

## Role of Carbon Substrates Added in the Transformation of Surplus Nitrate to Organic Nitrogen in a Calcareous Soil<sup>\*1</sup>

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### ABSTRACT

Excessive amounts of nitrate have accumulated in many soils on the North China Plain due to the large amounts of chemical N fertilizers or manures used in combination with low carbon inputs. We investigated the potential of different carbon substrates added to transform soil nitrate into soil organic N (SON). A 56-d laboratory incubation experiment using the <sup>15</sup>N tracer (K<sup>15</sup>NO<sub>3</sub>) technique was carried out to elucidate the proportion of SON derived from accumulated soil nitrate following amendment with glucose or maize straw at controlled soil temperature and moisture. The dynamics and isotopic abundance of mineral N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) and SON and greenhouse gas (N<sub>2</sub>O and CO<sub>2</sub>) emissions during the incubation were investigated. Although carbon amendments markedly stimulated transformation of nitrate to newly formed SON, this was only a substitution effect of the newly formed SON with native SON because SON at the end of the incubation period was not significantly different ( $P > 0.05$ ) from that in control soil without added C. At the end of the incubation period, amendment with glucose, a readily available C source, increased nitrate immobilization by 2.65 times and total N<sub>2</sub>O-N emission by 33.7 times, as compared with maize straw amendment. Moreover, the differences in SON and total N<sub>2</sub>O-N emission between the treatments with glucose and maize straw were significant ( $P < 0.05$ ). However, the total N<sub>2</sub>O-N emission in the straw treatment was not significantly ( $P > 0.05$ ) greater than that in the control. Straw amendment may be a potential option in agricultural practice for transformation of nitrate N to SON and minimization of N<sub>2</sub>O emitted as well as restriction of NO<sub>3</sub>-N leaching.

**Key Words:** available C source, carbon amendments, greenhouse gases, N immobilization, <sup>15</sup>N tracer

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### INTRODUCTION

It is well documented that the potential risk of nitrogen eutrophication of aquatic ecosystems is due mainly to nitrate (NO<sub>3</sub><sup>-</sup>) accumulation in agricultural soils after excessive nitrogen fertilizer application (Ju *et al.*, 2009). The denitrification of nitrate N (NO<sub>3</sub>-N) is an efficient pathway to decrease excessive NO<sub>3</sub>-N accumulation in soils. However, N<sub>2</sub>O is an important greenhouse gas that can be generated by heterotrophic microorganisms through denitrification and substantial amounts of CO<sub>2</sub> may be emitted through

microbial respiration (Murray *et al.*, 2004; Wan *et al.*, 2009). Therefore, the immobilization of excessive NO<sub>3</sub>-N into soil organic nitrogen (SON) pools by microbial assimilation contributes to soil fertility and also ameliorates the negative environmental effect of soil NO<sub>3</sub>-N (Zogg *et al.*, 2000).

Immobilization of NO<sub>3</sub><sup>-</sup> may be controlled by the ammonium (NH<sub>4</sub><sup>+</sup>) concentration in soil and by carbon (C) amendments. High NH<sub>4</sub><sup>+</sup> concentrations tend to inhibit NO<sub>3</sub>-N immobilization (Myrold and Posa-vatz, 2007) because NH<sub>4</sub><sup>+</sup> has been reported to be the preferential N form assimilated by heterotrophic soil

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microorganisms (Recous *et al.*, 1990). C sources can increase  $\text{NO}_3^-$  immobilization (Burger and Jackson, 2003) by stimulating microbial growth and the subsequently immobilized  $\text{NO}_3\text{-N}$  in live or dead microbial cells can enter the stable SON pool. About 7% of applied  $\text{NO}_3\text{-N}$  entered the soil organic matter after four months because substantial amounts of easily decomposable C sources were present in a forest soil (Zogg *et al.*, 2000). Amendment with C substrates usually also provides electron donors for denitrification (Šimek and Kalčík, 1998; Matheson *et al.*, 2002; Kim *et al.*, 2008).  $\text{NO}_3\text{-N}$  immobilization can be promoted by increasing availability of C sources (Myrold and Posa-vatz, 2007). The corresponding generation of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  *via* denitrification also increases as shown by de Catanzaro and Beauchamp (1985) in a comparison between lignin and glucose amendments. Hence, in order to promote the immobilization of accumulated  $\text{NO}_3\text{-N}$  at the lowest environmental cost, it is necessary to understand N fluxes in soil with excessive  $\text{NO}_3\text{-N}$  accumulation following amendment with different available C sources.

