertilizer application.

The purpose of the present study was thus to investigate the relative importance of biotic and abiotic N immobilization in an acidified agricultural soil, and how these patterns were altered by long-term organic fertilizer application. We also addressed an inconsistency where there is higher biotic N immobilization in soil treated with crop straw due to a higher C/N ratio compared to animal manure, while higher stimulation of microbial activity in soil treated with animal manure due to a higher capacity to alleviate acidification.

2. Material and methods

2.1. Site description and long-term fertilization experiment design

The long-term field experiment was established in 1990 and conducted on red soil at Qiyang Experimental Station (26°45′12″ N, 111°52′32″ E) at the Chinese Academy of Agricultural Sciences, Qiyang, Hunan Province, China. The site is representative of the typical local agriculture that applies a wheat-maize rotation system. The location has a subtropical monsoon climate with an annual rainfall of 1300 mm and annual average temperature of 18 °C (40-year average). The soil is classified as Ferralic Cambisol with a silty clay texture. In the region, soil acidification is a natural process due to intensive leaching. The intense crop production and increased input of chemical N fertilizers for high yields are expected to accelerate the process (Cai et al., 2015).

In the present study, seven fertilizer treatments were selected: CK, unfertilized control; NPK, mineral NPK fertilizer; NPKS, mineral NPK fertilizer plus straw; NPKSL, mineral NPK fertilizer plus straw and quicklime; NPKL, mineral NPK fertilizer plus quicklime; NPKM, mineral NPK fertilizer (30% of applied N) plus pig manure (70% of applied N) applied each crop season; M, manure only. For all treatments receiving fertilizer, the total amount of N (manure + urea) applied was the same each year. The manure and urea were applied to the soil together. The

pig manure was obtained from local farms annually, and the average water content was 70%. Half of the crop residues were incorporated into the soil each year. Urea was applied at 300 kg N ha^{-1} year^{-1} for all N treatments, and superphosphate and potassium chloride were applied at 53 kg P and 100 kg K ha^{-1} year^{-1}, respectively, for all P or K treatments. Annually, 30% of the fertilizers were applied in the wheat season and 70% in the maize season. Due to high soil acidification in plots subjected to annual inputs of NPK fertilizers since 1990, in 2010, half of the plots were amended with 2250 kg ha^{-1} quicklime based on the same fertilization protocol to alleviate soil acidification followed by the addition of 1500 kg ha^{-1} quicklime in 2014 (Wang et al., 2017b). Quicklime powder was broadcasted when the field was fallow and mixed with the soil through subsequent manual ploughing. Prior to the application of the fertilization treatments, the field had been under a wheat-corn rotation for 3 years without fertilization to achieve low and uniform nutrient levels. Fresh soil samples from the plough layer (0–20 cm) were collected in October 2015 after maize had been harvested from each plot and pooled together to form a composite sample. The soil samples were sieved (2 mm) and stored at 4 °C for one week for the incubation studies. The properties of the soil under the various fertilization treatments are shown in Table 1.

2.2. Laboratory ^{15}N tracer experiment

Each fresh soil sample (20 g of fresh soil on an oven-dried basis) was placed in a 250 mL flask and sealed. The flasks were then pre-incubated in the dark at 25 °C in the laboratory for 24 h. After pre-incubation, 2 mL of either the ^{15}N-enriched $^{15}NH_4NO_3$ or the $NH_4^{15}NO_3$ solution (10 atom% ^{15}N excess) were applied to each soil sample by pipetting the solutions uniformly over the soil surface, which was equivalent to adding 50 mg of NH_4^+-N and 50 mg of NO_3^--N kg^{-1} to the soil. Subsequently, the final moisture content of each labeled sample was adjusted to 60% WHC by adding deionized water. The flasks were then sealed with rubber stoppers and incubated at 25 °C in the dark for 6 d. During the incubation period, the flasks were opened for 30 min each day to refresh the atmosphere in each flask. The moisture content of the incubated soil samples was maintained by adding water every 3 days. Soil samples were extracted destructively at 0.5 h, 2, 4, and 6 d after the ^{15}N labeling by using a 100 mL solution of 2 M KCl to determine the concentrations and isotopic compositions of NH_4^+ and NO_3^-. After the KCl extraction, the residual soil was washed with 150 mL deionized water three times, oven-dried at 60 °C to a constant weight, and ground to pass through a 0.15 mm sieve for the ^{15}N analysis of insoluble organic N.

Table 1
Soil properties of the varied fertilized treatments after a 25-year experiment in an upland red soil in Qiyang (QY), China.

