Abstract

Excessive amounts of nitrate have accumulated in many soils on the North China Plain due to the large amounts of chemical N fertilizer used combined with low carbon inputs. The present study investigated the promotion of soil nitrate transformation to soil organic N by different carbon amendments. A laboratory incubation experiment using ^{15}N tracer ($K^{15}NO_3$) was employed to elucidate the proportion of soil organic N derived from accumulated soil nitrate following amendment with glucose (G) or maize straw (S) at controlled soil temperature and moisture content. During the 56-d incubation period we determined the dynamics and isotopic abundance of mineral N (NO_3^- and NH_4^+) and soil organic N and greenhouse gas (N_2O and CO_2) emissions. Carbon amendment markedly stimulated transformation of nitrate to soil organic N. Mineralization of native soil organic N was also enhanced because soil organic N at the end of the incubation period was not significantly different from that in the control without added C. Glucose had a greater effect on nitrate-N immobilization (25.0 mg kg^{-1}) than maize straw (9.4 mg kg^{-1}) and glucose addition also led to highest greenhouse gas emissions. With glucose and straw amendment total N_2O-N emissions were 1.01 and 0.03 mg kg^{-1}, respectively. Similarly, glucose amendment lowered the concentration and abundance of NO_3^--N and increased that of NH_4^+-N more than maize straw. In agricultural practice straw amendment may be an effective strategy to deplete the excess NO_3^--N pool and restrict leaching, to promote nitrate-N immobilization and to decrease greenhouse gas emissions.

Key words: C source availability, greenhouse gases, soil organic N, soil surplus nitrate

1. Introduction

Large amounts of nitrate (NO_3^-) accumulated in soil profile due to excessive mineral fertilizer or manure application in intensively managed cropping system on the North China Plain (NCP) (Ju et al., 2007). This NO_3^--N has a high potential to leach out of the root zone or to denitrify and thus to contribute to the eutrophication of aquatic ecosystems or the emission of greenhouse gases (N_2O). The immobilization of nitrate in the soil is therefore a major challenge to decrease its potentially negative environmental effects (Zogg et al, 2000; Burger and Jackson, 2003). For the formation of soil organic N in arable soils with low ammonium (NH_4^+) concentration, NH_4^+ immobilization is preferred over NO_3^- by soil heterotrophic microorganisms (Burger and Jackson, 2003). Thus, NO_3^- immobilization may be controlled by NH_4^+ concentrations (Myrold and Posavatz, 2007).

Carbon (C) amendments are known to increase nitrate immobilization (Burger and Jackson, 2003) by stimulating microbial growth. Dead microbial cells with previously assimilated NO_3^- can enter the stable soil organic N pool. In addition, added C substrate usually acts as an electron donors for denitrification (Matheson et al, 2002). During denitrification N_2O can be generated by heterotrophic microorganisms and at the same time substantial amounts of CO_2 may be emitted through microbial respiration. The availability of C sources may thus play an important role in NO_3^- immobilization and denitrification (Myrold and Posavatz, 2007), readily available C sources can promote NO_3^- immobilization and stimulate more N_2O and CO_2 generation via denitrification pathway.

Low soil organic C content is the main factor limiting nitrate immobilization in soils on the North China Plain (Wan et al, 2009; Murray et al, 2004) and N_2O is largely derived

from nitrification of ammonium fertilizer (Wan et al, 2009). This provides the theoretical basis for nitrate immobilization by C substrate amendment to decrease pollution from nitrate leaching and N_2O emission. In this context the objective of the present study was to elucidate the degree of immobilization of excessive accumulated nitrate-N into the stable soil organic N pool and loss via denitrification with different C availability amendments using ^{15}N isotopic signature.

2. Materials and methods

2.1 Soil

On 4 August 2007 surface soil (0-20 cm) was collected from a field at Dongbeiwang Agricultural Experimental Station (40.08°N, 116.28°E, 40 m above sea level) near Beijing. The cropping system is winter wheat-summer maize rotation. Freshly collected soil was sieved (< 2mm) to remove stones, roots and crop residues prior to the onset of the experiment. The soil contained 27 % sand, 57 % silt and 61 % clay, is of typical calcareous alluvial (Fluvaquents) texture. It has 1.24 % soil organic carbon, 1.12 % total N, pH 8.0, a bulk density of 1.34 g cm^{-3}, 0.14 mg kg^{-1} NH_4^+-N and 9.86 mg $kg^{-1}NO_3^-$-N.