Large amounts of nitrate ( $\text{NO}_3^-$ ) have accumulated in the soil profiles on the North China Plain (NCP) due to excessive application of mineral fertilizers or manures in the intensively managed agricultural systems (Ju *et al.*, 2006; Wan *et al.*, 2009). For example, the amount of  $\text{NO}_3\text{-N}$  in the top 90 cm of the soil profile reached  $700 \text{ kg N ha}^{-1}$  after four years (*i.e.*, eight seasons) of successive crops under conventional N fertilizer applications ( $600 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) in a field experiment (Zhao *et al.*, 2006). Agricultural soils on the NCP have typical properties of low soil organic C content, low  $\text{NH}_4^+$  concentration (around  $1 \text{ mg kg}^{-1}$ ), high pH (around 8), and high nitrification potential (Wan *et al.*, 2009). Low soil organic C content is the main factor limiting nitrate immobilization (Murray *et al.*, 2004; Wan *et al.*, 2009) and  $\text{N}_2\text{O}$  is derived largely from the nitrification of ammonium fertilizers (Wan *et al.*, 2009; Ju *et al.*, 2011). According to Burger and Jackson (2003), when the concentration of  $\text{NH}_4^+$  was low in an arable soil in the US, immobilization of  $\text{NO}_3^-$  was much greater than that of  $\text{NH}_4^+$  due to strong competition for  $\text{NH}_4^+$  by nitrifiers. Therefore, copious  $\text{NO}_3\text{-N}$  immobilization (Burger and Jackson, 2003) and the high nitrification potential of the soils on the NCP (Wan *et al.*, 2009; Ju *et al.*, 2011) provide a theoretical hypothesis for accumulated  $\text{NO}_3^-$  immobilization by C substrate amendment to control pollution from nitrate leaching and  $\text{N}_2\text{O}$  emission.

The objective of the present study was to elucidate the immobilization of accumulated  $\text{NO}_3\text{-N}$  into stable

SON pools and  $\text{N}_2\text{O}$  emission after amendment with organic materials with contrasting C availabilities. A  $^{15}\text{N}$  isotope tracer technique was employed to study N transformations under conditions of controlled soil temperature and moisture.

## MATERIALS AND METHODS

### Soil

On August 4, 2007, surface (0–20 cm) soil samples were collected from a field at the Dongbeiwang Agricultural Experimental Station ( $40.08^\circ \text{ N}$ ,  $116.28^\circ \text{ E}$ , 40 m above sea level) near Beijing, where optimum N fertilizer application had been practiced based on the mineral nitrogen test (Zhao *et al.*, 2006). The cropping system at this site was a winter wheat-summer maize rotation and urea fertilizer was applied at  $130 \text{ kg N ha}^{-1}$  for each winter wheat season and  $97 \text{ kg N ha}^{-1}$  for each summer maize season over the previous 8 years. Full details of the experimental site were provided by Zhao *et al.* (2006). Freshly collected soil samples were sieved ( $< 2 \text{ mm}$ ) to remove stones, roots, and crop residues prior to the onset of the experiment. The soil is a calcareous soil classified as a Fluvaquent (USDA Soil Taxonomy), which contains 27% sand, 57% silt and 16% clay and has  $12.4 \text{ g kg}^{-1}$  soil organic carbon,  $1.2 \text{ g kg}^{-1}$  total N, a pH of 8.0, a bulk density of  $1.34 \text{ g cm}^{-3}$ ,  $0.14 \text{ mg kg}^{-1} \text{ NH}_4\text{-N}$ , and  $9.86 \text{ mg kg}^{-1} \text{ NO}_3\text{-N}$ .

### Experimental design

The sieved soil samples were divided into two groups. One was uniformly sprayed with  $\text{K}^{15}\text{NO}_3$  solution at 60.25%  $^{15}\text{N}$  enrichment with a volume of  $\text{K}^{15}\text{NO}_3$  solution equivalent to an amendment of  $100 \text{ mg N kg}^{-1}$  oven-dried soil. The soil water content was then adjusted to 45% water-filled pore space (WFPS). The other was sprayed with deionized water to 45% WFPS as a control. Both groups were pre-incubated aerobically at  $18^\circ \text{ C}$  for 2 weeks in the dark so that exchange between the  $\text{NO}_3\text{-N}$  amendment and native soil N could take place.

A 56-d incubation experiment at  $18^\circ \text{ C}$  in the dark was carried out using 1-L glass jars containing  $300 \text{ g}$  pre-incubated soil. Before the incubation, glucose and straw were added uniformly to the pre-incubated  $\text{K}^{15}\text{NO}_3$ -labelled soil samples. The glucose was added in aqueous solution and the maize straw had an organic C content of  $474 \text{ g kg}^{-1}$  and a total N content of  $10 \text{ g kg}^{-1}$  and was finely ground with a ball mill (Retsch Model MM200, Germany). All treatments were adjusted to 50% WFPS with deionized water and the soil bulk density was adjusted to  $1.3 \text{ g cm}^{-3}$ . The experi-

ment comprised four treatments (Table I): (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 9 sampling dates, giving a total of 96 jars. The samples were taken after 0 (the end of pre-incubation), 1, 3, 7, 14, 21, 28, 42, and 56 d of incubation. During the 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. The concentration and  $^{15}N$  abundance of SON and mineral N in the  $K^{15}NO_3$ -treated soil and control soil at the end of pre-incubation (0 d of incubation) are shown in Table II.