Treatment	pH	Total C	Total N
		g kg^{-1} soil	
CK	5.49 (0.03)c	7.75 (0.06)g	1.03 (0.02)e
NPK	4.09 (0.01)d	9.50 (0.13)f	1.24 (0.07)d
NPKS	4.19 (0.02)d	11.8 (0.03)d	1.71 (0.02)c
NPKL	6.45 (0.02)a	10.6 (0.14)e	1.33 (0.04)d
NPKSL	5.96 (0.03)b	12.3 (0.05)c	1.29 (0.10)d
NPKM	5.72 (0.10)b	14.6 (0.03)b	1.98 (0.03)b
M	6.64 (0.00)a	19.0 (0.03)a	2.14 (0.02)a

CK, unfertilized control; NPK, mineral NPK fertilizer; NPKS, mineral NPK fertilizer plus straw; NPKSL, mineral NPK fertilizer plus straw and quicklime; NPKL, mineral NPK fertilizer plus quicklime; NPKM, mineral NPK fertilizer (30% of applied N) plus swine manure (70% of applied N) each crop season; M, manure only. Different letters within the same column indicate significant differences between treatments for each site at $P < 0.05$ (Duncan-test). Values in parentheses denote the standard deviation ($n = 3$).
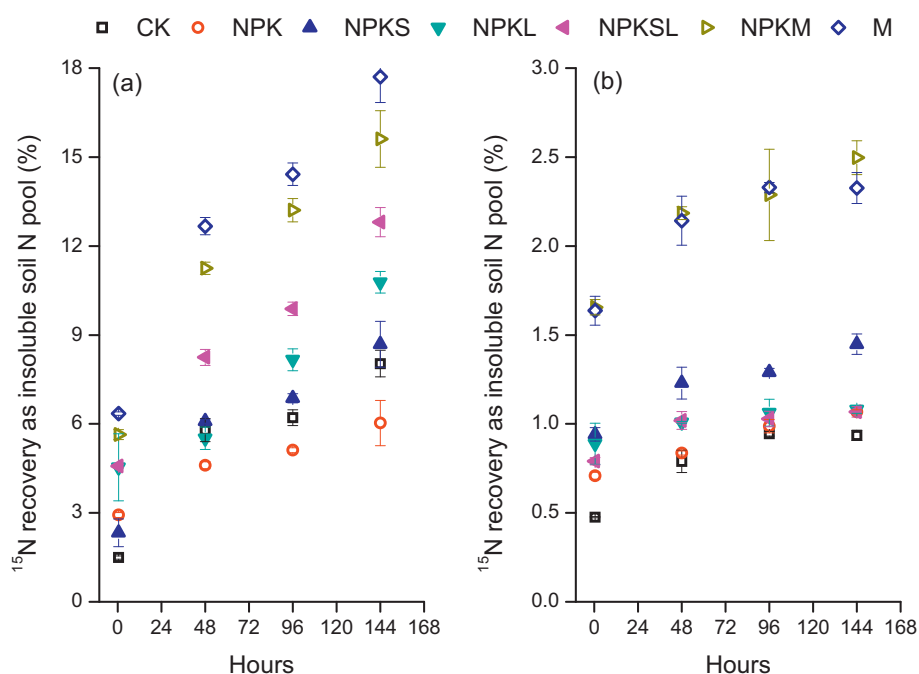
Fig. 1. Percentage of immobilized NH_4^+-^{15}N (a) and NO_3^--^{15}N (b) vs. time under different fertilization treatments after a 25-year fertilizer experiment. CK, unfertilized control; NPK, mineral NPK fertilizer; NPKS, mineral NPK fertilizer plus straw; NPKSL, mineral NPK fertilizer plus straw and quicklime; NPKL, mineral NPK fertilizer plus quicklime; NPKM, mineral NPK fertilizer (30% of applied N) plus swine manure (70% of applied N) each crop season; M, manure only. Error bars are standard deviations of the means ($n = 3$). The invisible error bars are smaller than the symbols.

2.3. Soil inorganic and organic nitrogen analysis

The concentrations of NH_4^+ and NO_3^- in the KCl extracts were determined using a continuous-flow analyzer (SA1000, Skalar, The Netherlands). NH_4^+ and NO_3^- were separated for ^{15}N measurements by distillation with magnesium oxide and Devarda's alloy, respectively (Bremner, 1996). The isotopic compositions of NH_4^+, NO_3^-, and insoluble organic N were determined using an automated C/N analyzer isotope ratio mass spectrometer (Europa Scientific Integra, Sercon 20-22, UK).

2.4. Calculations and statistical analysis

We employed the organic ^{15}N recovery method to calculate biotic and abiotic N immobilization (Murphy et al., 2003; Corre et al., 2007; Sotta et al., 2008; Remero et al., 2015; Cheng et al., 2017). In organic ^{15}N recovery, abiotic NH_4^+-N and NO_3^--N immobilization are expressed as ^{15}N recovered in the KCl washed soil residue divided by the labeled $^{15}NH_4^+$-N and $^{15}NO_3^-$-N added 0.5 h after ^{15}N addition, respectively. Abiotic NH_4^+-N immobilization has been attributed to physical condensation reactions with phenolic compounds (Nömmik, 1970) and fixation in clay minerals (Davidson et al., 1991). However, abiotic NO_3^--N immobilization was probably due to abiotic conversion of NO_3^- to soluble or insoluble organic N (Corre et al., 2007). An earlier study has demonstrated that the process consuming added ^{15}N was abiotic within minutes of adding the label due to lack of a significant effect of sterilization (Davidson et al., 1991). They also found that the abiotic consumption occurred within the first 15 min because of no significant difference between the amount of ^{15}N extracted at 15 min and 24 h after ^{15}N addition to sterilized soils. Therefore, an initial ^{15}N recovery (15 min to 1 h) was often determined to approximately represent abiotic N immobilization (Corre et al., 2007; Sotta et al., 2008). We would rather not use gamma irradiation, autoclaving, and Hg addition to distinguish abiotic and biotic N immobilization because such sterilization procedures generally contribute to large errors in estimates of N immobilization (Barrett et al., 2002; Fricks et al., 2009). For instance, significantly greater insoluble ^{15}N recovery in ir-radiated samples compared with live samples has been observed (Fricks et al., 2009), and the use of $HgCl_2$ may suppress abiotic N uptake and biological N immobilization (Barrett et al., 2002). Conversely, biotic

