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ABSTRACT

A 56-day aerobic incubation experiment was performed with 15-nitrogen (N) tracer techniques after application of wheat straw to investigate nitrate-N (NO_3-N) immobilization in a typical intensively managed calcareous Fluvaquent soil. The dynamics of concentration and isotopic abundance of soil N pools and nitrous oxide (N_2O) emission were determined. As the amount of straw increased the concentration and isotopic abundance of total soil organic N and newly formed labeled particulate organic matter (POM-N) increased while NO_3-N decreased. When $^{15}NO_3$-N was applied combined with a large amount of straw at 5000 mg carbon (C) kg^{-1}

only 1.1 ± 0.4 mg kg^{-1} NO_3-N remained on the 56th day. The soil microbial biomass N (SMBN) concentration and newly formed labeled SMBN increased significantly ($P<0.05$) with increasing amount of straw. Total N_2O-N emissions were at levels of only micrograms kg^{-1} soil. The results indicate that application of straw can promote the immobilization of excessive nitrate with little emission of N_2O.

Keywords: NO_3-N immobilization, N pools, straw amendment, N_2O emission

INTRODUCTION

In agricultural soils substantial nitrate nitrogen (NO_3-N) accumulation in the soil profile and pollution of groundwater can result from excessive nitrogen (N) application rates or the lack of synchronization between the N supply and crop demand for N (Ju et al., 2006, 2009). Leaching or runoff of soil nitrate leads to the eutrophication of aquatic ecosystems and denitrification produces the long-lived and powerful greenhouse gas nitrous oxide (N_2O) (Conley et al., 2009; Ravishankara et al., 2009; Dungait et al., 2012). Therefore, understanding the processes of soil NO_3-N transformation is important for high N use efficiency and low N loss. Stimulating immobilization of excessive accumulated NO_3-N into soil organic N pools by microbial assimilation may contribute to both increasing soil fertility and ameliorating its negative environmental impacts (Ju et al., 2009, 2011; Dungait et al., 2012).

Carbon (C) amendments often increases nitrate $(NO_3)^-$ immobilization by stimulating microbial growth (Sakala et al., 2000; Burger and Jackson, 2003) with the concomitant production of N_2O and carbon dioxide (CO_2), especially in soils with excessive NO_3-N and C-limitations.

Amendment with complex C compounds such as straw leads to low NO_3-N immobilization (Chaves et al., 2008; Miller et al., 2008) with minor N_2O and CO_2 emissions in comparison with readily decomposed C sources such as glucose (de Catanzaro and Beauchamp, 1985; Miller et al., 2008; Qiu et al., 2013), because straw has much low available lignin-C. Correspondingly the quantity of low molecular available C for microorganism increases if straw amendment rate increased. However, little information is available on both NO_3-N immobilization and N_2O emission with increasing rate of amendment with straw.

Ammonium (NH_4^+) coexists with NO_3^- in aerobic soils (Burger and Jackson, 2003) and is the preferred N form for assimilation by microorganism (Recous et al., 1990), while Burger and Jackson (2003) reported that microorganism assimilated more NO_3^- than NH_4^+ because of low NH_4^+ concentration and strong nitrifiers competition in a arable soil. Ammonium is mainly derived from microbial turnover, soil organic N mineralization, and application of NH_4^+ based fertilizers to agriculture soils, and nitrate is mainly from NH_4^+ rapidly oxidized to NO_3^-, especially in nitrified dominant soils such as on North China Plain (Wan et al., 2009; Ju et al., 2011). However, it is still unclear how NH_4-N responds to NO_3-N immobilization with different straw amendment rate in the high accumulated NO_3-N soil.

The immobilized N in live or dead microbial cells can enter different soil organic N pools and thus N immobilization by the soil microbial biomass can increase the soil N storage capacity and thereby reduce N loss through NO_3-N leaching (Perelo et al., 2006; Ju, 2014). Microbial metabolism regulates dissolved organic N dynamics because it is derived partly from dead microorganisms and provides an N source for living microorganisms (Kalbitz et al., 2000; Park et

al., 2002). Microorganisms can also bind particulate organic matter (POM) with microbial tissues or secretions (Mendham et al., 2004) and further promote the N reservoir in aggregates because of the role of POM as a nucleus for aggregation (Bongiovanni and Lobartini, 2006; Marriott and Wander, 2006a, b). Microorganisms can also assimilate C sources from POM (Christensen, 2001; Marriott and Wander, 2006a, b). Therefore, soil microbial biomass N, dissolved organic N and POM-N can reflect short-term turnover in the soil N reservoir.

Large amounts of nitrate (NO_3^-) have accumulated in the soil profiles on the North China Plain (NCP) due to excessive application of mineral fertilizers with ca. 300 kg N ha^{-1} per season applied in conventional farming practice in intensively managed winter wheat and summer maize systems (Ju et al., 2006; Zhao et al., 2006; Wan et al., 2009). For example, the amount of NO_3-N in the top 90 cm of the soil profile reached 700 kg N ha^{-1} after four years (i.e. eight growing seasons) of successive crops under conventional N fertilizer applications (600 kg N ha^{-1} yr^{-1}) in a field experiment (Zhao et al., 2006). Moreover, agricultural soils on the NCP have typical properties of low soil organic C content, low NH_4^+ concentration (around 1 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 NO_3^- immobilization (Murray et al., 2004; Wan et al., 2009).

Therefore, the main objectives of the present study on this typical high NO_3-N calcareous Fluvaquent soil were to investigate the immobilization of accumulated NO_3-N into various soil N pools and N_2O emissions with different amounts of amendment with wheat straw C source. ^{15}N isotope tracer techniques were employed for these studies under conditions of controlled soil temperature and moisture.