TABLE I

Amounts of added C and N in the treatments of the 56-day incubation experiment with a calcareous soil from the North China Plain

Treatment <sup>a)</sup>	Carbon mg C kg <sup>-1</sup>	NO <sub>3</sub> -N mg N kg <sup>-1</sup>
Control	0	0
$K^{15}NO_3$	0	100
G + $K^{15}NO_3$	1 000	100
S + $K^{15}NO_3$	1 000	100

<sup>a)</sup>G = glucose; S= maize straw.

TABLE II

Concentrations and abundance of soil organic N (SON) and mineral N in the  $K^{15}NO_3$ -treated and the control calcareous soil from the North China Plain at the end of pre-incubation

N pool	$K^{15}NO_3$ -treated soil		Control soil	
	Concentration	Abundance	Concentration	Abundance
	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%
SON	868.3±7.5 <sup>a)</sup>	0.40±0.00	1 053.8±43.7	0.37±0.00
NO <sub>3</sub> -N	109.2±0.5	51.10±0.01	12.3±0.5	1.41±0.01
NH <sub>4</sub> -N	0.03±0.00	0.70±0.05	0.36±0.00	0.37±0.02

<sup>a)</sup>Mean ± standard error of three replicates.

### Sampling and analysis

Fresh soil samples were collected at each sampling date. NO<sub>3</sub>-N and NH<sub>4</sub>-N were extracted with 1 mol L<sup>-1</sup> KCl using a soil:water ratio of 1:5 (W/V). The  $^{15}N$  abundance and concentration of NO<sub>3</sub>-N and NH<sub>4</sub>-N were determined using the SPINMAS technique introduced by Stange *et al.* (2007) and Wan *et al.* (2009) with a quadruple mass spectrometer (QMS) after the reduction of NO<sub>3</sub><sup>-</sup> to NO with VCl<sub>3</sub> and the oxidation of NH<sub>4</sub><sup>+</sup> to N<sub>2</sub> with NaBrO, respectively. After extraction of mineral N, the soil slurry was washed once again with 1 mol L<sup>-1</sup> KCl at a soil:water ratio of 1:2.5 (W/V), then centrifuged and washed twice

with deionized water at the same soil:water ratio as above. The washed residue, which was regarded as the SON form, was dried at 60 °C and finely ground for total N and  $^{15}N$  analysis by Kjeldahl digestion and mass spectrometry (DELTA Plus XP, Thermo Finnigan, Germany), respectively.

An airtight lid was used to seal each incubation jar on the day before sampling for 24 h of incubation and 60-mL gas samples were taken using a gas-tight syringe connected to a two-way valve. N<sub>2</sub>O and CO<sub>2</sub> were analyzed with a gas chromatograph (6890N, Agilent Technologies, USA) equipped with a  $^{63}Ni$  electron capture detector (ECD) and a hydrogen flame ionization detector (FID). CO<sub>2</sub> was reduced by H<sub>2</sub> to CH<sub>4</sub> in a nickel catalytic converter at 375 °C and then detected by FID.

Daily fluxes of N<sub>2</sub>O-N and CO<sub>2</sub>-C were calculated using the equation  $PV = nRT$ , where  $P$  is the standard atmospheric pressure, 101.3 Pa,  $V$  is the residue volume with subtraction of incubated soil in 1 L incubation bottle, 0.81 L,  $n$  is the amount of substance,  $R$  is a constant, 8.314, and  $T$  is the absolute temperature, 291 K in our study, with the concentrations of N<sub>2</sub>O and CO<sub>2</sub> in the atmosphere based on the report of the International Panel on Climate Change (IPCC) (2007).

### Statistical analysis

Data were expressed on an oven-dry basis. One-way analysis of variance was conducted with the SPSS version 11.0 software package and the mean values were compared using a least significant difference (LSD) test at the 5% level. Data are reported as mean value ± one standard error of the mean (SEM).