NH_4^+-N and NO_3^--N immobilization during incubation are expressed as the difference in ^{15}N recovered in the KCl washed soil residue between 0.5 h and 6 d after ^{15}N addition divided by the labeled $^{15}NH_4^+$-N and $^{15}NO_3^-$-N added, respectively (Murphy et al., 2003; Remero et al., 2015). Organic ^{15}N recovery method has been demonstrated to reliable in estimating biotic N immobilization (Cheng et al., 2017). Assuming that what we did not recover from the insoluble organic N, NH_4^+-N, and NO_3^--N pools as a soluble organic N pool, this fraction could be overestimated due to possible gases losses from denitrification and ammonia volatilization.

One-way analysis of variance (ANOVA) with a least significant difference (LSD) test was used to assess differences in soil properties, percentage recovery of ^{15}N in various soil N pools, and abiotic and biotic N immobilization among the different fertilization treatments. Pearson's correlation analysis was employed in evaluating the relationships between soil pH, total C and N concentrations, and abiotic and biotic N immobilization among treatments. All statistical analyses were performed in SPSS 16.0 (SPSS Inc., Chicago, IL, USA). All results were reported on a soil dry weight basis.

3. Results

3.1. Soil properties

Compared with the CK treatment, long-term mineral NPK application significantly decreased soil pH from 5.49 to 4.09 (Table 1). The combined application of crop residue and mineral NPK fertilizer (NPKS treatment) did not change soil pH in when compared with mineral NPK fertilizer application alone. In contrast, soil pH was significantly higher in the NPKL treatment (6.45) than in the NPKSL treatment (5.96); both were significantly higher than values for the NPK treatment. Similarly, manure application resulted in a significant increase in soil pH, with the increase being more pronounced in the M treatment (6.64) than in the NPKM treatment (5.72). Long-term field fertilizer application resulted in significant accumulation of soil total C and N, which increased in the following order: CK < NPK < NPKL < NPKS < NPKSL < NPKM < M and CK < NPK ≤ NPKSL ≤ NPKL < NPKS < NPKM < M, respectively (Table 1).
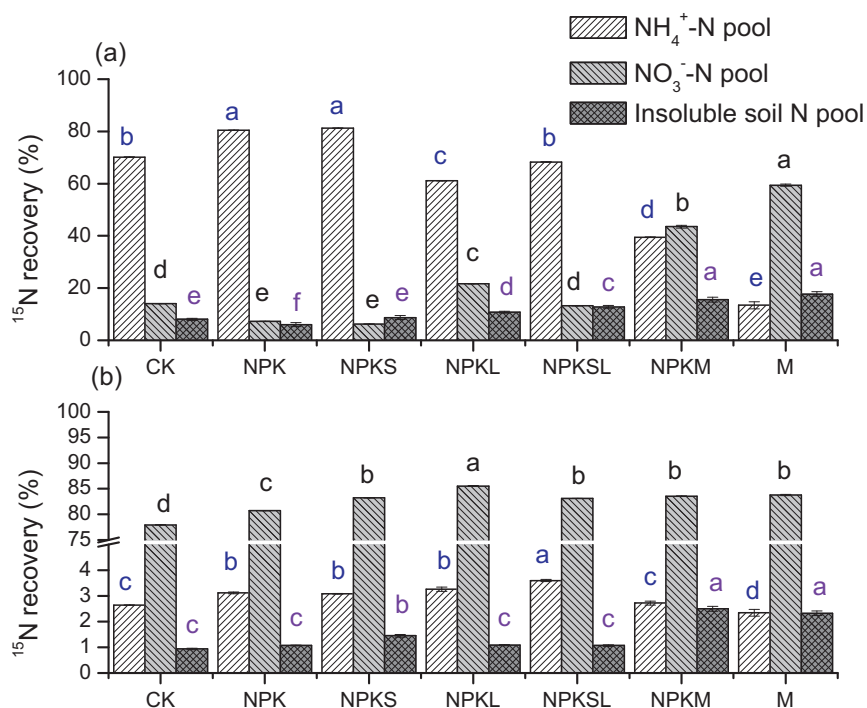
Fig. 2. Percentage recovery of ^{15}N in soil N pools during a 144-h incubation period under different fertilization treatments after the 25-year fertilizer experiment: (a) ^{15}NH$_4^+$-labeled samples and (b) ^{15}NO$_3^-$-labeled samples. CK, unfertilized control; NPK, mineral NPK fertilizer; NPKS, mineral NPK fertilizer plus straw; NPKSL, mineral NPK fertilizer plus straw and quicklime; NPKL, mineral NPK fertilizer plus quicklime; NPKM, mineral NPK fertilizer (30% of applied N) plus swine manure (70% of applied N) applied each crop season; M, manure only. Error bars are standard deviations of the means (n = 3). Invisible error bars are smaller than the symbols. Similar letters in the bars of the same N pool indicate that the recovery of ^{15}N is not significantly different at the P = 0.05 level.
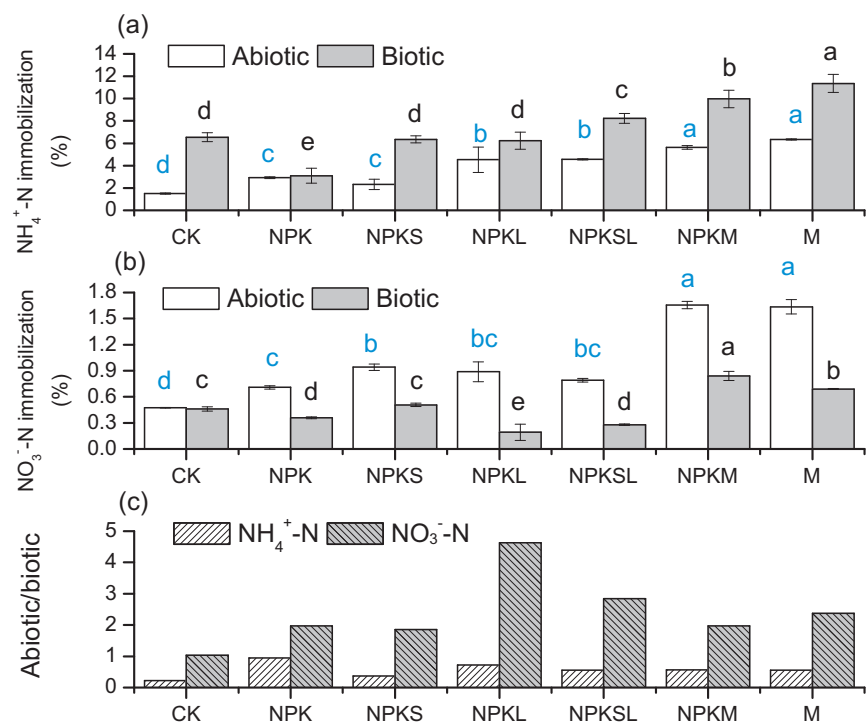