MATERIALS AND METHODS

Soil

At the beginning of October 2007 surface soil (0-20 cm) after the summer maize harvest was collected from a field at Dongbeiwang Agricultural Experimental Station (40.08° N, 116.28° E, 40 m above sea level) near Beijing, in which optimum N fertilizer application had been practised based on the soil mineral N test (Zhao et al., 2006). The cropping system at this site was a winter wheat-summer maize rotation and urea fertilizer had been applied at a rate of 130 kg N ha^{-1} for each winter wheat season and 97 kg N ha^{-1} for each summer maize season over the preceding 8 years. Full details of the experimental treatments were provided by Zhao et al. (2006). Freshly collected soil was removed stones, roots and crop residues, and sieved (< 2 mm) for uniform and storied in 4 ºC refrigerator for the incubation experiment late on. A little mixed soil sub-sample was air dried for soil properties analysis. The soil contained 27 % sand, 57 % silt and 16 % clay and had 1.24 % soil organic carbon, 0.12 % total N, a pH of 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. We used low initial NO_3-N soil for creating high $^{15}NO_3$-N soil during the incubation experiment.

Experimental Design and Procedures

The moisture of the sieved field fresh soil was determined by oven drying an aliquot at 105 ºC the day before the potassium, nitrate ($K^{15}NO_3$) solution and deionized water were applied by spraying. The sieved field fresh soil was firstly divided into two portions so that one portion was treated

as $^{15}NO_3$-N labeled soil and the other as unlabeled and unfertilized control soil. In order to attain high $^{15}NO_3$-N soil, one portion of 27.5 kg fresh soil (equivalent to 24.0 kg oven-dried soil) was uniformly sprayed with 84.0 ml $K^{15}NO_3$ solution at 60.21 % ^{15}N abundance and 2.78% N concentration, which was equivalent to an amendment with 100 mg N kg^{-1} (ca. 300 kg N ha^{-1}) oven-dried soil, and the soil moisture was adjusted to 45 % water filled pore space (WFPS) on the basis of 1.3 g cm^{-3} soil bulk density. In detail, 2.29 kg sieved field fresh soil (equivalently to 2.00 kg oven-dried soil) spread out on a 1 × 1 m^2 plastic film, was uniformly sprayed four times with 7.0 ml $K^{15}NO_3$ solution and 31.41 g deionized water using a 100 ml atomizer and mixed thoroughly by hand. In total, the procedure above was replicated 12 times and all of the $^{15}NO_3$-N soils were mixed again by hand on a 2 × 2 m^2 plastic film. Similarly, the other portion of 9.17 kg sieved field fresh soil was sprayed with deionized water as a control. Then, both soil portions were pre-incubated aerobically at 18 ºC for 2 weeks in the dark so that the balance of the $^{15}NO_3$-N amendment and native soil N pools could be achieved.

The experiment then comprised four treatments as follows in a randomized complete design: (1) control (no fertilized, no labeled N , CK), (2) wheat straw (W) plus $K^{15}NO_3$ at a C amendment rate of 1000 mg kg^{-1} soil (W_{10} + $K^{15}NO_3$), (3) wheat straw plus $K^{15}NO_3$ at a C amendment rate of 2500 mg kg^{-1} soil (W_{25} + $K^{15}NO_3$), and (4) wheat straw plus $K^{15}NO_3$ with at a C amendment rate of 5000 mg kg^{-1} soil (W_{50} + $K^{15}NO_3$). The details of the treatments are shown in Table 1. Three replicates of each treatment were destructively sampled at each of 8 sampling dates, giving a total of 144 jars. The samples were taken after 1, 3, 7, 14, 21, 28, 42 and 56 d incubation. The soil sampled at the end of pre-incubation was regarded as the 0 d sample and the 0 d ^{15}N abundance and

N concentration of total soil organic N and mineral N in the $K^{15}NO_3$ treated soil and control soil are shown in Table 2.

The incubation experiment was carried out in 1-L glass jars containing 300 g pre-incubated soil at 18 ºC for 56 d in the dark and the soil moisture was adjusted to 50 % WFPS with deionized water. Soil bulk density in each incubated jar was adjusted to 1.3 g cm^{-3}. During incubation the lids of the jars were closed to prevent soil water loss and were opened for 15 min each day to maintain aerobic conditions.

The wheat straw was oven-dried at 60 ºC and finely ground with a ball mill (Retsch Model MM200, Germany) after coarse grinding, and was added uniformly according to the different treatments described above and mixed thoroughly with pre-incubated soil. The moisture content of pre-incubated ^{15}N soil and control soil was determined at 105 ºC the day before the straw and deionized water were added. The wheat straw addition and adjustment of soil moisture to 50 % WFPS were similar to $K^{15}NO_3$ solution sprayed above. In detail from treatments (2) to (4), 2052.54 g pre-incubated $^{15}NO_3$-N (equivalent to 1800 g oven-dried soil) 4.16, 10.40 and 20.79 g finely ground wheat straw was added uniformly, 90.0 g deionized water sprayed, and the soil and straw mixed thoroughly on a plastic film. In each treatment, the procedure of straw and/or deionized water addition was replicated four times and mixed again on a plastic film. The added wheat straw had an organic C concentration of 43.3 %, a total N content of 0.95 % and a ^{15}N abundance of 0.36%, and the amounts of wheat straw added to each jar were 0.59, 1.46, and 2.93 g in treatments (2) to (4).

Sampling and Analysis

150 NO_3-N and NH_4-N were extracted with 1 M potassium chloride (KCl) using a soil:water ratio of 1:5 (w/v) (Bremner and Keeney, 1966; Wan et al., 2009) and the soil and KCl suspension was 152 shaken for 1 hour at 180 rpm in a reciprocating shaker and filtered using a medium-speed ashless filter paper (Shuangquan brand, China) 11 cm in diameter and with 30-50 μm pore diameter. 154 Before filtering the filter paper was prewashed three times with the filtrate to remove NH_4^+. The ^{15}N abundance and concentration of NO_3-N and NH_4-N were determined using the 156 SPINMAS technique described by Stange et al. (2007) and Wan et al. (2009). The ^{15}N abundance and concentration of NO_3^- were determined by quadruple mass spectrometry (QMS) 158 when NO_3^- was reduced to NO with vanadium (III) chloride (VCl_3), and NH_4^+ was oxidized to N_2 with sodium bromate (NaBrO).