## RESULTS AND DISCUSSION

### Soil organic nitrogen (SON)

During the first 28 d of incubation, the glucose amendment had a greater effect than the maize straw amendment in increasing SON concentration (Fig. 1a), which was possibly because of the stimulation of growth of soil microorganisms by the readily available C source (glucose). After 42 d of incubation, SON did not differ significantly ( $P > 0.05$ ) among the four treatments (Fig. 1a), indicating a trade-off between the mineralization of native SON and the newly formed SON in the C-amended treatments. The increase in  $^{15}N$  abundance in the C-amended treatments indicates that the labeled NO<sub>3</sub>-N ( $K^{15}NO_3$ ) can transform to SON (Fig. 1b) although the transformation in the

maize straw treatment was slow compared with that of the glucose treatment. The SON abundance in the glucose treatment (Fig. 1b) remained constant from the third day to the end of incubation (56th day) while the SON abundance in the straw treatment increased gradually during incubation. This phenomenon may be dependent on the contribution of different microbial populations induced by C sources with different availabilities. According to Blagodatsky and Richter (1998), soil microbial biomass comprises active and dormant parts, with the active part acting only as the “intermediate” which affects C and N immobilization or mineralization by the dormant part. The growth of active soil microorganisms can be rapidly stimulated by readily available C sources (*e.g.*, glucose) and subsequently the N assimilated by the active microorganisms is transformed to the dormant part. In our study, the ratio of C source to  $K^{15}NO_3$  (10:1) was conducive to the mineralization of native SON and the priming effect was induced by C sources and N fertilizer amendment (Kuzyakov *et al.*, 2000); as a result, a small part of native SON became active. Therefore, the change in SON concentration and  $^{15}N$  abundance (Fig. 1) over the whole incubation period showed a substitution effect of the newly formed SON with the native SON in the C-amended treatments although labeled  $NO_3-N$  could transform to newly formed SON. Without C amendment, the presence of  $K^{15}NO_3$  resulted in the mineralization of native SON (Fig. 1a). The similar  $^{15}N$  abundance in the  $K^{15}NO_3$  treatment and CK ( $P > 0.05$ ) indicates little transformation of labeled  $NO_3-N$  to SON (Fig. 1b). This further suggests the priming effect of N fertilizer on native SON and the important effect of C amendment on SON.

#### Mineral nitrogen ( $NO_3-N$ and $NH_4-N$ )

C substrate amendment led to a clear decrease in the concentration of  $NO_3-N$  during the incubation (Fig. 2a), and the  $NO_3-N$  lost in the C-amended treatments may transform to SON or denitrify to gaseous forms. The concentration of  $NO_3-N$  in the glucose treatment decreased predominantly during the first day of incubation from  $109.2 \pm 0.47$  (Table II) to  $51.7 \pm 1.21$   $mg\ N\ kg^{-1}$  (Fig. 2a) and remained constant in the later stages of incubation. In the straw treatment, the concentration of  $NO_3-N$  decreased gradually. The concentration of  $NO_3-N$  in the  $K^{15}NO_3$  treatment remained constant throughout the incubation period. In contrast to the CK, the abundance of  $NO_3-N$  in the three treatments decreased over the whole incubation period (Fig. 2b). This may be attributed to the dilution effect of the mineralization of native SON, and this effect accelerated after C amendment and increased with increasing availability of the C source. In the control, the natural abundance of  $NO_3-N$  exceeded 1% during the incubation period (Fig. 1b) and this may be related to the time of soil sampling. Choi *et al.* (2002) reported a similar result that the  $^{15}N$  of  $NO_3-N$  with only urea amendment increased from 1.13% to 1.30% during 30 to 50 d of maize growth.

On the first day of incubation the concentration and abundance of  $NH_4-N$  in the glucose treatment reached maximum values (Fig. 2c, d), and 59.4% of  $NH_4-N$  (Fig. 2c, d) was derived from the labeled  $NO_3-N$  (Fig. 2a, b). The  $NH_4-N$  may have been mainly derived from dissimilatory nitrate reduction to ammonium (DNRA) (Azam *et al.*, 2002; Wan *et al.*, 2009) although microbial metabolism can also contribute to