Fig. 3. Immobilization of NH$_4^+$-N (a) and NO$_3^-$-N (b) and abiotic/biotic immobilization ratio (c) under different fertilization treatments after a 25-year fertilizer experiment. CK, unfertilized control; NPK, mineral NPK fertilizer; NPKS, mineral NPK fertilizer plus straw; NPKSL, mineral NPK fertilizer plus straw and quicklime; NPKL, mineral NPK fertilizer plus quicklime; NPKM, mineral NPK fertilizer (30% of applied N) plus swine manure (70% of applied N) applied each crop season; M, manure only. Error bars are standard deviations of the means (n = 3). Similar letters in bars of the same variable indicate that the value is not significantly different at the P = 0.05 level.

3.2. ^{15}N recoveries in the NH$_4^+$, NO$_3^-$, and insoluble organic N pools

From both ^{15}NH$_4^+$ and ^{15}NO$_3^-$-labeled samples, ^{15}N recovery in the insoluble organic N pool increased gradually over the entire incubation period, regardless of fertilizer treatments (Fig. 1). In the ^{15}NH$_4^+$-labeled samples, 70.2%, 14.1%, and 8.0% of added ^{15}N was recovered in the NH$_4^+$, NO$_3^-$, and insoluble organic N pools in the CK treatment at the end of the incubation, respectively (Fig. 2a). In comparison with the CK treatment, ^{15}N recoveries in the NH$_4^+$ pool were significantly enhanced by the application of NPK and NPKS, but were significantly reduced by the application of NPKM and M, and to a less degree by NPKL application (Fig. 2a). Similarly, ^{15}N recovery in the NO$_3^-$ pool was significantly reduced by the application of NPK and NPKS, but was significantly increased by the application of NPKM and M, and to a less degree by NPKL application. In contrast, the application of NPKSL did not affect ^{15}N recovery in the NH$_4^+$ and NO$_3^-$ pools when compared with the CK treatment. Compared to the CK treatment, the application of NPK significantly decreased ^{15}N recovery in the insoluble organic N pool, but the application of NPKS did not alter ^{15}N recovery. The application of lime and manure resulted in a significant increase in ^{15}N recovery in the insoluble organic N pool with the increase being more pronounced in the NPKM and M treatments than in

the NPKL and NPKSL treatments.

In the $^{15}NO_3^-$-labeled samples, ^{15}N recovery in the NH_4^+ and insoluble organic N pools ranged from 2.3% to 3.6% and from 0.9% to 2.5%, respectively, and were generally < ^{15}N recovery in the NO_3^- pool (> 78%) (Fig. 2b). ^{15}N recovery in the insoluble organic N pool was significantly greater in the NPKM and M treatments than in the NPKS treatment, and both were significantly greater than those in the CK, NPK, NPKL, and NPKSL treatments (Fig. 2b). In general, ^{15}N recovery in the insoluble organic N pool in the $^{15}NO_3^-$-labeled samples was significantly lower than in the $^{15}NH_4^+$-labeled samples (6.0%–17.7%), irrespective of fertilizer treatments (Fig. 2a and b).

