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Alterations of pathways in fertilizer N conservation and supply in soils treated with dicyandiamide, hydroquinone and glucose

Wantai Yu[a], Feifei Pan[a,b], Qiang Ma[a,*], Jing Wang[c], Hua Zhou[a], Chunming Jiang[a], Yonggang Xu[a]

[a] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Shenyang No. 1 High School, Shenyang, 110042, PR China

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ABSTRACT

Nitrogen (N) immobilization by microorganisms and NH_4^+ fixation by soil minerals are common reactions responsible for fertilizer N retention in soils. However, the relationship between microbial immobilization and NH_4^+ fixation remains unclear to date, and the availability of immobilized or fixed fertilizer N has yet to be compared. Accordingly, we conducted a 96-day incubation experiment to study the effects of the nitrification inhibitor dicyandiamide (DCD), the urease inhibitor hydroquinone (HQ), and glucose adding on ^{15}N-labeled urea-N partitioning in different N pools and on the subsequent remineralization of immobilized N and release of fixed NH_4^+. Glucose significantly increased N retention in soil but decreased the availability of urea-derived N because a great proportion of urea-derived N was transformed into soil microbial necromass N (SMNN). In the non-glucose treatments, the effects of the fixed NH_4^+ pool on the conservation and supply of urea-derived N were 1.6-fold and 2.7-fold greater on average than those of the organic N pool (including soil microbial biomass N and SMNN), respectively, from the 12th day to the end of the incubation. In the glucose treatments, the corresponding effects of the organic N pool were 3.7-fold and 3.0-fold greater than those of the fixed NH_4^+ pool. Both inhibitors raised urea-derived fixed NH_4^+ but exhibited different influences on urea-derived organic N; in particular, DCD input increased urea-derived organic N, whereas HQ input decreased this parameter. The combination of glucose and DCD further decreased the availability of urea-derived N, but HQ alleviated the decline of urea-derived N availability induced by glucose input. Microbial immobilization and nitrification comparably contributed to the release of urea-derived fixed NH_4^+ in soil treated with urea alone. In the presence of glucose, microbial immobilization was the principal driving force of fixed NH_4^+ release, and this tendency was further enhanced by DCD but mitigated by HQ. The partitioning of released fixed NH_4^+ between the organic N pool (immobilization) and the mineral N pool (nitrification) can be clarified by comparing the path coefficients under different conditions. These results provide valuable information for combining the abiotic and biotic processes in N cycling after fertilizer N application and for quantifying N transformation in soils.

1. Introduction

Nitrogen (N) occupies a unique position among the elements essential for plant growth because of the rather large amount required by most agricultural crops (Fageria and Baligar, 2005). Agricultural systems thus rely heavily on synthetic N to meet the N requirements of plants (Ma et al., 2012). The combined N in soil is largely converted into organic forms by microbial immobilization or fixed by mineral materials, which can prevent N losses and significantly influence the subsequent availability of conserved N (Liu et al., 2008; Sugihara et al., 2012). Therefore, clarification of these processes is necessary to maximize N use efficiency and minimize environmental pollution (Nieder et al., 2011; Singh et al., 2011).

Some of the fertilizer N applied to a crop–soil system is rapidly incorporated into soil microbial biomass, thereby conserving fertilizer N temporarily, especially when the timing of organic carbon (C) amendment is appropriate (Sugihara et al., 2012). Several investigations focused on the importance of soil microbial biomass N (SMBN) and its beneficial role in agricultural production

* Corresponding author.
E-mail address: qma@iae.ac.cn (Q. Ma).

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(Nannipieri and Paul, 2009; Singh et al., 2011). However, a great portion of microbial-immobilized N can transform into soil microbial necromass N (SMNN, including microbial metabolites and residues), which contributes to soil organic matter formation (Fan and Liang, 2015; Miltner et al., 2012). The long turnover time of SMNN results in the temporary decline of N availability and even in the reduction of crop yield (Liang and Balser, 2011; Rutherford and Juma, 1992). This phenomenon is a particular challenge for organic farming, where large quantities of organic substrates probably return and the inorganic N supply of the system heavily depends on microbial net N mineralization in soil (Stevenson, 1982). In addition to microbial immobilization, applied-NH_4^+ can be fixed rapidly by soil minerals, especially for soil rich in 2:1 clay minerals (Nieder et al., 2011). Recently fixed NH_4^+ has a relatively high availability and may be gradually released in the subsequent weeks or growing seasons (Lu et al., 2010; Matsuoka and Moritsuka, 2011). Moreover, NH_4^+ fixation/defixation reduces NH_3 volatilization, declines nitrification, and subsequently decreases nitrate leaching and nitrous oxide (N_2O) emissions (Drury et al., 1991; San Francisco et al., 2011). Therefore, NH_4^+ fixation should not be considered as an entirely unfavorable reaction from the agronomic point of view. Under certain soil and climatic conditions, NH_4^+ fixation may prevent N losses and ensure an even supply of N throughout the growing season.

The use of nitrification and urease inhibitors efficiently reduces N losses. These inhibitors increase crop N use efficiency by delaying ammonium oxidation (nitrification inhibitors) or urea hydrolysis (urease inhibitors), consequently altering the temporal pattern of fertilizer N remaining in its cationic form (Barneze et al., 2015; Gioacchini et al., 2002). Soil microorganisms preferentially assimilate NH_4^+ because of the high energy costs associated with biological NO_3^- assimilation (Recous et al., 1990; Winsor, 1958). Thus, inhibitor addition can promote the microbial immobilization of N (Williamson et al., 1996; Zhou et al., 1992), although this strategy may inhibit target microorganisms or enzymes (Bremner and Douglas, 1971; Kleineidam et al., 2011). In addition, inhibitor application can increase NH_4^+ concentration and, consequently, its fixation by soil minerals, thereby enhancing the effects of fixed NH_4^+ on the conservation and supply of fertilizer N (Juma and Paul, 1983). In these scenarios, clarification of fertilizer N partitioning among different soil N pools and the subsequent release process is critical to assess N availability, improve N use efficiency, and decline N losses to the environment.

