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Effect of glucose addition on the fate of urea-^{15}N in fixed ammonium and soil microbial biomass N pools

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

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

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ABSTRACT

This study aims to determine the effect of glucose addition on the fate of urea-^{15}N in fixed NH_4^+ and soil microbial biomass N (SMBN) pools in a 96-day incubation experiment. The contributions of organic N (including SMBN and soil microbial necromass N) and fixed NH_4^+ pools to mineral N were also assessed. Glucose addition significantly improved ^{15}N recovery but decreased the availability of urea-derived N. A great portion of urea-derived N was immobilized and then transformed into microbial necromass. The effect of fixed NH_4^+ on the conservation and supply of urea-derived N were greater than those of organic N pool in the non-glucose treatment, in contrast to that in the glucose treatment. From the sixth day to the end of the incubation, the amount of urea-derived fixed NH_4^+ release was 1.8-fold higher than that of urea-derived organic N mineralization in the treatment without glucose. By contrast, the latter was 3.1-fold higher than the former under glucose addition. Moreover, path coefficient analysis showed that the release of fixed NH_4^+ was promoted by microbial immobilization and nitrification in the absence of glucose, whereas the release was primarily induced by microbial immobilization in the presence of glucose. The partitioning of the released fixed NH_4^+ in the mineral or organic N pools was identified using path coefficients. This study provides very helpful information for quantification of fertilizer N transformation in soil and combines the abiotic and biotic processes in the N cycle.

1. Introduction

After fertilizer application, N can be conserved by biotic and abiotic processes and subsequently mineralized or released to meet the N demand of crops [1,2]. Microbial immobilization and ammonium fixation by clay minerals are the major forms of biotic and abiotic processes, respectively; both processes can minimize N losses to the environment and modulate the temporal pattern of N supply [3,4].

Soil microorganisms drive N transformation while serving as source and sink of N [1,5]. Addition of readily degradable C can stimulate N immobilization by soil microorganisms and probably decrease N availability temporarily, depending on the availability and application rate of external C [6]. Rutherford and Juma [7] found that glucose addition decreased barley biomass by 45.1%. Correspondingly, soil microbial biomass N (SMBN) and total ^{15}N recovery in the barley–soil system increased by 15.3% and 62.0%, respectively. Ammonium can be rapidly fixed by clay minerals, particularly for 2:1 clay minerals; thus, N losses are reduced [2]. Ahmad et al. [8] reported that less than 10% to more than 70% of applied fertilizer N can be fixed in soils from the West Indies, depending on the type of constituent soil minerals. More than 70% of the recently fixed NH_4^+ was released in several weeks after fertilization or in subsequent growing seasons [2,9].

Kaye and Hart [6] inferred that competition for NH_4^+ exists between microbial immobilization and mineral fixation. However, the relationship between microorganisms and fixed NH_4^+ remains unclear, thereby limiting our understanding on the effects of these factors on N conservation and supply under different conditions. In the present study, a laboratory incubation experiment was conducted for 96 days by using ^{15}N-labeled urea, which was the most important N fertilizer form in China [10]. This experiment aimed to (1) elucidate the effect of glucose addition on the fate of urea-derived N in SMBN and fixed NH_4^+ pools, (2) compare the

* Corresponding author.
** Corresponding author.
E-mail addresses: qma@iae.ac.cn (Q. Ma), iae_yfxh@sina.com (W. Yu).

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contributions of both pools to urea-derived mineral N, and (3) clarify the microbial effects on the release of fixed NH_4^+ in treatments with or without glucose.

2. Materials and methods

The experiment was conducted at the Shenyang Experimental Station of the Institute of Applied Ecology [11]. The test soil is Alfisol, which is the main soil type for agricultural production in the study region. Soil samples (0–10 cm) were collected from the treatment that received no fertilizer in the past 20 years (Table S1).

The incubation experiment included three treatments: (1) control without addition (CK), (2) soil + ^{15}N-labeled urea (10.33 atom% ^{15}N) (U), and (3) soil + ^{15}N-labeled urea + glucose (UC) (Fig. S1). The application rates of N and C were 188.9 mg N kg^{-1} soil (200 kg N ha^{-1}, the common application rate for cereal crop production in the study region) and 3228 mg C kg^{-1} soil, respectively. The added nutrients were mixed homogeneously with the soil. The soil was sieved to <5 mm, and the soil moisture was maintained at 50% of the water-holding capacity. Fresh soil samples (equivalent to 150 g oven-dry weight) were placed in polyethylene bottles (500 ml) with screw caps and incubated at 25 °C in the dark after preincubation for 2 weeks. Moisture loss during incubation was monitored by weighing the samples every 3 days and then replenished by adding equivalent amounts of distilled water. Twenty-one bottles were employed for each treatment, and three replications of each treatment were randomly collected after 0.5, 3, 6, 12, 24, 48, and 96 days of incubation.