2.2 Experimental design

Sieved soil was uniformly sprayed with $K^{15}NO_3$ solution at 60.25 % ^{15}N enrichment with the volume of $K^{15}NO_3$ solution equivalent to an amendment of 100 mg N kg^{-1} oven-dried soil and the soil water content was adjusted to 45 % water filled pore space (WFPS), the soil was pre-incubated aerobically at 18 ºC for 2 weeks in the darkness so that the exchange between

NO_3^--N amendment and native soil N become balance. The treated steps of control soil were the same above except for deionized water instead of $K^{15}NO_3$ solution.

The formal incubation experiment was carried out in 1-L glass jars containing 300g 50% WFPS pre-incubated soil at 18 ºC for 56 d in the dark. The experiment comprised four treatments (Table 1): (1) control (no fertilizer, CK); (2) $K^{15}NO_3$ (no carbon amendment); (3) glucose (G) plus $K^{15}NO_3$; and (4) maize straw (S) plus $K^{15}NO_3$. There were three replicates of each treatment at each of 8 sampling dates, yielding a total of 96 jars. The samples were taken after 0, 1, 3, 7, 14, 21, 28, 42 and 56 d. During incubation the lids of the jars were closed to create gas-tight conditions and were opened for 10 min each day to maintain aerobic conditions. Before the incubation the glucose was added as a solution and all treatments were adjusted to 50% WFPS with deionized water, the soil bulk density was adjusted to 1.3 g cm^{-3}, the finely grounded maize straw had 47.35 % organic C and 1.00% total N. At 0 d, the content and abundance of mineral N in the $K^{15}NO_3$ treated soil were: NO_3^--N: 109.2 mg kg^{-1} and 51.14 %; NH_4^+-N: 0.03 mg kg^{-1} and 0.70 %; and those in the control soil were: NO_3^--N: 12.27 mg kg^{-1} and 1.41 %; NH_4^+-N: 0.36 mg kg^{-1} and 0.37 %.

2.3 Sampling and analysis

Fresh soil was destructively taken on each sampling date. The content and ^{15}N abundance of NO_3^--N and NH_4^+-N was determined using the SPINMAS technique introduced by Stange et al. (2007). The soil slurry after mineral N extracted was washed once again with 1 M KCl at a soil:water ratio of 1:2.5 (W/V), then centrifugation and washing twice with deionized water at the same soil:water ratio as above. The washed residue was dried at 60 ºC and finely ground for total N and ^{15}N analysis by Kjeldahl digestion and mass spectrometry (DELTA

Plus XP, Thermo Finnigan, Germany), respectively. Total N of the washed residue soil was regarded as soil organic N. N_2O and CO_2 were measured with a gas chromatograph (6890N, Agilent Technologies, USA) similarly as Wan et al (2009).

2.4. Statistical analysis

Data were adjusted to oven-dried soil weight. One-way analysis of variance was conducted with SPSS version 11.0 and means compared using least significant difference (LSD) at the 5 % level. In the text data are reported as mean ± one standard error of the mean (SEM).

3 Results

3.1 Soil organic nitrogen (SON)

Glucose amendment had a greater effect than maize straw in increasing soil organic N concentration. The presence of $K^{15}NO_3$ with no added C source resulted in the mineralization of native soil organic N (Figure 1a). At the end of the incubation (56 d), soil organic N did not differ significantly among the four treatments. The ^{15}N abundance indicated that the accumulated NO_3^--N ($K^{15}NO_3$) was mainly transformed to soil organic N after C source amendment (Figure 1 b) and simultaneously some of the native soil organic N was replaced by the accumulated NO_3^--N which would benefit crop N uptake.