3.3. Abiotic and biotic N immobilization

Abiotic NH_4^+-N immobilization was significantly enhanced by long-term field fertilizer application compared with the CK treatment (mean recovery 1.5%) (Fig. 3a). Abiotic NH_4^+-N immobilization was highest in the NPKM and M treatments (5.6%–6.3%), followed by the NPKL and NPKSL treatments (4.5%–4.6%), and lowest in the NPK and NPKS treatments (2.3%–2.9%). In contrast, biotic NH_4^+-N immobilization in the CK treatment (6.5%) was significantly reduced by NPK application (3.1%) and not affected by the application of NPKL and NPKS (6.2%–6.4%) (Fig. 3a). While biotic NH_4^+-N immobilization in NPKSL (8.2%), NPKM (10.0%), and M (11.4%) treatments was significantly greater than in the CK treatment. Generally, biotic NH_4^+-N immobilization exceeded abiotic NH_4^+-N immobilization across treatments. Therefore, the ratio of abiotic NH_4^+-N immobilization to biotic NH_4^+-N immobilization was < 1 (Fig. 3c).

Long-term field fertilizer application resulted in a significant increase in abiotic NO_3^--N immobilization in the following order: CK < NPK ≤ NPKSL ≤ NPKL ≤ NPKS < NPKM ≈ M (Fig. 3b). In contrast, biotic NO_3^--N immobilization was significantly reduced by the application of NPK, NPKL, and NPKSL, enhanced by the application of NPM and M, and not affected by the application of NPKS when compared with the CK treatment (Fig. 3b). Generally, biotic NO_3^--N immobilization was less than abiotic NO_3^--N immobilization across treatments. Therefore, the ratio of abiotic NO_3^--N immobilization to biotic NO_3^--N immobilization was greater than one (Fig. 3c). In addition, both abiotic and biotic NH_4^+-N immobilization were generally greater than their corresponding NO_3^--N immobilization across treatments (Fig. 3a and b). Soil pH accounted for 41% and 38% of the variance in the estimates of biotic and abiotic NH_4^+-N immobilization, respectively (Fig. 4). Soil organic C content was positively correlated with abiotic NO_3^--N immobilization and biotic and abiotic NH_4^+-N immobilization (Fig. 5). Soil total N content was positively correlated with biotic and abiotic NO_3^--N immobilization, and biotic and abiotic NH_4^+-N immobilization (Fig. 6).

4. Discussion

Microbial immobilization of N is considered a key mechanism for soil N retention. Soil microbes generally prefer NH_4^+ over NO_3^- for their growth because of the additional energy consumption associated with NO_3^- reduction and assimilation (Lindell and Post, 2001), and because NH_4^+ can suppress biotic NO_3^--N immobilization even at relatively low concentrations (Rice and Tiedje, 1989). Similarly, our study showed that biotic NO_3^--N immobilization was responsible for not > 1.0% of ^{15}N added, and was generally less than biotic NH_4^+-N immobilization (3.1%–11.4%) across fertilizer treatments in the acidified agricultural soil. The results are consistent with previous reports that microbial immobilization of NO_3^--N is not a critical process in the regulation of NO_3^- concentrations in most agricultural soils (Shi and Norton, 2000; Shi et al., 2004; Wang et al., 2017a). Nevertheless, some studies have demonstrated the significance of microbial NO_3^--N immobilization in forest and grassland soils (Stark and Hart, 1997; Hatch et al., 2000). Compared with forest and grassland soils, agricultural

soils are relatively C limited but N sufficient. Therefore, the available C content in agricultural soils is probably not sufficient to support the energy demands of NO_3^- reduction and assimilation processes. In addition, in the present study, the application of NPKS and NPKM significantly increased both biotic NH_4^+-N and NO_3^--N immobilization compared with NPK treatment in the acidified agricultural soil. The results indicated that long-term input of exogenous C could enhance microbial immobilization of N, suggesting that microbial N immobilization in agricultural soils is somewhat C limited.

Not all exogenous C input may enhance biotic NO_3^--N immobilization. Several studies have reported cases where animal manure addition did not stimulate microbial NO_3^--N immobilization in agricultural soils (Shi and Norton, 2000; Shi et al., 2004; Wang et al., 2017a). The above findings suggest C limitation of the heterotrophic microorganisms even in animal manure treated soil. A meta-analysis further revealed that microbial NO_3^--N immobilization was not stimulated by animal manure application regardless of the application rate (Cheng et al., 2017). Animal manure, which often has a low C/N ratio, can provide sufficient N to meet microbial growth demands. Consequently, its application fails to stimulate microbial NO_3^--N immobilization. In contrast, our results indicated that both microbial NH_4^+-N and NO_3^--N immobilization were significantly increased by the application of pig manure, either alone or in combination with NPK, and to a less extent by NPKS application, when compared with the NPK treatment in the acidified agricultural soil. The results addressed our question regarding which class of organic fertilizer between animal manure and crop straw has greater stimulation effects on biotic N immobilization in acidified agricultural soils. The findings also suggest that microbial NO_3^--N immobilization might be regulated by other factors in addition to the C/N ratio of the organic material in the acidified agricultural soil.