160 After extraction of mineral N as described above, the soil slurry was washed once again with 1 M KCl at a soil:water ratio of 1:2.5 (w/v) then centrifuged and washed twice with 162 deionized water at the same soil : water ratio as above. The washed residue, which was regarded as total soil organic N, was oven dried at 60 ºC for determination of total soil organic N and ^{15}N 164 analysis.

SMBN was determined by the chloroform ($CHCl_3$) fumigation-extraction (FE) method 166 (Brookes et al., 1985). The N in fumigated and unfumigated samples in 0.5 M potassium sulfate (K_2SO_4) solution (1:4, w/v) was determined by Kjeldahl digestion. SMBN was calculated as: 168 SMBN = (total N in fumigated extracts - total N in unfumigated extracts)/K_E, where K_E = 0.57

(Jenkinson, 1988). DON was the difference between NH_4-N and total dissoluble N in unfumigated extracts (Cookson et al., 2007; Ghani et al., 2007).

POM was determined as described by Bronson et al. (2004). Briefly, 25 g air-dried incubated soil (< 2 mm) was dispersed in 100 ml sodium hexametaphosphate solution (5 $g \cdot L^{-1}$) for 1 h on a reciprocal shaker and the mixed suspension was washed over a 53 μm sieve until the rinsing water was clear. The remaining material (after removal of visible stones and roots on the sieve) was oven-dried in a beaker at 60 °C.

N concentrations of total soil organic N and POM were determined with a CN analyzer (Vario Max CN, Elementar, Germany) and those ^{15}N abundances were determined by mass spectrometry (DELTA Plus XP, Thermo Finnigan, Germany) after they passed a 0.15-mm sieved. Mineral N and SMBN were analyzed using 2-mm sieved fresh samples, and N concentrations of fumigated and unfumigated samples were quantified by titration with 0.005 N H_2SO_4. ^{15}N abundances of 0.005 N H_2SO_4-neutralized solution of fumigated samples and unfumigated samples as described above were determined by diffusion into a trapping acid GD/F glass fibre filter paper with magnesium oxide (MgO)as described by Brooks et al. (1989) and the filter paper was analyzed directly by mass spectrometry before oven-drying at 60 °C. The newly formed labeled N fraction was calculated using the following equation:

$$Ndff_p = [content_p \times ape_p\ (\%)]/ape_c\ (\%)$$

where Ndff is N derived from labeled $^{15}NO_3$-N, ape is ^{15}N atom percent excess, subscript p is the soil N pool, and subscript c is the applied $^{15}NO_3$-N.

The newly formed labeled SMBN was calculated using a similar equation:

190 $[content_f \times ape_f\ (\%) - content_u \times ape_u\ (\%)]/0.57/ape_c\ (\%)$,

where subscripts f and u are fumigated N and unfumigated N, respectively.

192 A 60-ml gas sample was taken from each replication of jar which had an airtight lid sealed for 24 h to the sampling day using a gas-tight syringe connected to a two-way valve. N_2O and CO_2 194 were then analyzed with a gas chromatograph (6890N, Agilent Technologies, Santa Clara, CA) equipped with a ^{63}Ni electron capture detector (ECD) and a hydrogen flame ionization detector 196 (FID). CO_2 was reduced by H_2 to CH_4 in a nickel catalytic converter at 375 °C and then detected by FID.

198 Daily fluxes of N_2O-N and CO_2-C were calculated using the equation PV = nRT using the concentrations of N_2O and CO_2 in an incubated 1-L empty glass jar. In the equation, P is standard 200 atmospheric pressure, 101.3 Pa; V is the residue volume with subtraction of incubated soil in a 1 L incubated bottle, 0.81 L; n is amount of substance; R is a constant, 8.314; and T is the absolute 202 temperature, 291 K in our study.

Statistical Analysis

204 Data were adjusted to an oven-dried soil weight basis. One-way analysis of variance was conducted with the SPSS version 11.0 software package and the mean values were compared 206 using least significant difference (LSD) at the 5 % level. Data are reported as mean value ± one standard error of the mean (SEM).

RESULTS

Total soil organic N concentration ranged from 1.1 to 1.5 g kg^{-1} across all treatments during incubation. Throughout the incubation period the greatest total soil organic N concentration was maintained in treatment $W_{50}+^{15}NO_3$, followed by $W_{25}+^{15}NO_3$, with $W_{10}+^{15}NO_3$ showing the lowest value (Figure 1a), and significant differences ($P < 0.05$) were found between $W_{50}+^{15}NO_3$ and $W_{10}+^{15}NO_3$ at day 14 and after day 21. There was no significant difference in total soil organic N concentration among the control and $W_{25}+^{15}NO_3$ treatments except at day 56 of the incubation period.

In all treatments (Figure 1b) the changes in ^{15}N abundance of total soil organic N after day 21 were much slower and so the immobilization of labeled $^{15}NO_3$ by the C source-induced microbial response occurred mainly within the first 21 days and then stabilized as incubation proceeded. The ^{15}N abundance of total soil organic N increased as the amount of C source; moreover, the difference of ^{15}N abundance in different treatments was significant ($P < 0.05$).