Levels of soil NH_4^+–N and NO_3^-–N were measured through extraction with 2 M KCl and distillation using MgO–Devarda's alloy [12]. The extracted soil was washed three times with 0.1 M KCl, air dried, and then sieved to <0.15 mm prior to determining fixed NH_4^+ content in accordance with a modified KOBr–HF procedure [13,14]. Briefly, organic N in 2 g of soil was destroyed by hypobromite oxidation. The residual soil was washed three times with 0.5 M KCl by using methods described by Silva and Bremner [13]. The residue was then digested by Kjeldahl procedure, and N content of the residue was regarded as fixed NH_4^+. Chloroform ($CHCl_3$) fumigation–extraction method was employed to estimate the amount of SMBN by using the equation: SMBN = E_N/K_{EN}, where E_N is the N extracted by 0.5 M K_2SO_4 from fumigated soil minus that extracted from non-fumigated soil, and K_{EN} is the conversion factor for SMBN (0.54) [15]. The residual soils were air dried and passed through a 0.15 mm sieve for the determination of total soil N by using a Vario EL III elemental analyzer (Elementary Corp. Germany). Soil microbial necromass N (SMNN) was calculated by subtracting the sum of urea-derived SMBN, NH_4^+–N, NO_3^-–N, and fixed NH_4^+ from the total urea-derived N in the soil. Urea-derived NH_4^+ fixed by organic matter was not separated from SMNN because the former was negligible in amount in similar soils [16]. The ^{15}N samples were prepared using a method proposed by Shen et al. [14], and $^{15}N/^{14}N$ ratio was measured by a stable isotope-ratio mass spectrometer (Delta plus XP).

The mass of urea-derived N in different N pools presented in Fig. 1 was calculated according to the Equation (1), and the ^{15}N atom % excess was corrected for the corresponding background abundance.

$$M_{AB} = \frac{P_B \cdot I_B}{I_A} \quad (1)$$

where M_{AB} is the urea-derived N in a given N pool (mg kg^{-1}); I_A is the ^{15}N atom % excess of urea, P_B is the N mass of the given N pool (mg kg^{-1}); and I_B is the ^{15}N atom% excess of the given N pool.

$$^{15}\text{N recovery} = 100 \cdot \frac{P_B \cdot I_B}{P_A \cdot I_A} \quad (2)$$

where ^{15}N recovery presented in Table S2 is the ^{15}N recovered in a given N pool (%); P_A is the application rate of urea-derived N [17].

Path coefficient analysis was adopted to analyze the relationships among urea-derived SMBN, NH_4^+–N, NO_3^-–N, and fixed NH_4^+. This method can partition simple correlation coefficients between independent and dependent variables into direct and indirect effects, which indicate the relative strength of the causal relationship [18]. In the present study, the dependent variable was urea-derived NO_3^-–N. The independent variables were urea-derived SMBN, NH_4^+–N, and fixed NH_4^+, which represent the effects of microbial immobilization–mineralization, nitrification, and fixed NH_4^+ release on urea-derived NO_3^-–N, respectively. Moreover, the result of the analysis can provide insights into predicting the partitioning of released fixed NH_4^+ between organic N (immobilization) and mineral N (nitrification) pools.

Significant differences among treatment means were determined using Duncan test at 0.05 probability. Statistical analyses were performed using the SPSS 11.0 package (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Addition of N and glucose significantly influenced urea-derived N partitioning among the four N pools (Fig. 1). The peaks of urea-derived SMBN were 16.9 and 54.7 mg kg^{-1} in the U and UC treatments, respectively (Fig. 1a), whereas the peaks of urea-derived fixed NH_4^+ were 91.0 and 56.1 mg kg^{-1} in the same treatments, respectively (Fig. 1b). In this study, fixed NH_4^+ exhibited a greater effect on the conservation of urea-derived N than that of SMBN in the absence of glucose. However, the effect of fixed NH_4^+ on the N cycle has not received significant research attention because of rapid fixation/defixation of NH_4^+ following fertilization [2]. Furthermore, most studies did not perform with sufficient sampling frequencies to detect the change in fixed NH_4^+ pool [19].

Urea-derived inorganic N (NH_4^+–N plus NO_3^-–N plus fixed NH_4^+) decreased by an average of 46.0% in the UC treatment compared with that in the U treatment (Fig. 1b–d). By contrast, urea-derived SMBN in the UC treatment was 3.5-fold higher than that in the U treatment (Fig. 1a). Microbial immobilization can prevent mineral N accumulation in soils, especially in the presence of available C [1]. In the present study, the amount of fixed NH_4^+ also decreased after glucose addition, indicating that the competition for NH_4^+ intensified between microbial immobilization and mineral fixation [6]. However, the addition of an organic substrate with a wide C/N ratio does not necessarily decrease the content of fixed NH_4^+ [20,21]. The discrepancies in the results among the studies can be attributed to different qualities, application rates and timing of organic substrates, in addition to various soil types and different levels of soil indigenous N [22–24]. Although organic substrates added as energy source generally enhance microbial proliferation, the extent of increase in SMBN is markedly influenced by the quality of substrates, which vary the N partitioning among different N pools [25,26]. The competitive ability of microorganisms to immobilize applied-NH_4^+ significantly reduced after cereal straw addition compared with that after glucose application [27]. The application rate of organic C can also affect the release of fixed NH_4^+, and a threshold possibly exists as suggested by Scherer and Werner [9]. Several researchers also applied fertilizer N and organic substrates at different time points to avoid competition for N [4,28]. Therefore, competition for NH_4^+ between microbial immobilization and mineral fixation is dependent on the availability, application rate, and