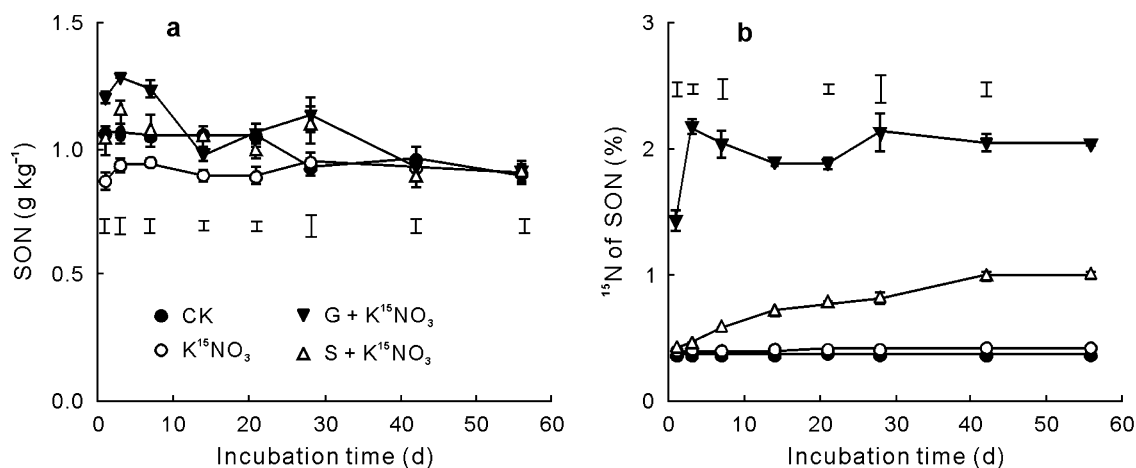


Fig. 1 Soil organic nitrogen (SON) in a calcareous soil from the North China Plain during 56-d incubation. G = glucose; S = maize straw. Data shown are mean value  $\pm$  one standard error of three replicates. Least significant difference values at the 0.05 level ( $LSD_{0.05}$ ) are denoted by vertical lines.

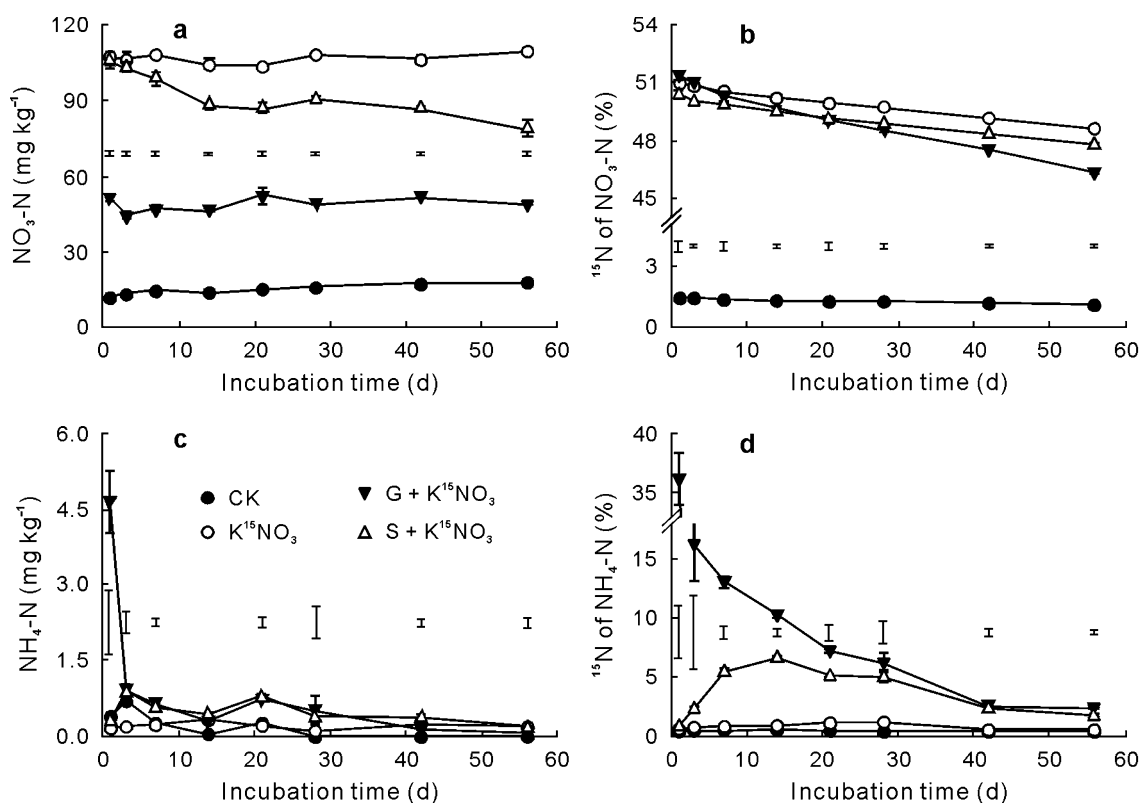


Fig. 2 Mineral nitrogen ( $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ ) in a calcareous soil from the North China Plain during 56-d incubation. G = glucose; S = maize straw. Data shown are mean value  $\pm$  one standard error of three replicates. Least significant difference values at the 0.05 level ( $\text{LSD}_{0.05}$ ) are denoted by vertical lines.