With the exception of the control, NO_3-N concentration and isotopic abundance showed a declining trend over the whole incubation period (Figures 2a, b). Moreover, as the amount of wheat straw increased the declining ranges of NO_3-N concentration and isotopic abundance increased. At the end of the incubation period (day 56) the NO_3-N concentration in $W_{50}+^{15}NO_3$ was only 1.1 ± 0.4 mg kg^{-1} and was significantly lower than the control ($P<0.05$). A significant difference ($P<0.05$) during the incubation was found in NO_3-N isotopic abundance as the C amount of wheat straw increased (Figure 2b).

228 The NH_4-N concentration (Figure 2c) was < 2 mg kg^{-1} during the course of the incubation and NH_4-N isotopic abundance (Figure 2d) increased with increasing amount of straw, with the
230 maximum value occurring at 28 days. A significant difference ($P<0.05$) during the incubation was found in NH_4-N isotopic abundance as the C amount of wheat straw increased (Figure 2d).

232 As the amount of wheat straw increased (Figure 3a), and especially in treatment $W_{50}+^{15}NO_3$, the SMBN concentration increased significantly during the incubation period ($P<0.05$). Moreover, the
234 decrease of SMBN concentration at the end of the incubation period might be because the available C source in the straw decreased. Similarly, newly formed labeled SMBN increased
236 during incubation as the amount of wheat straw increased (Figure 3b), and newly formed labeled SMBN in $W_{50}+^{15}NO_3$ treatment was significantly ($P<0.05$) larger than the other two treatments
238 after the 3rd day.

The peak DON concentration in $W_{50}+^{15}NO_3$ treatment was noticeably greater than the other two
240 treatments (Figure 4a). The lower DON concentrations in $W_{10}+^{15}NO_3$ and $W_{25}+^{15}NO_3$ compared to the control may be attributed to low microbial assimilation under the smaller amount of
242 available wheat straw during the first 7 days. The DON concentrations tended to stabilize after 28 days and remained at < 7 mg kg^{-1} in all treatments. In all treatments (Figure 4b) the newly formed
244 labeled DON was generally < 1mg kg^{-1} except for the maximum in $W_{50}+^{15}NO_3$. $W_{50}+^{15}NO_3$ showed no newly formed labeled DON after 14 days.

246 Throughout the incubation period the POM-N concentration (Figure 5a) was < 200 mg kg^{-1} in all treatments. Except for $W_{25}+^{15}NO_3$, the POM-N concentration in all treatments at the end of
248 incubation tended to be larger than at the start because of the N contribution from NO_3^- and added

wheat straw but the difference was not significant. However, the newly formed labeled POM-N (Figure 5b) at the end of the incubation period increased significantly ($P<0.01$) in comparison with begin of incubation. As the amount of wheat straw increased, the newly formed labeled POM-N increased (Figure 5b).

On the first day (Figure 6a) the N_2O-N emissions in $W_{50}+^{15}NO_3$ and $W_{25}+^{15}NO_3$ were significantly greater than in the other two treatments ($P<0.05$) as a result of stimulation by the adequate supply of available C. After incubation for 28 days the N_2O emission in all treatments tended to stabilize. Throughout the incubation period (Figure 6b) the CO_2-C emissions in $W_{50}+^{15}NO_3$ and $W_{25}+^{15}NO_3$ were significantly greater than in the other treatments ($P<0.05$). The maximum CO_2-C emission in all wheat straw occurred on day 7.

The$^{15}NO_3$-N recovery at the start and end of the incubation period indicates little loss of $^{15}NO_3$-N during the 56-day incubation experiment (Table 3). As the amount of wheat straw C increased the final total immobilized labeled N (newly formed total soil organic N) increased significantly ($P < 0.05$) and the final labeled mineral N decreased significantly ($P < 0.05$) at the end of incubation. The labeled N recovery in other pools except for total soil organic N and mineral N was 18.4-25.3 mg kg^{-1}. Total N_2O-N emissions were only of microgram magnitude per kilogram of soil and were much smaller than the other transformation processes, and total N_2O-N emissions in $W_{50}+^{15}NO_3$ and $W_{25}+^{15}NO_3$ treatments was significantly larger ($P < 0.05$) than $W_{10}+^{15}NO_3$ and control treatments.

DISCUSSION

The quantity, availability and intrinsic nature of the C source (Ocio et al., 1991; Barrett and Burke, 2000; Myrold and Bottomley, 2008) can affect the transformation of NO_3-N into various soil N pools as shown by the ^{15}N abundance or newly formed N in different soil N pools in Figs 1-5 and the small amount of N_2O emissions (Figure 6). Straw decomposition has two phases, an initial rapid decomposition with the utilization of the easily decomposed C fractions and a subsequent slower phase with the decomposition of the more recalcitrant fractions (Duong et al., 2009). As the amount of straw amendment increases the quantity of easily decomposed C fractions in the straw increases and there are corresponding increases in the labeled NO_3-N immobilization in the total soil organic N, SMBN and POM pools and N_2O emission, and the dynamics of NO_3-N concentrations and their isotopic abundance showed different trends during the incubation (Fig 1-6, Table 3). For example, NO_3-N isotopic abundance in $W_{25}+^{15}NO_3$ treatment showed a linear trend and in $W_{50}+^{15}NO_3$ followed a curvilinear trend (Figure 2b). The greatest DON concentration in $W_{50}+^{15}NO_3$ and the lowest DON concentration in $W_{25}+^{15}NO_3$ occurred during the first incubated 28^{th} days because DON was closely linked with SMBN turnover according to the amount and availability of C and N sources in the straw (Figure 3, 4). Even so, the N_2O emission in $W_{50}+^{15}NO_3$ in the present study was much lower than was found by Qiu et al. (2013) in the same soil with glucose amendment because the molecular weight of the easily decomposed fraction in the straw markedly higher than that of glucose.