Microbial immobilization and NH_4^+ fixation are common reactions in many arable soils. However, the relationship between these reactions, especially in the presence of inhibitors and available C, remains poorly understood to date. Accordingly, we conducted a 96-day incubation experiment to clarify urea-derived N partitioning in various soil N pools with nitrification inhibitor (DCD), urease inhibitor (HQ), and organic C (glucose) additions, and to assess the availabilities of fixed or immobilized fertilizer N in subsequent release or remineralization processes, respectively.

2. Materials and methods

2.1. Site and soil description

This study was conducted at the Shenyang Experimental Station of the Institute of Applied Ecology, Chinese Academy of Sciences, approximately 30 kms south of Shenyang, Liaoning Province, China (41°32′N latitude, 123°23′E longitude) (Yu et al., 2011). A bulk soil sample (0–10 cm) classified as an Alfisol, which is primarily a combination of 2:1 clay minerals, was collected, air-dried, and then sieved (<5 mm). The soil properties are presented in Table 1.

Table 1
Physicochemical properties of test soil.

Chemical properties	
Organic C (g kg^{-1})	11.09
Total N (g kg^{-1})	0.96
Available P (mg kg^{-1})	3.50
Exchangeable K (mg kg^{-1})	74.36
Fixed NH_4^+ (mg kg^{-1})	160.3
pH	6.7
Physical properties	
Bulk density (0–10 cm) (g cm^{-3})	1.06
Water holding capacity (%)	42.5
Sand 50–2000 μm (%)	16.2
Silt 2–50 μm (%)	59.6
Clay 2 μm (%)	24.1
Clay mineral composition (<2 μm, %)	
Smectite	12
Vermiculite	17
Hydromica	21
Kaolinite	24
Chlorite	23
Quartz	1
Feldspars	2

2.2. Experimental design and sampling schedule

Seven treatments were used in the 96-day incubation experiment: (1) control with no addition (data not shown); (2) ^{15}N-labeled urea (10.33 atom% ^{15}N) (+U); (3) ^{15}N-urea and glucose (+U + G); (4) ^{15}N-urea and DCD (+DCD); (5) ^{15}N-urea, DCD, and glucose (+DCD + G); (6) ^{15}N-urea and HQ (+HQ); and (7) ^{15}N-urea, HQ, and glucose (+HQ + G). The application rates of urea and glucose were 188.9 mg N kg^{-1} soil (200 kg N ha^{-1}) and 3228 mg glucose-C kg^{-1} soil, respectively. Dicyandiamide and HQ were added at the rates of 6% and 2% of applied urea-derived N (*w*/*w*), respectively. The amendments were added as powder according to the treatments and were mixed thoroughly with soil. Fresh soil (150 g oven-dry weight) was incubated in polyethylene bottles (500 ml) with screw caps at 25 °C in the dark after 2 weeks of pre-incubation. Soil moisture was adjusted to 50% water-holding capacity (WHC). The bottles were opened and aerated at an interval of 3 days; meanwhile, the loss of moisture during incubation was replenished by weighing and adding distilled water. Three replication samples (bottles) of one treatment were randomly collected after 0.5, 3, 6, 12, 24, 48, and 96 days of incubation.

2.3. Analytical methods

Soil organic C and total N were determined by an elemental analyzer (Elementary vario EL III). Soil particle-size composition, available P, exchangeable K and pH were determined by the methods: pipette method (Jones, 2001), Olsen method, flame photometer method, and a glass electrode pH meter with a soil/water ratio of 1/2.5, respectively (Lu, 2000). Clay types were determined by X-ray diffraction (Whitting, 1965). Soil microbial biomass N was determined using the chloroform–fumigation extraction method. Soil samples were fumigated with ethanol-free chloroform for 24 h, and both fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 by shaking for 30 min. Soil microbial biomass N was the difference between the extracted N from fumigated and non-fumigated soils. The factor used to convert the extracted N to SMBN was 0.54 (Brookes et al., 1985). Soil mineral-N were extracted by 2 M KCl, and NH_4^+-N and NO_3^--N in the KCl extracts were analyzed by steam distillation after MgO and Devarda's alloy additions (Lu, 2000). The extracted soil was