Bacteria rather than fungi have the greatest potential for immobilizing NO_3^--N (Myrold and Posavatz, 2007). Acidic soil, therefore, could be associated with lower bacterial diversity (Fierer and Jackson, 2006). NPK fertilizer-induced severe soil acidification (pH = 4.09) could have inhibited soil microbial activity and decreased both biotic NH_4^+-N and NO_3^--N immobilization compared with the CK treatment. The application of animal manure not only provided a C source but also alleviated soil acidification, which enhanced soil microbial activity (Table 1). Therefore, the animal manure-induced increase in biotic NH_4^+-N and NO_3^--N immobilization could be attributed to enhanced soil microbial activity following an increase in soil pH. However, the application of lime also alleviated soil acidification and increased biotic NH_4^+-N immobilization, but did not increase biotic NO_3^--N immobilization. The results suggest that the input of exogenous C from animal manure may also control biotic NO_3^--N immobilization, in addition to soil pH. In contrast, crop straw application did not alleviate soil acidification but enhanced biotic NH_4^+-N and NO_3^--N immobilization, since plant residues, which have high C/N ratios, facilitated greater heterotrophic activity resulting in higher indigenous soil N demand, and enhanced NO_3^- immobilization (Cheng et al., 2017).

The distinct advantage of microbial N assimilation is that the N stored in microbial biomass could be temporarily immobilized and subsequently re-mineralized and made available to crops in the current or the following growing season (Remero et al., 2015). Soil NO_3^- accumulation is associated with N losses via runoff, leaching, and denitrification, which are very common globally, and particularly in China (Erisman et al., 2013; Zhou et al., 2016). Strategies that enhance biotic NO_3^--N immobilization through the input of specific C sources could be useful in the reduction of NO_3^- accumulation in soils. However, it should be noted that biotic NO_3^--N immobilization was only responsible for 1.0% of ^{15}N added across fertilizer treatments in the acidified agricultural soil, which suggest that the stimulation of biotic NO_3^--N immobilization by animal manure was still limited. In addition, ^{15}N recovery in the NO_3^- pool was significantly increased by the
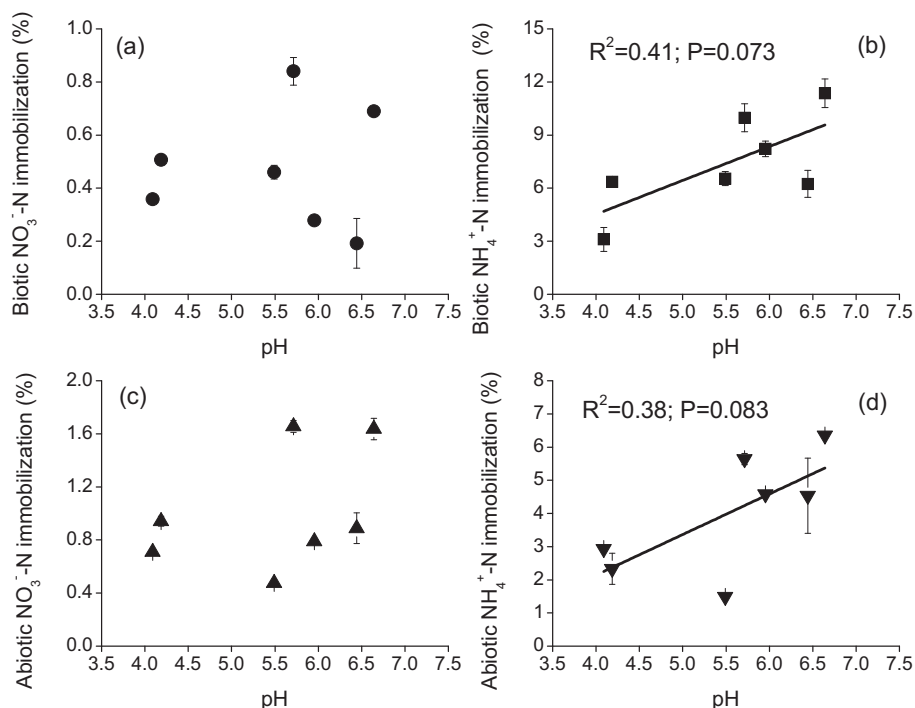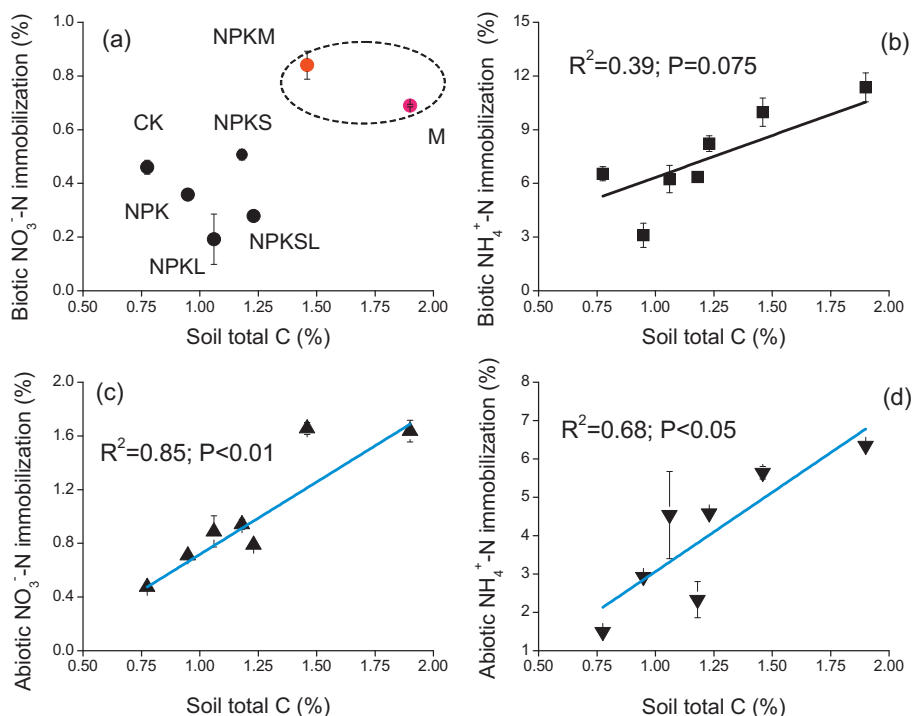
Fig. 4. Scatter plot and potential linear regression of (a) biotic NO_3^--N immobilization, (b) biotic NH_4^+-N immobilization, (c) abiotic NO_3^--N immobilization, and (d) abiotic NH_4^+-N immobilization vs. soil pH under different fertilization treatments after a 25-year fertilizer experiment. Error bars are standard deviations of the means (n = 3).