the formation of  $\text{NH}_4\text{-N}$  via ammonification in the  $\text{NO}_3^-$ -dominated soil. Because the largest SON by glucose amendment appeared on the 3rd day (Fig. 1) and a decline of 29.4 % of newly formed SON occurred from the 3rd to the 14th day, the ammonification rate could not have contributed to the higher newly formed  $\text{NH}_4\text{-N}$  on the 1st day in the glucose treatment (Fig. 2c, d). In addition, C amendment stimulated the growth of denitrifiers and increased microbial activity; increasing microbial respiratory demand for  $\text{O}_2$  can lead to the formation of anaerobic microsites and thus further promote denitrification and DNRA in the  $\text{O}_2$ -limited zone (Azam *et al.*, 2002). The occurrence of anaerobic microsites under unsaturated conditions was also reported by Wolf and Russow (2000). The DNRA pathway might be much favored by the properties of our soil, for example the high pH (Schmidt *et al.*, 2011; Stevens *et al.*, 1998) and ratio of C/ $\text{KNO}_3$  (Schmidt *et al.*, 2011), and the optimum sand content and bulk density (Schmidt *et al.*, 2011). Even so, in the first day in the glucose treatment, denitrification of  $\text{NO}_3\text{-N}$  should be the dominant pathway because the disappearance of labeled  $\text{NO}_3\text{-N}$  ( $33.3 \pm 0.61 \text{ mg N kg}^{-1}$ ) was much larger than the formation of  $\text{NH}_4\text{-N}$  ( $2.8 \pm 0.26 \text{ mg N kg}^{-1}$ ). The concentration of  $\text{NH}_4\text{-N}$  decreased

rapidly from the 1st to the 3rd day (Fig. 2c) as a result of the nitrification effect in the nitrification-dominated soil (Wan *et al.*, 2009; Ju *et al.*, 2011). When  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were added simultaneously to the soil of our study under incubation at moisture of 40% or 60% WFPS, more than 80% of  $\text{N}_2\text{O}$  was derived from the nitrification effect (Wan *et al.*, 2009). After the third day, the  $\text{NH}_4\text{-N}$  concentration in the glucose treatment declined to a low concentration similar to those of the other three treatments with the rapid nitrification of  $\text{NH}_4\text{-N}$  in the nitrification-dominated soil (Fig. 2c) (Wan *et al.*, 2009; Ju *et al.*, 2011), and there was a gradual decline in  $\text{NH}_4\text{-N}$  abundance resulting from the mineralization of native SON (Fig. 2d). The slow decomposition characteristics of maize straw may have contributed to the gradual increase in  $\text{NH}_4\text{-N}$  abundance to a maximum value on the 14th day and dilution by the mineralization of native SON may have occurred in the straw treatment (Fig. 2d).

#### $\text{N}_2\text{O}$ and $\text{CO}_2$

Glucose amendment markedly increased  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions in the soil with excessive accumulation of  $\text{NO}_3\text{-N}$  before the 7th day of incubation (Fig. 3a, b). During the first day of incubation, the emission of

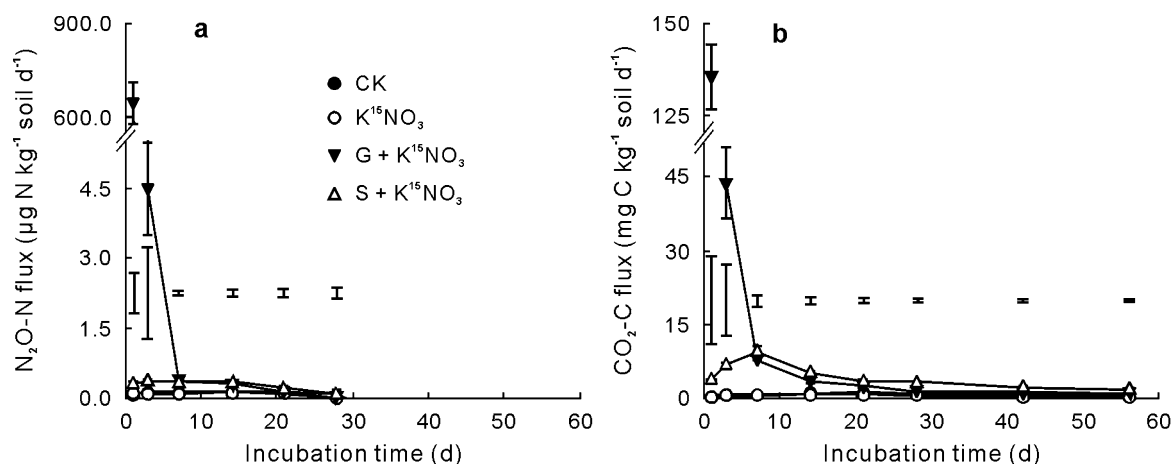


Fig. 3 Daily fluxes of  $\text{N}_2\text{O-N}$  and  $\text{CO}_2\text{-C}$  from a calcareous soil from the North China Plain during 56-d incubation G = glucose; S = maize straw. Data shown are mean value  $\pm$  one standard error of three replicates. Least significant difference values at the 0.05 level ( $\text{LSD}_{0.05}$ ) are denoted by vertical lines.