The immobilization of NO_3-N is due mainly to microbial turnover when C sources are applied, but the determined factor was maybe the quantity of available C source in the straw because the net

turnover time of newly formed SMBN from 14th d to 56th d in W_{25}+$^{15}NO_3$ and W_{50}+$^{15}NO_3$ treatments was 0.38 yr, 0.38 yr and it in W_{10}+$^{15}NO_3$ treatment was 1.05 yr, according to the equation of the net turnover rate ($B_t = B_0e^{-kt}$) and net turnover time $(T = 365k)^{-1}$ (Perelo et al., 2006), The difference of the net turnover rate of newly formed SMBN between W_{25}+$^{15}NO_3$ and W_{50}+$^{15}NO_3$ treatments attributed mainly to the B_0 value, it was 26.0 and 18.3 in W_{25}+$^{15}NO_3$ and W_{50}+$^{15}NO_3$ treatments, respectively. In addition, the ferrous wheel hypothesis is an important mechanism for NO_3-N abiotic immobilization (Dividson et al., 2003); N fixation in clay minerals might be also important for N retention, especially in the 2:1 type clay mineral soil in our study (Ju et al., 2004). However, the calcareous soil with pH > 7 used in our study can hinder ferrous/ferric ion activity the low molecular organic compounds from straw decomposition can hold back NH_4-N fixation in 2:1 type clay minerals (Qiu et al., 2012). In the present study the dynamics of NH_4-N concentration and ^{15}N abundance indicate that it (Figure 2c,d) was mainly generated from the mineralization of soil native organic N, DON and SMBN, because NH_4-N is the preferential N form over NO_3-N by microbial assimilation (Recous et al., 1990) and it can be rapidly oxidized in our nitrification-dominated soil during aerobic incubation (Wan et al., 2009; Ju et al., 2011). Dissolved organic N or newly formed labeled DON (Figure 4) was maintained at low concentrations, it is possible that DON was only an intermediate product during microbial metabolism and was a preferred form of N for microorganisms similar to NH_4-N (Figure 2c, d). The labeled N recovery in other pools (Table 3) should be readily leached N (after washing three times) in the weakly fixed mineral N and dissolved small-molecular-weight SMBN pools. Another source would be the cumulative errors in the measurement of labeled N in total soil organic N and labeled mineral N.

312 POM-N might be an important fraction in total soil organic N but the newly formed labeled POM-N accounted for only 16.3-28.6 % of the newly formed labeled total soil organic N (Figure1,
314 5) at the end of the incubation period. Therefore, a large proportion of labeled N might be immobilized into the fine fraction. Angers et al. (1997) also reported that the majority of ^{15}N was
316 found in the fine fraction (<50 μm) after an 18-month experiment in situ. It is possible that the fine fraction has a larger surface area than POM, can fix N in soil clay minerals and is therefore more
318 likely to immobilize the newly formed labeled N.

Straw N is not account for calculation of C/N ratio in the current study but it also plays an
320 important role in soil N immobilization-mineralization. Treatment $W_{10}+^{15}NO_3$ supplied only 21.9 mg kg^{-1} of wheat straw N but $W_{50}+^{15}NO_3$ supplied 109.7 mg kg^{-1} and the straw N in $W_{50}+^{15}NO_3$
322 was sufficient to influence the transformation of NO_3-N and soil native N, with a difference in POM-N between the start and end of the incubation period (Figure 5a). Eagle et al. (2000) also
324 found that net N mineralization occurred when the straw N concentration exceeded 0.54 %. Therefore, the availability of straw N needs to be taken into account for further study.

326 In addition, during the incubation period the lower total soil organic N concentration in $W_{10}+^{15}NO_3$ in comparison with the control may be explained by mineralization of the soil native
328 organic N or by NO_3-N amendment induced during the pre-incubation stage (Qiu et al., 2013), with slight NO_3-N immobilization induced by low available C amount of wheat straw in
330 $W_{10}+^{15}NO_3$ treatment (Figure 2, Table 3). Compared to the isotopic abundance of total soil organic N, the smaller difference in total soil organic N concentration (Figure 1) may be attributed
332 to a substitution effect between the immobilization of labeled fertilizer N and the mineralization of

soil native N, with the same phenomenon occurring in POM and newly formed labeled POM during the incubation period.

In summary, increasing straw incorporation clearly stimulated the immobilization of excessive accumulated NO_3-N together with minor N_2O emissions by microbial assimilation. As the amount of straw amendment increased, straw N must be regarded as an important N source. In the field on NCP, straw return might be an efficient management practice to reverse excessive soil NO_3-N accumulation and leaching but the risk of competition for available N between microbial assimilation and crop uptake from excessive straw application needs to be considered.

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REFERENCES

Angers, D.A., S. Recous, and C. Aita. 1997. Fate of carbon and nitrogen in water-stable aggregates during decomposition of $^{13}C^{15}N$-labelled wheat straw in situ. European Journal of Soil Science 48: 295-300.

Barrett, J.E. and I.C. Burke. 2000. Potential nitrogen immobilization in grassland soils across a soil organic matter gradient. Soil Biology and Biochemistry 32: 1707-1716.

Bongiovanni, M.D. and J.C. Lobartini. 2006. Particulate organic matter, carbohydrate, humic acid contents in soil macro- and microaggregates as affected by cultivation. Geoderma 136: 660-665.

Bremner, J.M., and D.R. Keeney. 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate, and nitrite by extraction–distillation methods. Soil Science Society of American Journal 30, 504–507.

Bronson, K.F., T.M. Zobeck, T.T. Chua, V. Acosta-Martinez, R.S. van Pelt and J.D. Booker. 2004. Carbon and nitrogen pools of southern high plains cropland and grassland soils. Soil Science Society of America Journal 68: 1695-1704.

Brookes, P.C., A. Landman, G. Pruden, D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry, 17: 837-842.

Brooks, P.D., J.M. Stark, B.B. Mclnteer and T. Preston. 1989. Diffusion Method To Prepare Soil Extracts For Automated Nitrogen-15 Analysis. Soil Science Society of America Journal 53: 1707-1711.