application of NPKM and M in the $^{15}NH_4^+$-labeled samples, indicating that the application of NPKM and M significantly stimulated soil nitrification rates and probably soil NO_3^- accumulation due to enhanced soil pH. It has been suggested that the co-application of nitrification inhibitor with the animal manure could decrease the risk of NO_3^- loss and N_2O emission without changing biotic NO_3^--N immobilization and remineralization rates (Zhu et al., 2016).

In addition to biotic N immobilization, abiotic NH_4^+-N and NO_3^--N immobilization also played a critical role in soil N retention, accounting for 22% to 73% and 103% to 463% of biotic NH_4^+-N and NO_3^--N immobilization, respectively. Abiotic NH_4^+-N immobilization is a

common phenomenon in soils and has been attributed to physical condensation reactions with phenolic compounds (Nömmik, 1970) and fixation in clay minerals (Davidson et al., 1991). In the present study, a positive and significant relationship between abiotic NH_4^+-N immobilization and soil organic C content (Fig. 5d) also suggested that there was an enhanced NH_4^+-N fixation potential through reactions with organic compounds following long-term straw and animal manure application. In addition, abiotic NH_4^+-N immobilization was positively correlated with soil pH (Fig. 4d). Such results are consistent with previous findings where reactions facilitating the abiotic stabilization of NH_3 and NH_4^+ through humic substances and substitution in clay

Fig. 5. Scatter plot and potential linear regression of (a) biotic NO_3^--N immobilization, (b) biotic NH_4^+-N immobilization, (c) abiotic NO_3^--N immobilization, and (d) abiotic NH_4^+-N immobilization vs. soil total C concentration under different fertilization treatments after a 25-year fertilizer experiment. Error bars are standard deviations of the means (n = 3).

Fig. 6. Scatter plot and linear regression of (a) biotic NO_3^--N immobilization, (b) biotic NH_4^+-N immobilization, (c) abiotic NO_3^--N immobilization, and (d) abiotic NH_4^+-N immobilization vs. soil total N concentration under different fertilization treatments after a 25-year fertilizer experiment. Error bars are standard deviations of the means (n = 3).

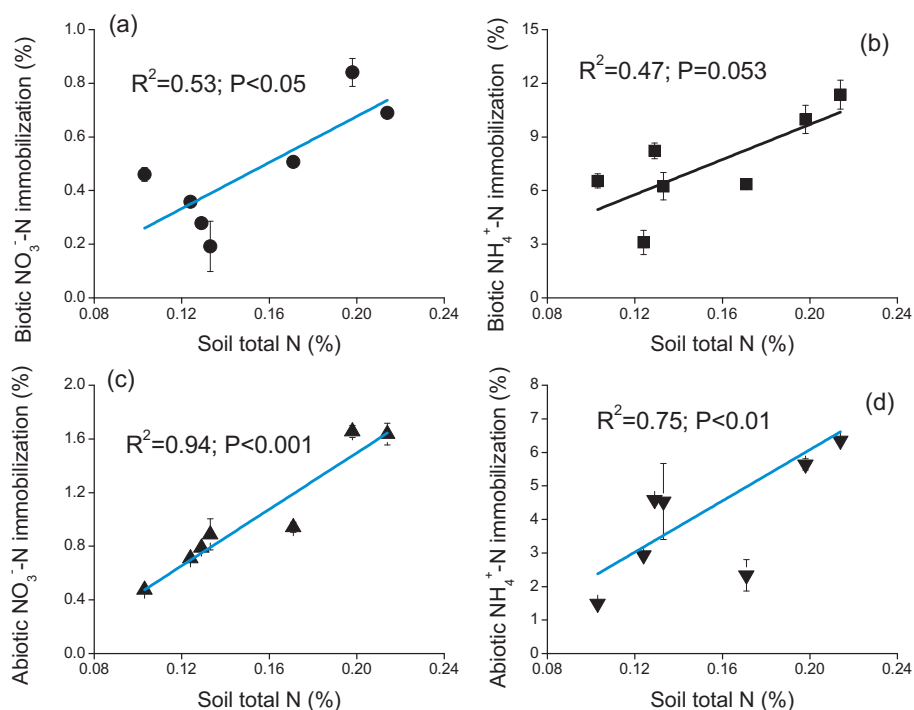minerals are favored at high soil pH (He et al., 1988; Stevenson, 1994). Consequently, increased abiotic NH_4^+-N immobilization following straw and animal manure application could be due to either increased soil pH or enhanced soil organic C content, or both. In contrast, Barrett et al. (2002) found a negative relationship between soil pH and abiotic NH_4^+-N immobilization in semiarid grassland soils. Schimel and Firestone (1989a, 1989b) observed that abiotic reactions accounted for as high as 20% of the NH_4^+-N retained in acidic (pH 4.3–4.5) forest soils. Nevertheless, differences in mineralogy and organic matter composition across gradients could be an additional source of variability and influence the relationship between abiotic NH_4^+-N uptake and pH (Barrett et al., 2002).