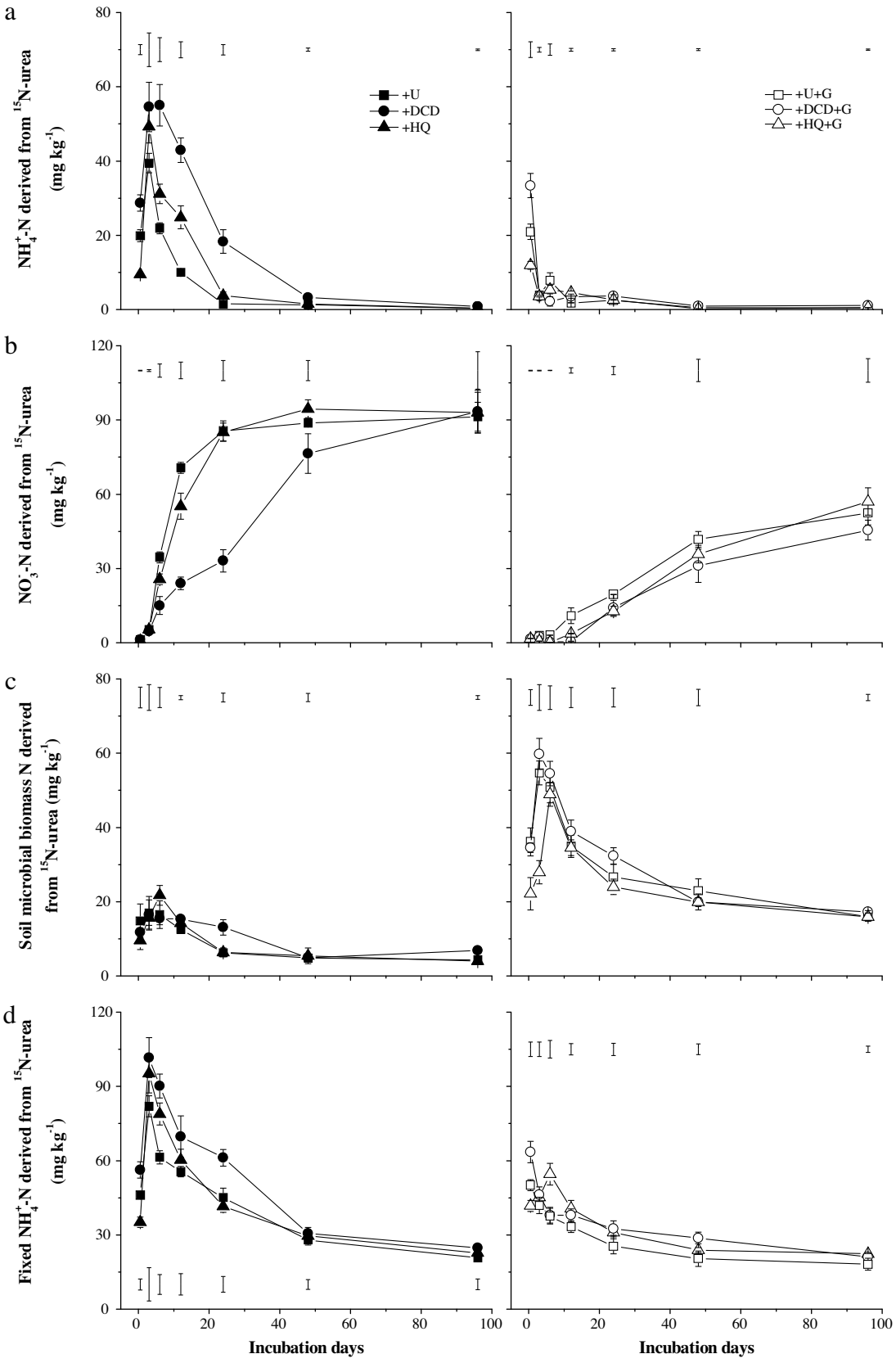

Fig. 1. Dynamics of urea-derived NH_4^+-N (a), NO_3^--N (b), soil microbial biomass N (c) and fixed NH_4^+ during 96-day incubation in different treatments. Error bar with each point represents standard error of the mean ($n = 3$), and separate bars represent LSD values ($t = 0.05$) for different treatments at the same incubation stage.

washed thrice with 0.1 M KCl, air-dried, and then sieved (<0.15 mm) to determine soil-fixed NH_4^+ in accordance with the method described by Silva and Bremner (1966), and modified by Shen et al. (1984). The ^{15}N samples were prepared as reported by Shen et al. (1984), and the $^{15}N/^{14}N$ ratio was measured by a stable isotope-ratio mass spectrometer (Delta plus XP). Urea-derived SMNN was calculated by subtracting the urea-derived SMBN, NH_4^+-N, NO_3^--N and fixed NH_4^+ from total urea-derived N in the soil (Rutherford and Juma, 1992).

2.4. Calculation and data analysis

Path analysis was performed to evaluate the effects of urea-derived NH_4^+-N (i.e., nitrification), SMBN (i.e., microbial immobilization–mineralization), and fixed NH_4^+ (i.e., fixed NH_4^+ release) on urea-derived NO_3^--N. Path analysis differentiates between correlation and causation by partitioning simple correlation coefficients between independent variables (NH_4^+-N, SMBN, and fixed NH_4^+) and dependent variable (NO_3^--N) into direct and indirect effects (Marinkovic, 1992). Direct effects represent the direct influences of independent variables on dependent variable, whereas indirect effects represent the influences of an independent variable on a dependent variable through other independent variables (Maphumulo et al., 2015). Path analysis can provide numerical values for both direct and indirect effects, thus indicating the relative strength of causal relationships (Zhang et al., 2005). Therefore, the contributions of nitrification and immobilization to fixed NH_4^+ release can be clarified by comparing the indirect effects of fixed NH_4^+ release on NO_3^--N through NH_4^+-N (nitrification) and SMBN (microbial immobilization). The partitioning of released fixed NH_4^+ between the mineral N and organic N pools can also be assessed on the basis of the results of path analysis.

The mass of urea-derived N recovered in various N pools was calculated by Eq. (1), and the ^{15}N atom% excess was corrected for the corresponding back ground abundances.

$$N_{dfu} = \frac{^{15}N\ atom\%\ excess_{pool}}{^{15}N\ atom\%\ excess_{fertilizer} \times N_{pool}} \quad (1)$$

Where N_{dfu} were N derived from urea in different soil N pools (mg kg^{-1}); $^{15}N\ atom\%\ excess_{pool}$ and $^{15}N\ atom\%\ excess_{fertilizer}$ were $^{15}N\ atom\%\ excess$ of soil N pool and ^{15}N-labeled urea, respectively; N_{pool} was the size of corresponding soil N pool (mg kg^{-1}).

The effect of substrate additions on dynamics of urea-derived N in different soil N pools was analyzed using a one-way ANOVA and the LSD method at a 95% confidence level ($P < 0.05$) in each sampling date. Statistical analyses were performed using a SPSS 11.0 software package. Figures were generated using the Origin 8.0 program.

3. Result

3.1. Dynamics of urea-derived N in soil

Urea-derived NH_4^+-N averagely increased by 15.6 mg kg^{-1} and 3.7 mg kg^{-1} in the +DCD and +HQ treatments, respectively, compared with the +U treatment (Fig. 1a). Glucose addition decreased urea-derived NH_4^+-N by 14.5 mg kg^{-1} on average. Urea-derived NH_4^+-N was higher in the treatment +DCD + G, but lower in the treatment +HQ + G, than in the treatment +U + G. The effects of the inhibitors on urea-derived NH_4^+-N were significantly eliminated by glucose addition. For urea-derived NO_3^--N, in the absence of glucose, DCD and HQ decreased NO_3^--N by 18.5 mg kg^{-1} and 2.5 mg kg^{-1} on average, respectively, especially in the first half of the incubation period (Fig. 1b). In the presence of glucose, urea-derived NO_3^--N decreased by 30.7 mg kg^{-1} on average, and the inhibitory effect of DCD and HQ on NO_3^--N was significant for 48 and 24 days, respectively.