Figure 1 a, b to be placed here

3.2 Mineral nitrogen (N_{min})

C substrate amendment led to a clear decrease in the concentration of NO_3^--N (Figure 2a) and the dynamics of abundance in NO_3^--N showed a consistent trend (Figure 2b) which is in

agreement with previous reports in the literature (Wan et al., 2009). The concentration of NO_3^--N in the glucose + $K^{15}NO_3$ treatment decreased predominantly during the first day of incubation (from 109.2 in 0d to 51.7 mg N kg^{-1} in 1st d) while that in the straw + $K^{15}NO_3$ treatment decreased more gradually during the incubation period as a result of slower decomposition of maize straw by the microbial biomass. Conversely, the ready availability of glucose contributed to a higher gross N flux rate in the soil and resulted in a higher ^{15}N abundance in the glucose + $K^{15}NO_3$ treatment during the first two weeks compared to the straw + $K^{15}NO_3$ treatment (Figure 2b). In the $K^{15}NO_3$ treatment ^{15}N abundance gradually decreased with incubation time and this is attributable mainly to the N dilution effect derived from the mineralization of soil organic N on account of $K^{15}NO_3$ amendment.

The increase in the ^{15}N abundance of NH_4^+-N significantly depended on the C source (Figure 2d). The dynamics of ^{15}N abundance in the glucose and straw treatments with $K^{15}NO_3$ was determined by the gross N flux rate derived from the difference in the availability of the C source. In particular, the ^{15}N abundance in the glucose + $K^{15}NO_3$ treatment reached 36.1 % on the first day. The DNRA pathway would have played an important role in NO_3^--N transformation to NH_4^+-N with the rapid assimilation of glucose by the microbial biomass. The concentration of NH_4^+-N remained low after the third day (Figure 2) and much of the NH_4^+ may have been assimilated or nitrified rapidly by microorganisms in this nitrification-dominant soil from the North China Plain, or fixed by soil clay minerals (Wan et al.,2009).

Figure 2 a, b, c, d to be placed here

3.3 N_2O and CO_2

Dinitrous oxide is the byproduct from the nitrification-denitrification process. Emissions of CO_2 are the results of microbial respiration and thus denote microbial activity. Glucose amendment markedly increased N_2O and CO_2 emissions in the soil with excessive accumulation of NO_3^--N (Figure 3). During the first day of incubation the emission of N_2O in the glucose + $K^{15}NO_3$ treatment reached 647 μg N kg^{-1} soil d^{-1} and CO_2 reached 116 mg C kg^{-1} soil d^{-1}. Due to the relatively low C availability of maize straw the peak of N_2O and CO_2 in the straw + $K^{15}NO_3$ treatment was much lower and showed a clear lag phase. Emissions of N_2O were negative after 28 d of incubation, but CO_2 emission was still significantly different between C amendment and no substrate addition on day 56

Figure 3 a, b to be placed here

3.4 Residue and loss of $^{15}NO_3^-$-N after C amended at the end of incubation

After 56 days of incubation the residual organic N and the loss of $^{15}NO_3^-$-N followed the sequence glucose + $K^{15}NO_3$ > straw + $K^{15}NO_3$ > $K^{15}NO_3$ (Table 2) and the differences between the treatments were significant. The addition of C substrate can effectively decrease NO_3^--N accumulation. A higher decomposition rate of C substrate (glucose) can lead to a maximum transformation rate of NO_3^--N to soil organic N at the price of maximum environmental pollution (N_2O and CO_2 emissions). In contrast, straw which contains lignin and cellulose with low C availability to microorganisms can increase soil organic N and also lower the risk of greenhouse gas emissions. For example, total N_2O emissions in the straw + $K^{15}NO_3$ treatment did not differ substantially from the $K^{15}NO_3$ treatment. In our study the loss of NO_3^--N ($K^{15}NO_3$) without any C amendment occurred mainly during the pre-incubation phase (NO_3^--N: 90.12 mg kg^{-1} at 0 d vs. 86.56 mg kg^{-1} after 56 d). Straw

amendment is therefore the better choice for minimizing environmental pollution and transforming nitrate to soil organic N.