$\text{N}_2\text{O}$  in the glucose treatment reached  $647.0 \mu\text{g N kg}^{-1} \text{ soil d}^{-1}$  and  $\text{CO}_2$  emission reached  $116.3 \text{ mg C kg}^{-1} \text{ soil d}^{-1}$ .  $\text{CO}_2$  is mainly derived from microbial respiration and indicates microbial activity (Murray *et al.*, 2004).  $\text{N}_2\text{O}$  is simultaneously produced by nitrification and denitrification (Kuenen and Robertson, 1994; Stevens *et al.*, 1997; Wan *et al.*, 2009). On the first day in the glucose treatment, nitrification should also contribute to the production of  $\text{N}_2\text{O}$  because higher  $\text{NH}_4\text{-N}$  concentrations (Fig. 2c) were found in our nitrification-dominant soil (Wan *et al.*, 2009; Ju *et al.*, 2011). Due to the relatively low C availability of maize straw, the  $\text{N}_2\text{O}$  emission in the straw treatment was only slightly higher than that in the  $\text{K}^{15}\text{NO}_3$  treatment and CK, and the peak of  $\text{CO}_2$  was much lower and showed a clear lag phase. Emissions of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  in the C-amended treatments occurred mainly during the first 7 d and remained at low levels as in the  $\text{K}^{15}\text{NO}_3$  treatment and CK at the later stages of incubation. This phenomenon further favored the limiting of denitrification of excessive  $\text{NO}_3\text{-N}$  in our soil by the low availability of soil organic C (Ju *et al.*, 2011).  $\text{NO}_3\text{-N}$  leaching is therefore the main pathway of N loss on

the North China Plain (Ju *et al.*, 2009).

#### *Residues and loss of $^{15}\text{NO}_3\text{-N}$ after C amendment at the end of incubation*

After 56 d of incubation, the residual organic N and the loss of  $^{15}\text{NO}_3\text{-N}$  in the three treatments followed the sequence of glucose > straw >  $\text{K}^{15}\text{NO}_3$  (Table III). The addition of the C substrate effectively decreased  $^{15}\text{NO}_3\text{-N}$  accumulation. A higher decomposition rate of the C substrate (glucose) can lead to a maximum transformation rate of  $^{15}\text{NO}_3\text{-N}$  to SON at the expense of maximum  $\text{N}_2\text{O}$  emission and total  $^{15}\text{NO}_3\text{-N}$  loss. In comparison, the transformation of  $^{15}\text{NO}_3\text{-N}$  to SON by straw with low C availability to microorganisms because of high lignin and cellulose content was less than that by glucose, and the  $\text{N}_2\text{O}$  emission was also very low. The loss of total  $^{15}\text{NO}_3\text{-N}$  in the straw treatment was significantly lower than that in the glucose treatment ( $P < 0.05$ ) but significantly larger than that in the  $\text{K}^{15}\text{NO}_3$  treatment. Moreover, the ratio of  $\text{N}_2\text{O-N}$  to total N loss by glucose amendment (2.6%) was larger than that by straw amendment (0.1%) and this may attributed to the rapid nitrifica-

TABLE III

Fate of  $^{15}\text{NO}_3\text{-N}$  with different C amendments to a calcareous soil from the North China Plain at the end of a 56-d incubation period

Treatment <sup>a)</sup>	Residual organic N	Residual $\text{NO}_3\text{-N}$	Residual total N <sup>b)</sup>	Total $\text{N}_2\text{O-N}$ emitted	Total N lost
	mg N kg <sup>-1</sup>				
$\text{K}^{15}\text{NO}_3$	$0.9 \pm 0.01^{\text{c,d)}$	$86.6 \pm 0.57\text{a}$	$87.4 \pm 0.57\text{a}$	$0.02 \pm 0.00\text{b}$	$12.6 \pm 0.57\text{c}$
G + $\text{K}^{15}\text{NO}_3$	$25.0 \pm 0.95\text{a}$	$36.9 \pm 0.36\text{c}$	$61.9 \pm 1.29\text{c}$	$1.01 \pm 0.12\text{a}$	$38.1 \pm 1.29\text{a}$
S + $\text{K}^{15}\text{NO}_3$	$9.4 \pm 0.60\text{b}$	$61.3 \pm 2.50\text{b}$	$70.7 \pm 2.78\text{b}$	$0.03 \pm 0.00\text{b}$	$29.3 \pm 2.78\text{b}$

a) G = glucose; S = maize straw.

b) Residual total N is the sum of residual organic N, residual  $\text{NO}_3\text{-N}$ , and residual  $\text{NH}_4\text{-N}$ .