Burger, M. and L.E. Jackson. 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biology and Biochemistry 35: 29-36.

Chaves, B., S.D. Neve, P. Boeckx, R. Dupont, O. Van Cleemput and G. Hofman. 2008. Manipulating the N release from 15N-labelled celery residues by using straw and vinasses in Flanders (Belgium). Agriculture, Ecosystems and Environment 123: 151–160.

Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science 52: 345-353.

Conley, D.J., H.W. Paerl, R.W. Howarth, D.F. Boesch, S.P. Seitzinger, K.E. Havens, C. Lancelot and G.E. Likens. 2009. Controlling eutrophication: Nitrogen and phosphorus. Science, 323: 1014-1015.

Cookson, W.R., M. Osman, P. Marschner, D.A.Abaye, I. Clark, D.V. Murphy, E.A. Stockdale and C.A. Watson. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biology and Biochemistry 39: 744-756.

Dividson, E.A., J. Chorover and D.B. Dail. 2003. Amechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Global Change Biology 9: 228-236.

de Catanzaro, J.B. and E.G. Beauchamp. 1985. The effect of some carbon substrates on denitrification rates and carbon utilization in soil. Biology and Fertility of Soils 1: 183-187.

Duong, T.T.T., K. Baumann and P. Marschner. 2009. Frequent addition of wheat straw residues to soil enhances carbon mineralization rate. Soil Biology and Biochemistry 41: 1475–1482.

Dungait, J.A.J., L.M. Cardenas, M.S.A. Blackwell, L. Wu, P.J.A. Withers, D.R. Chadwick, R. Bol, P.J. Murray, A.J. Macdonald, A.P. Whitmore and K.W.T. Goulding. 2012. Advances in the understanding of nutrient dynamics and management in UK agriculture. Science of the total Environment 434: 39-50.

Eagle, A.J., J.A. Bird, W.R. Horwath, B.A. Linquist, S.M. Brouder, J.E. Hill and C. van Kessel, 2000. Rice yield and nitrogen utilization efficiency under alternative straw management practices. Agronomy Journal 92:1096-1103.

392 Ghani, A., M. Dexter, R.A. Carran and P.W. Theobald. 2007. Dissolved organic nitrogen and carbon in pastoral soils: The New Zealand experience. European Journal of Soil Science 58: 394 832-843.

Jenkinson, D.S., 1988. Determination of microbial biomass carbon and nitrogen in soil. In: 396 Wilson, J.R. (ed) Advances in nitrogen cycling in agricultural ecosystems. CAB International, Wallingford, UK, pp 368-386.

398 Ju, X.T. 2014. Direct pathway of nitrate produced from surplus nitrogen inputs to the hydrosphere. Proceedings of National Academy of Sciences, USA 111: E416.

400 Ju, X., X. Lu, Z. Gao, X. Chen, F. Su, M. Kogge, V. Römheld, P. Christie and F. Zhang. 2011. Processes and factors controlling N_2O production in an intensively managed low carbon 402 calcareous soil under sub-humid monsoon conditions. Environmental Pollution 159: 1007-1016.

Ju, X.T., C.L. Kou, F.S. Zhang, and P. Christie. 2006. Nitrogen balance and groundwater nitrate 404 contamination: Comparison among three intensive cropping systems on the North China Plain. Environmental Pollution 143 : 117-125.

406 Ju, X.T., X.J. Liu and F.S. Zhang. 2004. Dynamics of soil fixed NH_4^+ and soil microbial biomass nitrogen in the growing season of winter wheat. Chinese Journal of Eco-Agriculture 12: 90-91. 408 (In Chinese.)

Ju, X.T., G.X. Xing, X.P. Chen, S.L. Zhang, L.J. Zhang, X.J. Liu, Z.L. Cui, B. Yin, P. Christie, 410 Z.L. Zhu and F.S. Zhang. 2009. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. Proceedings of the National Academy Sciences U.S.A. 412 106: 3041-3046.

Kalbitz, K., S. Solinger, J.H. Park, B. Michalzik and E. Matzner. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science 165: 277-304.

Marriott, E.E. and W. Wander. 2006a. Qualitative and quantitative differences in particulate organic matter fractions in organic and conventional farming systems. Soil Biology and Biochemistry 38: 1527-1536.

Marriott, E.E. and M.M. Wander. 2006b. Total and labile soil organic matter in organic and conventional farming systems. Soil Science Society of America Journal 70: 950-959.

Mendham, D.S., E.C. Heagney, M. Corbeels, A.M. O'Connell, T.S. Grove, R.E. McMurtrie. 2004. Soil particulate organic matter effects on nitrogen availability after afforestation with Eucalyptus globulus. Soil Biology and Biochemistry 36: 1067-1074.

Miller M.N., B.J. Zebarth, C.E. Dandie, D.L. Burton, C. Goyer and J.T. Trevors. 2008. Crop residue influence on denitrification, N_2O emissions and denitrifier community abundance in soil. Soil Biology and Biochemistry 40: 2553–2562.

Murray, P.J., D.J. Hatch, E.R. Dixon, R.J. Stevens, R.J. Laughlin, S.C. Jarvis. 2004. Denitrification potential in a grassland subsoil: effect of carbon substrates. Soil Biology and Biochemistry, 36: 545-547.

Myrold, D.D. and P.J. Bottomley. 2008. Nitrogen mineralization and immobilization. In: Schepers, J.S., Raun, W.R. (eds). Nitrogen in Agricultural Systems. Agronomy Monograph 49. ASA, CSSA, and SSSA, Madison, WI. 157-172.

Ocio, J.K., P.C. Brookes and D.S. Jenkinson. 1991. Field incorporation of straw and its effects on soil microbial biomass and soil inorganic N. Soil Biology and Biochemistry 23: 171-186.