Table 2 to be placed here

4. Conclusions

In the intensively managed agricultural soils of the North China Plain addition of C substrates can effectively promote the transformation of accumulated excessive soil NO_3^--N to soil organic N. With increasing availability of C substrate, the amount of soil organic N formed from the immobilization of accumulated soil nitrate will not only increase but also greatly stimulate the mineralization of soil native organic N and the emission of greenhouse gases. However, a slowly decomposing C source (straw) amendment can increase the amount of soil organic N with a minimum of greenhouse gases emissions, thus providing theoretical support for the return of straw biomass in these nitrification-dominant soils.

Acknowledgments

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Table 1. Amount of added C and N in the treatments of the incubation experiment (G: glucose; S: maize straw)

Treatment	Carbon (mg C kg^{-1})	NO_3^--N (mg N kg^{-1})
Control	0	0
$K^{15}NO_3$	0	100
G + $K^{15}NO_3$	1000	100
S + $K^{15}NO_3$	1000	100

Table 2. Fate of $^{15}NO_3^-$-N (mg kg^{-1}) with added different available C source at the end of incubation (G: glucose; S: maize straw).

Treatment	Residual organic N	Residual NO_3^--N	Residual total N*	Total emitted N_2O-N	Total loss of N
$K^{15}NO_3$	0.86 (0.01)c	86.56(0.57)a	87.42(0.57)a	0.02(0.0004)b	12.58(0.57)c
G + $K^{15}NO_3$	25.04 (0.95)a	36.86(0.36)c	61.90(1.29)c	1.01 (0.12)a	38.10(1.29)a
S + $K^{15}NO_3$	9.38 (0.60)b	61.30(2.50)b	70.69(2.78)b	0.03(0.0007)b	29.31(2.78)b

*Residual total N is the sum of residual organic N, residual NO_3^--N and residual NH_4^+-N. Brackets: standard errors of means of three replicates.

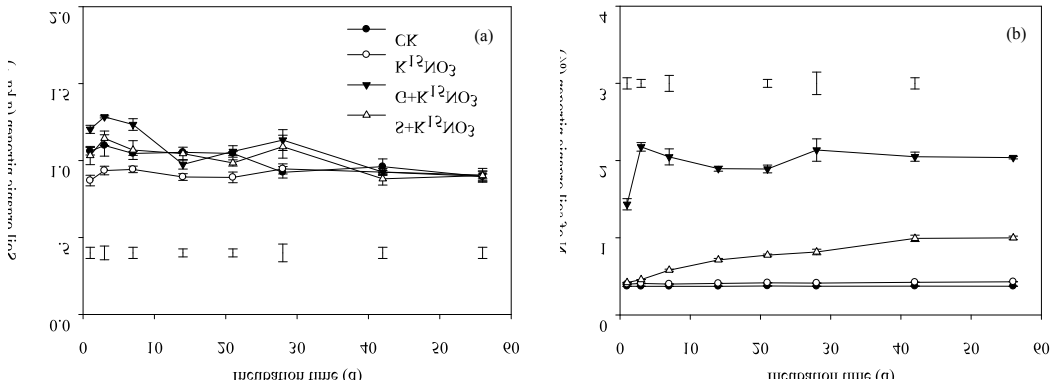

Figure 1. Mean soil organic nitrogen (SON) and its abundance in calcareous agricultural soil during a 56 d incubation experiment. Data show means and ± one standard error. $LSD_{0.05}$ values are denoted by vertical lines.