Urea-derived SMBN followed a similar single-peak curve in all treatments and reached maximum levels on the third or sixth day (Fig. 1c). In the non-glucose treatments, both DCD and HQ slightly increased urea-derived SMBN, especially for DCD. Urea-derived SMBN was 2.9-fold greater on average in the glucose treatments than in the non-glucose treatments. Combined addition of DCD and glucose further increased urea-derived SMBN by 2.2 mg kg^{-1}, whereas it decreased by 7.0 mg kg^{-1} in the +HQ + G treatment, compared with the +U + G treatment.

Urea-derived fixed NH_4^+ peaked at 82.0 mg kg^{-1}, 101.6 mg kg^{-1}, and 95.2 mg kg^{-1} in the treatments +U, +DCD and +HQ, accounting for 43.4%, 53.8%, and 50.4% of fertilizer N, respectively (Fig. 1d). When the glucose was added, urea-derived fixed NH_4^+ significantly decreased during the incubation period, except at the first and last sampling dates. It directly peaked at 50.1 and 63.5 mg kg^{-1} on the first day in the +U + G and +DCD + G treatments, respectively, but it peaked on the sixth day in the +HQ + G treatment. On average, both DCD and HQ increased urea-derived fixed NH_4^+, regardless of the presence of glucose.

3.2. Recovery of urea-derived N

At the end of the incubation period, the application of the inhibitors alone slightly increased urea-^{15}N recovery. Glucose addition, especially the combination of glucose and DCD, significantly improved ^{15}N recovery (Table 2). This result was primarily attributed to the increase in urea-derived organic N, including SMBN and SMNN. Correspondingly, glucose decreased urea-derived inorganic N, including NH_4^+-N, NO_3^--N, and fixed NH_4^+ (Fig. 2). Meanwhile, compared with that in the non-glucose treatments, the decrease in urea-derived inorganic N was 63.4 mg kg^{-1} on average in the glucose treatments across the incubation period. Correspondingly, urea-derived SMBN increased by 21.7 mg kg^{-1} on average in the glucose treatments, only accounting for 34.2% of the decrease in inorganic N.

3.3. Remineralization of urea-derived organic N and release of urea-derived fixed NH_4^+

Both size and ratio of urea-derived fixed NH_4^+ release (70.2 mg kg^{-1} and 75.5%) were greater on average than those of urea-derived SMBN remineralization (13.3 mg kg^{-1} and 71.6%) in the absence of glucose (Fig. 1c and d). When glucose was added, the former (35.5 mg kg^{-1} and 63.2%) was lower than the latter (38.8 mg kg^{-1} and 70.9%).

On the basis of the partitioning of fertilizer N in different N pools, the contributions of urea-derived fixed NH_4^+ and organic N (including SMBN and SMNN) to the conservation of fertilizer N and

Table 2
^{15}N Recovery (%) in various N pools for different treatments at the end of the incubation.

Treatment	SMBN	Fixed NH_4^+	NH_4^+-N	NO_3^--N	SMNN	Total
+U	2.29b	10.93ab	0.19a	48.32a	11.55c	73.28b
+U + G	8.42a	9.62b	0.24a	27.75bc	37.09b	83.12a
+DCD	3.63b	13.09a	0.44a	49.45a	9.73c	76.34b
+DCD + G	9.16a	11.13ab	0.58a	24.09c	42.79a	87.74a
+HQ	2.09b	12.06ab	0.16a	49.20a	10.57c	74.07b
+HQ + G	8.44a	11.84ab	0.28a	30.21b	33.89b	84.66a

Means of three replicates; the same lowercase letter indicates no significant difference between treatments ($P < 0.05$). SMBN indicates soil microbial biomass N; SMNN indicates soil microbial necromass N.

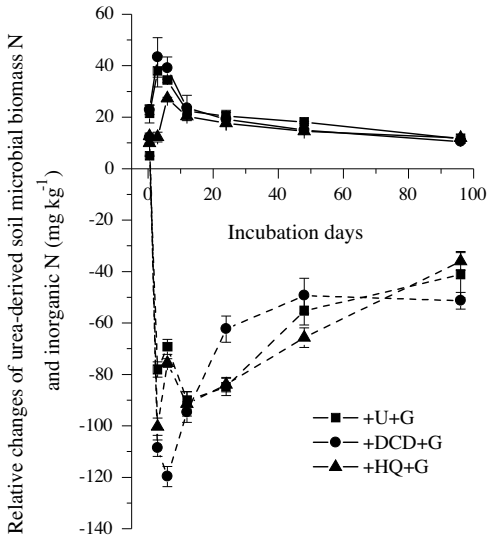

Fig. 2. Relative changes in the size of urea-derived soil microbial biomass N (solid line) and inorganic N (dashed line) during the incubation period in different treatments.
Relative change = $N_{glucosetreatment} - N_{non\text{-}glucosetreatment}$.
Inorganic N=NH_4^+-N + NO_3^--N + fixed NH_4^+-N.

supply of urea-derived mineral N were assessed from the 12th day to the 96th day (Table 3). In the non-glucose treatments, the effects of the fixed NH_4^+ pool on the conservation and supply of urea-derived N were 1.6-fold and 2.7-fold greater than those of the organic N pool, respectively. However, the corresponding effects of the urea-derived organic N pool were 3.7-fold and 3.0-fold greater than those of the fixed NH_4^+ pool in the glucose treatments. In the absence of glucose, both the absolute and proportional remineralization of urea-derived SMBN (8.9 mg kg^{-1} and 63.7% on average) were significantly higher than those of urea-derived SMNN (5.4 mg kg^{-1} and 21.2% on average). When glucose was added, the remineralization ratio of SMBN (54.6%) was still higher than that of SMNN (29.9%), whereas the size of SMBN remineralization (19.7 mg kg^{-1}) was lower than that of SMNN remineralization (30.3 mg kg^{-1}).