c) Data shown are mean value  $\pm$  standard error of three replicates.

d) Within columns, means with different lowercase letters are significantly ( $P < 0.05$ ) different.

tion of the high  $\text{NH}_4\text{-N}$  concentration in the glucose treatment (Fig. 2c) in the nitrification-dominant soil studied, despite the highly available C source being conducive to  $\text{N}_2\text{O}$  reduction (Murray *et al.*, 2004). Gaseous emissions ( $\text{N}_2$ ,  $\text{N}_2\text{O}$ , and  $\text{NO}$ ) formed the main pathway of  $\text{NO}_3\text{-N}$  reduction in the present study.  $\text{NO}$  emission is mainly derived from nitrification and generated at low soil moisture (< 50% WFPS) (Bouwman, 1998), and decreased with increasing soil pH (> 7) (Yamulki *et al.*, 1997).  $\text{N}_2$  is the major gas produced at pH 7–8 during denitrification (Yamulki *et al.*, 1997; Šimek *et al.*, 2002; Dannenmann *et al.*, 2008). Moreover, Ju *et al.* (2011) reported that denitrification would occur when soil moisture was more than 55% WFPS in the soil of our study, so  $\text{NO}$  might be much lower than that found by Bouwman (1998) in a soil of high pH (8.0). Straw amendment was therefore a potentially useful option to transform nitrate to SON and minimize  $\text{N}_2\text{O}$  emission.

Average N mineralization and immobilization between the beginning and end of incubation were calculated. The apparent mineralization of SON in all four treatments reached about  $150 \text{ mg N kg}^{-1}$  over the whole incubation period (Table IV) and decreased after C amendment. Furthermore, the apparent mineralization of SON decreased with glucose amendment more than with straw amendment. The net immobilization of  $^{15}\text{NO}_3\text{-N}$  into SON was much less than the apparent mineralization of SON (Table IV), and was very low without C source amendment. These results indicate that the readily available C source decreased the mineralization of SON and also replenished the native SON pool and the same trend can be found in residual organic N as shown in Table III. N mineralization and immobilization occur in soil simultaneously. In the present study, the larger N mineralization

during the incubation period may be attributed to a lack of adequate available C source with the result that the mineralized N can not be immobilized again. The incubation results require further testing under field conditions.

## CONCLUSIONS

Addition of C substrates (glucose and straw) to the intensively managed agricultural soils of the North China Plain could effectively promote the transformation of accumulated excessive soil  $\text{NO}_3\text{-N}$  to SON. Increasing availability of the C substrate increased the immobilization of accumulated soil nitrate and also greatly stimulated the mineralization of native SON and the emission of greenhouse gases. However, amendment of a slowly decomposing C source such as straw could increase the amount of  $\text{NO}_3\text{-N}$  transformed to SON with a minimum of greenhouse gas emissions. Therefore, the argument for returning straw biomass was strengthened in these nitrification-dominated soils, especially when deeper rooting varieties of crops were employed to increase the interception of  $\text{NO}_3\text{-N}$  before it could be leached to the subsoil.

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TABLE IV

Apparent mineralization of soil organic N (SON) and net immobilization of  $^{15}\text{NO}_3\text{-N}$  in SON with different C amendments to a calcareous soil from the North China Plain between 0 and 56 d of incubation

Treatment <sup>a)</sup>	Apparent mineralization rate of SON <sup>b)</sup>		Net immobilization rate of $^{15}\text{NO}_3\text{-N}^c)$	
	Amount	Rate	Amount	Rate
	$\text{mg N kg}^{-1}$	%	$\text{mg N kg}^{-1}$	%
CK	158.5	15.0	–	–
$\text{K}^{15}\text{NO}_3$	156.7	14.9	0.4	0.5
G + $\text{K}^{15}\text{NO}_3$	147.8	14.0	24.6	27.2
S + $\text{K}^{15}\text{NO}_3$	151.7	14.4	9.0	9.9

<sup>a)</sup>G = glucose; S = maize straw.

<sup>b)</sup>Apparent mineralization of SON is the difference in SON between 0 and 56 d of incubation, and the apparent mineralization rate of SON is the apparent mineralization of SON divided by SON at 0 d.

<sup>c)</sup>Net immobilization of  $^{15}\text{NO}_3\text{-N}$  is the difference in  $^{15}\text{NO}_3\text{-N}$  in SON between 0 and 56 d of incubation, and the net immobilization rate of  $^{15}\text{NO}_3\text{-N}$  is the net immobilization of  $^{15}\text{NO}_3\text{-N}$  divided by the total N from  $^{15}\text{NO}_3\text{-N}$  in soil at 0 d ( $90.57 \text{ mg N kg}^{-1}$  in the  $\text{K}^{15}\text{NO}_3$  treatment).

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