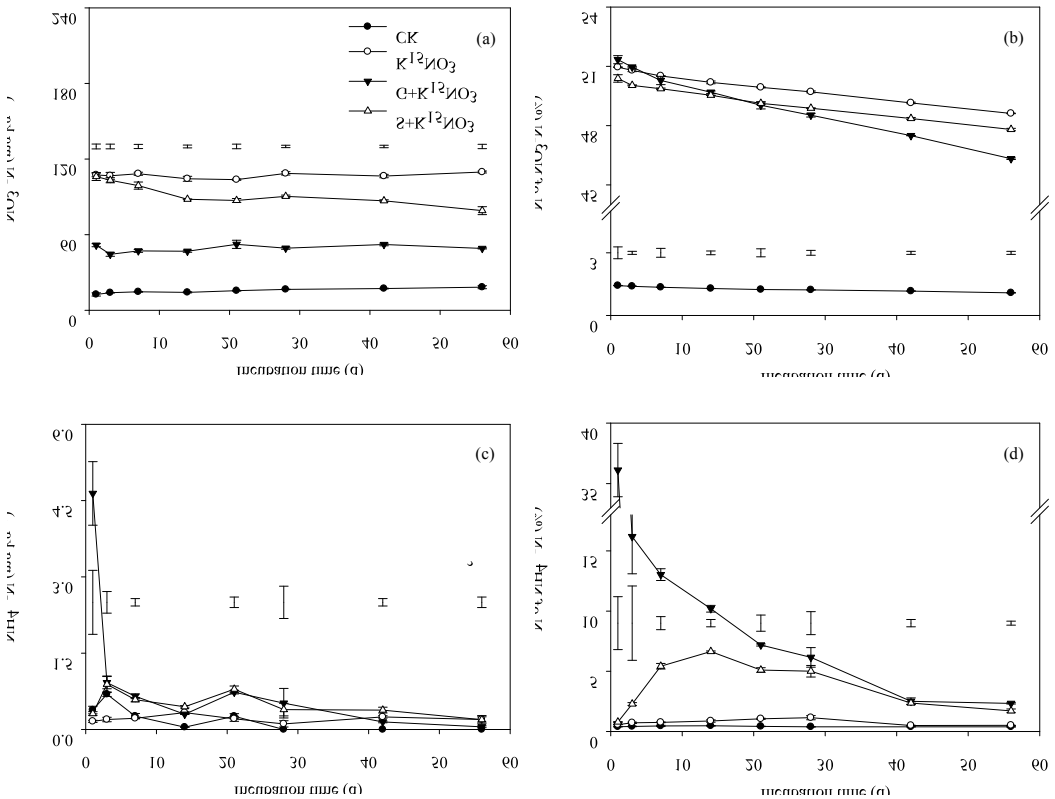

Figure 2. Mean mineral nitrogen (NO_3^--N and NH_4^+-N) and their abundance in calcareous agricultural soil during a 56 d incubation experiment. Data show means and ± one standard error. $LSD_{0.05}$ values are denoted by vertical lines.

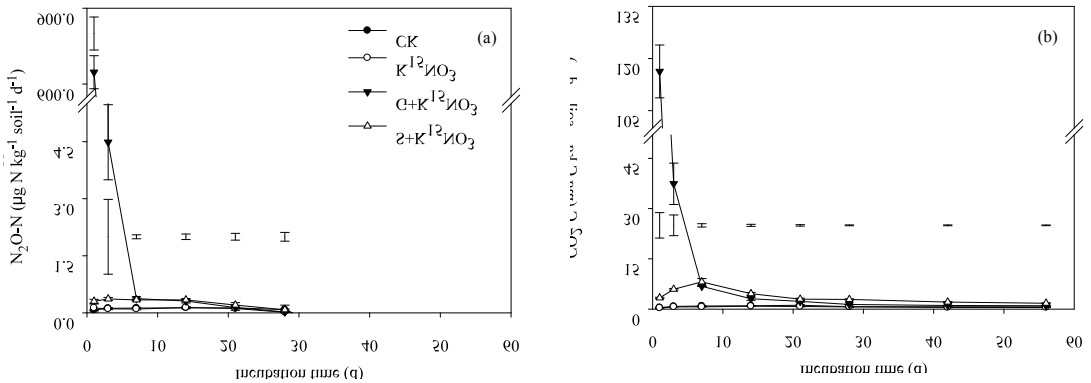

Figure 3. Daily fluxes of greenhouse gases from calcareous agricultural soil during a 56 day incubation experiment. Data show means and ± one standard error. $LSD_{0.05}$ values are denoted by vertical lines. The values were calculated according to the equation PV=nRT, the concentrations of N_2O and CO_2 in the atmosphere based on the IPCC (2007) report.