Dicyandiamide and HQ exhibited different influences on the partitioning of urea-derived N among the various N pools. Both inhibitors increased urea-derived fixed NH_4^+ and retarded its release. Dicyandiamide input increased urea-derived organic N, especially in the presence of glucose, whereas HQ input decreased this parameter. Moreover, urea-derived organic N remineralization was slightly promoted in the +DCD treatment but was prevented in the +DCD + G treatment. However, the addition of HQ consistently increased urea-derived organic N remineralization marginally.

3.4. Microbial effects on the release of urea-derived fixed NH_4^+

The results of path analysis showed that the microbial effects on the release of urea-derived fixed NH_4^+ were markedly influenced by the addition of inhibitors and glucose (Fig. 3). The direct effects of urea-derived fixed NH_4^+ on urea-derived NO_3^--N were marginal in all treatments, whereas the indirect effects were significant. In the +U treatment, the indirect effects (path-coefficients) of fixed NH_4^+ through SMBN (microbial immobilization) and NH_4^+-N (nitrification) were comparable, indicating similar effects of microbial immobilization and nitrification on fixed NH_4^+ release. The combined addition of urea and glucose significantly enhanced the role of SMBN in fixed NH_4^+ release and correspondingly diminished the effect of nitrification. When DCD was added, the effect of nitrification on fixed NH_4^+ release declined, and the effect further decreased in the +DCD + G treatment. Meanwhile, the effect of microbial immobilization on fixed NH_4^+ release increased. Hydroquinone addition slightly strengthened the effect of nitrification on fixed NH_4^+ release. Although the effect of microbial immobilization on fixed NH_4^+ release enhanced in the +HQ + G treatment, the increasing magnitude of immobilization effect was weakened with HQ addition.

4. Discussion

4.1. Conservation of urea-derived N in soil

Microbial immobilization and mineral fixation for fertilizer N are important processes in N conservation in soils (Aber et al., 1998; Nieder et al., 2011). However, the fact that most N in soils is associated with organic matter has led many soil scientists to assume that N retention in soils is controlled almost exclusively by biological processes (Kaye and Hart, 1997; Riha et al., 1986). Although NH_4^+ fixation is a common reaction in many arable soils, abiotic processes controlling N conservation in soils and their agronomic significance remain poorly understood (Nieder et al., 2011). Many studies showed that the fixed NH_4^+ pool is an important N sink conserving applied N in soils containing 2:1 clay minerals (Liu et al., 2008; Scherer and Mengel, 1986). Johnson et al. (2000) also pointed out that the abiotic effect on N conservation is relatively more important than the biotic effect in soils with N saturation due to fertilization, atmospheric deposition, or natural processes.

Available C addition effectively stimulated the activity of soil microorganisms and subsequent microbial immobilization of fertilizer N, which correspondingly decreased urea-derived fixed NH_4^+. Consequently, the effect of organic N pool on the conservation of fertilizer N enhanced, whereas that of fixed NH_4^+ pool declined. This result agrees with the finding reported by Qiu et al. (2012), in which returning straw significantly decreased fixed NH_4^+ and increased SMBN. However, organic substrate addition is unnecessary to reduce NH_4^+ fixation by minerals

Table 3
Conservation and supply of urea-derived N by various N pools in different treatments from the 12th day to the end of the incubation.

Treatments	Fixation/Immobilization of urea-derived N on the 12th day (mg kg^{-1})			Release/remineralization of fixed/immobilized urea-derived N (mg kg^{-1})			Release/remineralization proportion (%)		
	SMBN	SMNN	Fixed NH_4^+	SMBN	SMNN	Fixed NH_4^+	SMBN	SMNN	Fixed NH_4^+
+U	12.6Cc	27.3Bb	55.5Ab	8.2Bb	5.4Cc	34.9Ab	65.08Ab	19.78Bc	62.88Aa
+U + G	35.0Ba	103.0Aa	33.3Bd	19.0Ba	32.9Aa	15.2Bc	54.29Ac	31.94Ca	45.65Bb
+DCD	15.3Cb	24.5Bb	69.7Aa	8.4Bb	6.2Bc	45.0Aa	54.90Bc	25.31Cb	64.56Aa
+DCD + G	38.9Ba	105.0Aa	38.0Bcd	21.6Aa	24.2Ab	16.9Bc	55.53Ac	23.05Cb	44.47Bb
+HQ	14.2Cbc	24.6Bb	60.3Ab	10.2Bb	4.6Cc	37.6Ab	71.83Aa	18.70Cc	62.35Ba
+HQ + G	34.5Ba	97.7Aa	40.7Bc	18.6Ba	33.7Aa	18.3Bc	53.91Ac	34.49Ca	44.96Bb

Means of three replicates; the same capital letter indicates no significant difference between various N pools; the same lowercase letter indicates no significant difference between treatments ($P < 0.05$). SMBN indicates soil microbial biomass N; SMNN indicates soil microbial necromass N.