434 Park, J.H., K. Kalbitz and E. Matzner. 2002. Resource control on the production of dissolved organic carbon and nitrogen in a deciduous forest floor. Soil Biology and Biochemistry 34:
436 813-822.

Perelo L.W., M. Jimenez and J.C. Munch. 2006. Microbial immobilisation and turnover of ^{15}N
438 labelled substrates in two arable soils under field and laboratory conditions. Soil Biology and Biochemistry 38: 912–922.

440 Qiu, S.J., Q.P. Peng, L. Li, P. He, Q. Liu, J.S. Wu, P. Christie, and X.T. Ju. 2012. Effects of applied urea and straw on various nitrogen fractions in two Chinese paddy soils with differing clay
442 mineralogy. Biology and fertility of soils, 48: 161-172

Qiu, S.J., X.T. Ju, J. Ingwersen, Z.D. Guo, C.F. Stange, R. Bisharat, T. Streck, P. Christie and
444 F.S. Zhang. 2013. Role of added carbon in the transformation of surplus soil nitrate-nitrogen to organic forms in an intensively managed calcareous soil. Pedosphere, 23: 205-212.

446 Ravishankara, A.R., J.S. Daniel and R.W. Portmann 2009. Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. Science, 326: 123-125.

448 Recous, S., B. Mary and G. Faurie. 1990. Microbial immobilization and ammonium and nitrate in cultivated soils. Soil Biology and Biochemistry 22: 913-922.

450 Sakala, W.D., Cadisch, G. and K.E. Giller. 2000. Interactions between residues of maize and pigeon pea and mineral N fertilizers during decomposition and N mineralization. Soil Biology and
452 Biochemistry 32: 679-688.

Stange, C.F., O. Spott, B. Apelt and R. Russow. 2007. Automated and rapid online determination
454 of ^{15}N abundance and concentration of ammonium, nitrite or nitrate in aqueous samples by the SPINMAS technique. Isotopes in Environmental and Health Studies 43: 277-236.

456 Wan, Y.J., X.T. Ju, J. Ingwersen, U. Shwarz, C.F. Stange, F.S. Zhang and T. Streck. 2009. Gross nitrogen transformations and related nitrous oxide emissions in an intensively used calcareous soil.
458 Soil Science Society of America Journal 73: 102-112.

Zhao, R.F., X.P. Chen, F.S. Zhang, H.L. Zhang, , J. Schroder and V. Römheld. 2006.
460 Fertilization and nitrogen balance in a wheat-maize rotation system in North China. Agronomy Journal 98: 938-945

462

Table 1 Quantities of added C and labeled N in the treatments of the 56-day incubation experiment.

Treatment[§]	Carbon (mg C per kg soil)	Nitrogen (mg N per kg soil)	Ratio of C to labeled N
Control, CK	0	0	0
W_{10} + $^{15}NO_3$	1000	100	10:1
W_{25} + $^{15}NO_3$	2500	100	25:1
W_{50} + $^{15}NO_3$	5000	100	50:1

464 [§] W: wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the ratio of added C to labeled N.

466

Table 2 Concentration and ^{15}N abundance of total soil organic N and mineral N in $K^{15}NO_3$ labeled and control soil at 0 d (i.e. at the end of pre-incubation)

	$K^{15}NO_3$ soil		Control soil (CK)	
	Concentration (mg kg^{-1})	Abundance (atm %)	Concentration (mg kg^{-1})	Abundance (atm %)
Total soil organic N	1.0±0.01 ($\times 10^3$)	0.41±0.04	1.2± 0.06 ($\times 10^3$)	0.37±0.00
NO_3-N	92.9±0.42	48.59±0.01	15.8±0.40	0.35±0.00
NH_4-N	1.3±0.00	1.18±0.05	1.2±0.12	0.69±0.02

470 Data are the mean ± standard error of three replicates.

472 Table 3 Labeled fertilizer N ($^{15}NO_3$-N) fate at the start and end of the 56-day incubation experiment

Treatment	Total N input (mg kg^{-1}) (1)	Initial labeled N recovery in soil total N (mg kg^{-1}) (2)	Final labeled N recovery in soil total N (mg kg^{-1}) (3)	Final total immobilized labeled N (mg kg^{-1}) (4)	Final labeled mineral N (NO_3-N+NH_4-N) (mg kg^{-1}) (5)	Labeled N recovery in other pools (mg kg^{-1}) (6)=(3)-(4)-(5)	Total N_2O-N emission (μg kg^{-1}) (7)
Control	0	0					5.4±0.6 b
W_{10}+$^{15}NO_3$	129.1	94.6	95.1±4.3 a	19.9±0.6c	56.8±1.8a	18.4±3.3a	6.0±1.9 b
W_{25}+$^{15}NO_3$	162.0	94.6	101.2±3.8a	46.1±1.9b	30.4±1.9b	24.6±4.0a	10.9±0.3a

$W_{50}+^{15}N$ O_3	216.9	94.6	99.0±3.0a	73.3±2.6a	0.4±0.1c	25.3±0.5a	12.0±3.7a

474 NB: Data are the mean ± standard error of three replicates; different lowercase letters denote differences by $LSD_{0.05}$ in the same column.

476 W: wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the ratio of added C to label

478

Fig. 1 Dynamics of total soil organic N and its ^{15}N abundance in different treatments during the 56-day incubation experiment.

NB: data are means of three replicates; $LSD_{0.05}$ values are denoted by vertical lines; W: wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the ratio of added C to labeled N.