Abiotic NO_3^--N immobilization is particularly puzzling compared to abiotic NH_4^+-N immobilization, because there is no known abiotic mechanism for the binding of nitrate to SOM (Davidson et al., 2003; Hell et al., 2016). Although abiotic NO_3^--N immobilization was challenged by Colman et al. (2007), most studies tend to support the existence of biotic NO_3^--N immobilization (Dail et al., 2001; Perakis and Hedin, 2001; Zhang et al., 2010). Davidson et al. (2003) put forward the 'ferrous wheel hypothesis' based on the rapid incorporation of nitrate into SOM. In this conceptual model, Fe^{3+} is reduced by SOM to Fe^{2+}, which in turn reduces NO_3^- to NO_2^-, and NO_2^- subsequently reacts with phenolic organic matter to form dissolved organic N compounds. Such a hypothesis emphasizes the vital role of SOM in regulating abiotic NO_3^--N immobilization. Similarly, we observed a significant and positive relationship between soil total C content and abiotic NO_3^--N immobilization (Fig. 5c). This implied that straw and animal manure application enhanced abiotic NO_3^--N immobilization by increasing soil organic C content. In addition, NO_2^- reactions with C-containing compounds may depend on the forms of reactive C, which may determine whether the $^{15}NO_3^-$ is recovered in soluble or insoluble organic N forms (Corre et al., 2007). Assuming that what we did not recover from the insoluble organic N, NH_4^+-N, and NO_3^--N pools as an extractable organic N pool, this fraction constituted 10.1 to 18.5% of added $^{15}NO_3^-$ in the acidified agricultural soil studied, while only 0.5 to 1.7% of added $^{15}NO_3^-$ was recovered in the insoluble organic N pool (Fig. 2b). Other studies reported that 30% of added $^{15}NO_3^-$ was detected in extractable organic N and 5% in insoluble N from deciduous

forest soils (Dail et al., 2001), and 11% in extractable organic N and 37% in the insoluble N from an unpolluted/N-limited evergreen forest soils (Perakis and Hedin, 2001). Different soil types could be associated with different SOM structures and decomposition dynamics, which may result in varied reactive C forms in different soils (Corre et al., 2007). In the present study, even in cases where the extractable organic N was not included in the estimates of abiotic NO_3^--N immobilization, the ratio of abiotic NO_3^--N immobilization to biotic NO_3^--N immobilization > 1 is sufficient evidence of the significant role of abiotic NO_3^--N immobilization in soil NO_3^--N retention.

5. Conclusions

Our results demonstrate that microbial NH_4^+-N assimilation rather than abiotic NH_4^+-N immobilization is a key contributor to the retention of NH_4^+-N following long-term straw and animal manure application in an acidified agricultural soil. In contrast, abiotic NO_3^--N immobilization plays a more significant role in NO_3^--N retention compared to microbial NO_3^--N assimilation under elevated C conditions. Both abiotic and biotic NH_4^+-N and NO_3^--N immobilization were significantly enhanced by animal manure application and to a less degree by straw return. Manure-induced increases in microbial NH_4^+-N and NO_3^--N immobilization could be attributed to increased C availability and enhanced soil microbial activity in increased soil pH conditions. In contrast, crop straw application did not alleviate soil acidification but enhanced biotic NH_4^+-N and NO_3^--N immobilization, since plant residues, which have high C/N ratios facilitated more heterotrophic activity with greater indigenous soil N demand, and in turn enhanced NO_3^- immobilization. Straw and animal manure application enhanced abiotic NH_4^+-N and NO_3^--N immobilization by increasing soil organic C content. Overall, the results of the present study show that long-term inputs of straw and animal manure to an acidified agricultural soil could enhance abiotic and biotic N immobilization and N retention capacity. The findings reinforce an increasing awareness of the significance of abiotic and biotic N immobilization in influencing N retention in acidified agricultural soils. In addition, we suggested that co-application of nitrification inhibitor with the animal manure should be carried out to inhibit nitrification rate and reduce NO_3^- loss risks

considering that the application of animal manure stimulated soil nitrification rate in acidified agricultural soils. Future studies should be conducted to identify the microbial community patterns and the related activity under long-term repeated organic amendments.

Acknowledgments

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