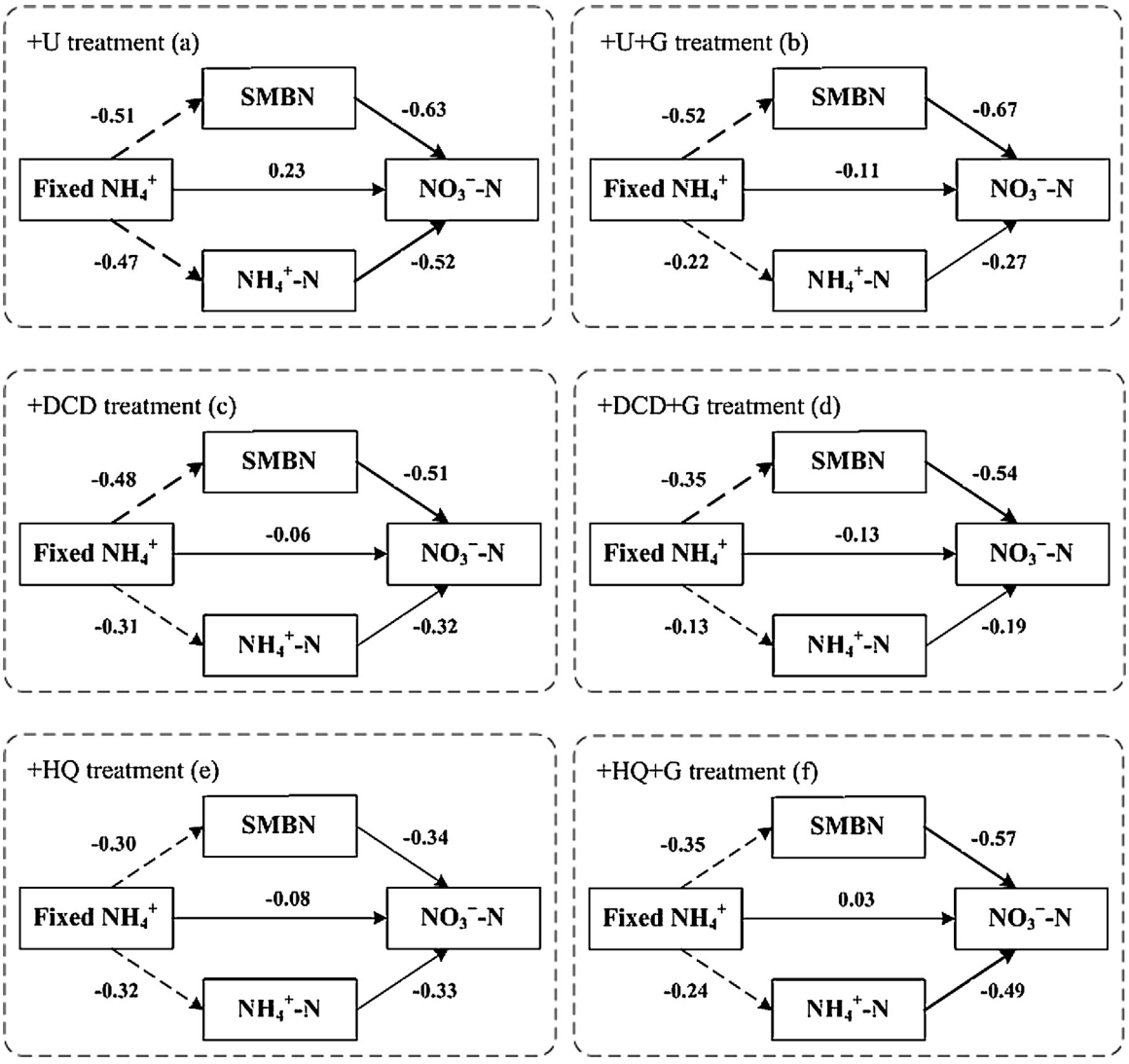

Fig. 3. Path analysis of effects of urea-derived NH_4^+-N, SMBN and fixed NH_4^+ on urea-derived NO_3^--N in different treatments.
The real lines and broken lines represent direct effects and indirect effects, respectively; the numbers beside the lines represent the direct or indirect path-coefficients; SMBN represents soil microbial biomass N.

(Liang et al., 2012; Lu et al., 2010). According to Tang et al. (2008), the addition of a mixed substrate with a narrow C/N ratio (5:1, i.e., 1000 mg glucose-C kg^{-1} and 200 mg N kg^{-1}) exerts little effect on the fixation/defixation of NH_4^+, whereas the addition of a substrate with a wide C/N ratio (50:1, i.e., 10000 mg C kg^{-1} and 200 mg N kg^{-1}) lowers the size of fixed NH_4^+ derived from fertilizer N and promotes the release of fixed NH_4^+. Moreover, the distribution of fertilizer N in various N pools was affected by the qualities of organic substrates. The straw-amended soil immobilized less N per unit of C added than either the glucose- or the cellulose-amended soils (Stevenson, 1982; Vinten et al., 2002). Consequently, the application rates and qualities of organic substrates significantly influenced the partitioning of applied N in different soil N pools and the subsequent release of these N pools (Scherer and Werner, 1996).

In the present study, the increase in urea-derived SMBN explained 34.2% of the decrease in inorganic N on average due to glucose addition. This result is in line with the finding of Vinten et al. (2002), who found that only approximately 30% of immobilized N is incorporated into microbial biomass. It implies that a great portion of fertilizer N was converted into SMNN, including microbial metabolites and residues, especially in the presence of glucose. Although living microbial biomass represents up to 1%–2% of soil organic C, the turnover of this biomass is a rapid and iterative process; thus, the microbial necromass in mineral soils can ultimately contribute up to 50%–80% of the C in stable soluble organic C fractions (Liang and Balser, 2011; Simpson et al., 2007). Rutherford and Juma (1992) also found that most fertilizer N remaining at harvest in a barley–soil system is present as "non-microbial organic N" regardless of the presence of glucose, but the recovery of fertilizer N in this pool is 3.4-fold greater in glucose-treated than in non-glucose-treated soil. Therefore, SMNN is an important soil N pool and mineralizable N source (Paul and Juma, 1981).