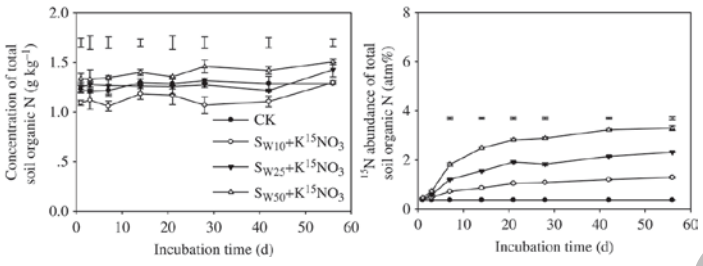


Fig. 2 Dynamics of mineral nitrogen (NO_3-N and NH_4-N) and their ^{15}N abundance in different treatments during the 56-day incubation experiment.

NB: data are the means of three replicates; $LSD_{0.05}$ values denoted by vertical lines; W: wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the ratio of added C to labeled N.

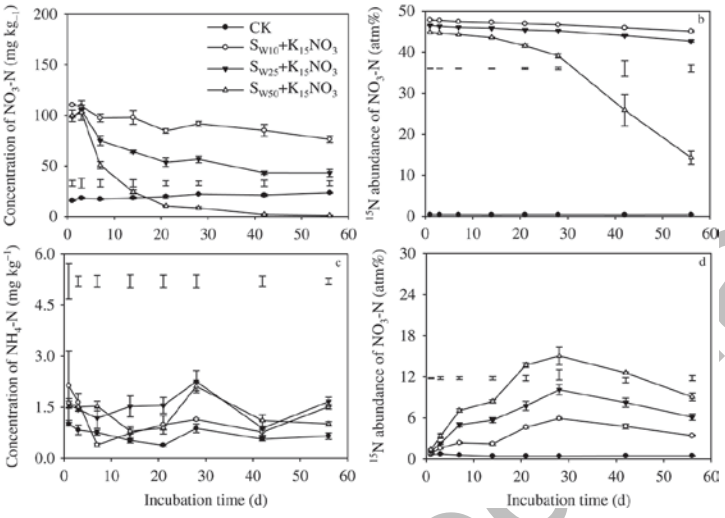
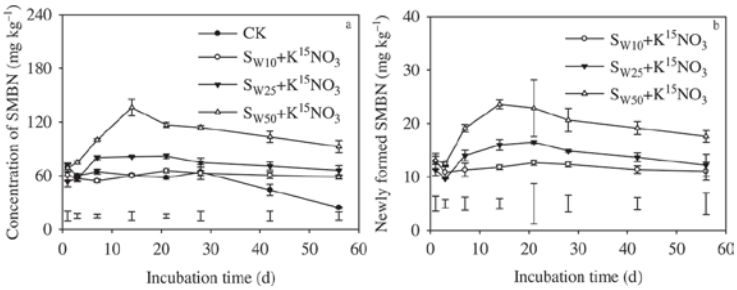


Fig. 3 Dynamics of soil microbial biomass nitrogen (SMBN) and newly formed labeled SMBN in
494 different treatments during the 56-day incubation experiment.

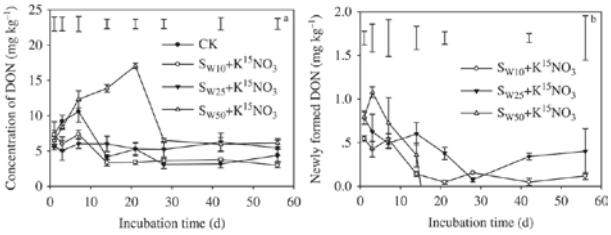
NB: data are means of three replicates; $LSD_{0.05}$ values are denoted by vertical lines; W: wheat
496 straw; superscript number denotes N isotopic signature; subscript number of straw represents the ratio of added C to labeled N.



498

500 **Fig. 4** Dynamics of dissolved organic nitrogen (DON) and newly formed labeled DON in different treatments during the 56-day incubation experiment.

502 NB: data are means of three replicates; $LSD_{0.05}$ values are denoted by vertical lines; W: wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the
504 ratio of added C to labeled N.

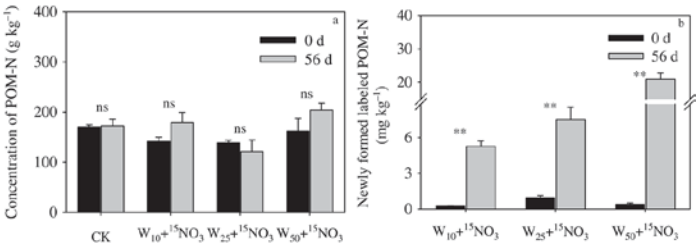


506

Fig. 5 Particulate organic matter nitrogen (POM-N) and newly formed labeled POM-N in different
508 treatments at the start (0 d) and end (56 d) of the 56-day incubation experiment.

NB: data are mean ± standard error; ns: not significant at P>0.05, **: significant at P<0.01; W:

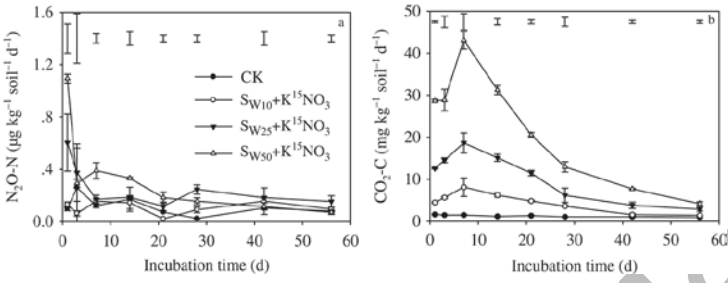
510 wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the ratio of added C to labeled N.



512

514 **Fig. 6** Dynamics of N_2O-N and CO_2-C in different treatments during the 56-day incubation experiment.

516 NB: data are means of three replicates; $LSD_{0.05}$ values are denoted by vertical lines; W: wheat straw; superscript number denotes N isotopic signature; subscript number of straw represents the 518 ratio of added C to labeled N.



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