Dicyandiamide favored N microbial immobilization regardless of the presence of organic substrate because DCD stimulates soil microbial activity by retaining the mineral N in its cationic form (Ali et al., 2009; Ernfors et al., 2014), which is preferentially immobilized by soil microorganisms (Recous et al., 1990; Winsor, 1958). To retain the fertilizer N as NH_4^+ with DCD input, urea-derived fixed NH_4^+ increased in both +DCD and +DCD + G treatments. Juma and Paul (1983) also reported that the recoveries of

fertilizer N in the fixed NH_4^+ pool in nitrification inhibitor-treated soils are between 5-fold and 8-fold greater than those in non-inhibitor soils. Meanwhile, the urease inhibitor HQ consistently enhanced urea-derived fixed NH_4^+ in all cases. However, urea-derived organic N decreased in both +HQ and +HQ + G treatments, although urea-derived SMBN slightly increased in the +HQ treatment. This result is primarily attributed to the asynchronism between microbial immobilization and urea hydrolysis with the utilization of HQ (Wang et al., 1991), whereas NH_4^+ fixation by soil minerals is highly sensitive to the changes in NH_4^+ concentration in soil solution (Nieder et al., 2011), resulting in the lower urea-derived organic N and higher fixed NH_4^+. Therefore, it can be concluded that both inhibitors increased urea-derived NH_4^+ fixation by soil minerals but exerted different effects on urea-derived N immobilization by soil microorganisms. In specific, DCD increased microbial immobilization, whereas HQ decreased this parameter.

4.2. Availability of urea-derived N

Both soil microbial biomass N and fixed NH_4^+ pools are important temporal N sinks and sources (Nannipieri and Paul, 2009; Nieder et al., 2011). In the absence of glucose, the contribution of fixed NH_4^+ to mineral N was significantly higher than that of SMBN. Liu et al. (2008) also reported that an available N pool can be built with an increasing fixed NH_4^+ pool to improve N use efficiency and decrease N losses into the environment. Inhibitor inputs, particularly DCD, increased the amount of urea-derived fixed NH_4^+ release but retarded fixed NH_4^+ release, thereby meeting the crop demand (Vilsmeier, 1991). Neglecting the effects of fixed NH_4^+ on the conservation and supply of N would overestimate the corresponding effects of microbial mineralization–immobilization turnover (Green et al., 1994).

Compared with fixed NH_4^+, SMBN plays a more important role in mineral N supply in the presence of glucose. This result suggests that the addition of organic substrates enhanced the capacity of soil microorganisms to compete for urea-derived N and magnified the effects of SMBN pool on N conservation and supply (Sugihara et al., 2012; Trehan, 1996). However, N availability probably lowered temporarily because a great portion of immobilized N transformed into SMNN when sufficient C was available, even resulting in yield reduction (Rutherford and Juma, 1992). In addition, the combined application of DCD and glucose further promoted the retention of urea-derived N in organic form and consequently decreased mineral N and N availability (Xu et al., 2000). However, HQ mitigated the decline of N availability by avoiding drastic immobilization with glucose addition (Wang et al., 1991). Therefore, we would pay considerable attention to the incorporation of returning organic C and addition of inhibitors into N management practices. Dicyandiamide should not be simultaneously added with easily degradable organic C to prevent the decrease in N availability, whereas HQ would be an alternative way to alleviate competition for N between crops and microorganisms. Nevertheless, the combined application of DCD and organic C may be an efficient way to increase N retention in soils and diminish N losses, such as in sandy soils (Martin et al., 1997).

Given their high growth and turnover rates, microorganisms can assimilate available N rapidly and convert this material into SMNN, which is also a transformation agent and source of N, especially when organic substrates are added (Paul and Juma, 1981; Vinten et al., 2002). The balance of urea-derived N was analyzed from the 12th day to the end of the incubation period by using the black-box approach to quantify the contribution of SMNN to mineral N (Oenema et al., 2003). The 12th day was selected as the beginning of the analysis because 3 or 6 days were too short to complete urea hydrolysis, especially in the presence of urease inhibitors (Wang et al., 1991), and because the remineralization of immobilized N began on the 12th day (Azam and Mueller, 2005; Rasul et al., 2009). In the absence of glucose, the remineralization rate of urea-derived SMBN and the release rate of fixed NH_4^+ were comparable but were significantly higher than the remineralization rate of SMNN. This result indicates the higher availability of N in living microorganisms than that in SMNN (Liang and Balser, 2011; Mayer et al., 2004). Consequently, the decrease in availability of the urea-derived organic N pool is mainly attributed to the low remineralization rate of SMNN. In the presence of glucose, although the remineralization size of SMNN was greater than that of SMBN, the remineralization rate of SMNN was significantly lower than that of SMBN, and a major portion of fertilizer N was transformed into SMNN, resulting in the further reduction of N availability (Rutherford and Juma, 1992). Therefore, the organic N pool should be split into different sub-pools for at least the SMBN and SMNN to precisely quantify N cycles in soil (Nannipieri and Paul, 2009).

In the incubation experiment, N removal by plant uptake or leaching was not performed to allow the loss of N primarily through NH_3 volatilization and N_2O emission (Xing and Zhu, 2000). The results of ^{15}N recovery indicated that the inhibitors slightly decreased N losses regardless of the presence of glucose. Although nitrification inhibitor was effective in reducing denitrification (Luo et al., 2010), it probably aggravated the NH_3 volatilization loss (Kim et al., 2012; Yu and Fu, 2009). Similarly, urease inhibitor decreased NH_3 volatilization but exerted minimal preventive effects on nitrification or denitrification in a long temporal scale (Gioacchini et al., 2002), especially in the incubation experiments without crop uptake. For organic substrate addition, several studies observed high rates of N losses as NH_3 in soils amended with organic substrates because urease activity is stimulated (Alkanani and Mackenzie, 1992). Emission of N_2O was also enhanced with organic substrate application because a high microbial proliferation results in O_2 deficiency after such an application (Gillam et al., 2008). However, some studies also reported lower (or similar) N losses as NH_3 and N_2O in soils amended with organic substrates than in soils with mineral fertilizers (Ma et al., 2009; Shahandeh et al., 1992). These discrepancies were primarily attributed to the different C/N ratios and C availabilities of organic substrates, and soil types. In general, organic substrates with low C/N ratio, such as compost, slurry and green manure, resulted in higher rate of N loss as N_2O (Dambreville et al., 2006; Thangarajan et al., 2013). Velthof et al. (1996) suggested that organic substrate input results in higher N_2O emission than mineral fertilizers only in soils with limited organic C; by contrast, the opposite was true in soils with sufficient available C. Microbial immobilization and NH_4^+ fixation, which were significantly influenced by available C addition and soil mineral composition, respectively, also exerted great and dominant effects on the reduction of NH_3 volatilization (Cameron et al., 2013; San Francisco et al., 2011). Nevertheless, inhibitor addition efficiently decreased N losses in gas form in the presence of organic substrates (Asing et al., 2008; Zaman et al., 2013). Compared with either inhibitor alone, the combination of urease inhibitor and nitrification inhibitor was more efficient in reducing N losses regardless of the presence of organic substrates (Thangarajan et al., 2013).

Admittedly, the results of laboratory studies cannot provide a complete picture of soil N transformation because laboratory conditions differ substantially from field conditions. However, the result of our complementary pot experiment showed that in the presence of straw ($10\,t\,ha^{-1}$), wheat yield was lowered in the treatment receiving DCD compared with the non-inhibitor treatment, whereas the yield reduction was alleviated with HQ input, implying that N availability was different under various conditions (data unpublished). These findings obtained in the

incubation and pot experiments corroborated each other, indicating the perspective provided by incubation experiment was valuable for quantifying N cycling and assessing N availability in soils, to a large extent.

4.3. Effects of microbial processes on fixed NH_4^+ release

In soils, nitrification and microbial immobilization are responsible for the consumption and removal of NH_4^+-N, consequently promoting fixed NH_4^+ release. However, information about the effects of these microbial processes on fixed NH_4^+ release is inconsistent (Green et al., 1994; Scherer and Werner, 1996). Green et al. (1994) reported that fixed NH_4^+ release is always coupled with nitrification, whereas Scherer and Werner (1996) pointed out that fixed NH_4^+ release is primarily promoted by microbial immobilization. Path analysis indicated that the effects of microbial immobilization and nitrification on fixed NH_4^+ release were comparable in the +U treatment. This result is partially in accordance with the finding obtained by Green et al. (1994), in which fixed NH_4^+ release was primarily driven by nitrification. In their study, however, an anaerobic incubation was carried out in the first 2 weeks of incubation, which seriously impaired N immobilization by soil microorganisms (Vinten et al., 2002) and lowered the effect of microbial immobilization on fixed NH_4^+ release.

Scherer and Werner (1996) observed that microbial immobilization is the principal driving force for fixed NH_4^+ release in the presence of glucose. This observation is corroborated by our result. Furthermore, the microbial requirement for K^+ in proliferation favors fixed NH_4^+ release (Stevenson, 1982). However, the results differed between the present study and the previous studies (Breitenbeck and Paramasivam, 1995; Scherer and Werner, 1996) in some aspects. Previous studies elucidated microbial effects on fixed NH_4^+ release by adding fertilizer N before organic substrate input for several days to complete the mineral fixation of applied-NH_4^+; as a result, microbial immobilization is triggered with glucose input, and fixed NH_4^+ release is subsequently promoted. Moreover, the size of fixed NH_4^+ release increased with the increasing application rates of glucose. In the present study, the combination of fertilizer N and glucose decreased the fixation of urea-derived NH_4^+ by soil minerals at the initial stage; consequently, the declining trends of fixed NH_4^+ in the glucose treatments were more even than those in the non-glucose treatments. Therefore, both the rates and the timing of organic substrate addition influence the partitioning of fertilizer N and the release of retained N in soils.

Inhibitor inputs also significantly influenced the microbial processes that promote the release of fixed NH_4^+. As expected, DCD diminished the effect of nitrification on fixed NH_4^+ release. The combined addition of DCD and glucose further decreased the corresponding effect of nitrification. In contrast to DCD, HQ relatively increased the effect of nitrification on fixed NH_4^+ release (Gioacchini et al., 2002). This phenomenon is mainly attributed to the retardation of urea hydrolysis with HQ addition, thereby mitigating microbial immobilization of fertilizer N (Wang et al., 1991). Despite glucose addition, HQ significantly enhanced the effect of nitrification on fixed NH_4^+ release compared with the corresponding non-inhibitor treatment. It suggested that DCD and HQ exhibit different effects on the microbial processes that prompt fixed NH_4^+ release. Although these processes are fundamentally different, they would not be treated separately because their relationships significantly influence the recently released fixed NH_4^+ partitioning between the organic N pool (immobilization) and the mineral N pool (nitrification). These results are also favorable to modify the N cycling models and to join the initial abiotic processes and the subsequent biotic processes after fertilizer N application.

5. Conclusion

In general, the addition of inhibitors and glucose significantly altered the pathways in N conservation and supply in the test soil. Fixed NH_4^+ and SMBN pools exerted important reservoirs and suppliers for fertilizer N, and the former was more effective in the absence of glucose, but the latter was more efficient in the presence of glucose. This result indicates that fertilizer N has different ways of conservation and supply under different conditions. A major portion of immobilized fertilizer N was transformed into SMNN, which was responsible for the increased retention of fertilizer N in soil and the decreased availability of fertilizer N, especially in the glucose-treated soils. Both inhibitors increased urea-derived fixed NH_4^+ but exhibited different effects on the microbial immobilization of urea-derived N. In particular, DCD increased but HQ decreased the microbial immobilization of urea-derived N. Correspondingly, the combination of DCD and glucose further decreased fertilizer N availability, whereas HQ alleviated the decline of fertilizer N availability induced by glucose addition to some extent. Moreover, microbial effects on fixed NH_4^+ release were significantly influenced by the inhibitors and glucose additions. Glucose and DCD inputs enhanced the effect of microbial immobilization on fixed NH_4^+ release, whereas HQ input relatively increased the corresponding effect of nitrification. These microbial processes markedly affected the partitioning of recently released fixed NH_4^+ between the organic N pool and the mineral N pool. Further studies are also required to determine the rates and timing of organic substrate application in different soils, especially when external organic C is incorporated with different inhibitors. The results of these studies will be helpful in optimizing N management practices and synchronizing N supply with crop demand.

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